probed the role of Presenilin (PS), also a key component in AD in axonal transport of APP. We found that reduction of PS and other gamma-secretase
all traits involved. Natural
canonical immune system. Pleiotropy between seemingly unrelated components if physiology is important because such linkages constrain the evolution of
proposed to be a central player in Alzheimer's disease and to regulate axonal transport by kinesin-1. We found that endogenous GSK-3 is a required negative
by dynein intermediate chain. We also analyzed mutations of the serine-threonine kinase Glycogen Synthase Kinase 3 (GSK-3), which has also been
behavior of these vesicles in humans appears to be part of the pathogenesis of Alzheimer’s Disease (AD). Computational analyses of a large number of
moving vesicles in defined genetic backgrounds enabled us to test models of in vivo motor activity and coordination. We discovered several previously
unknown features of in vivo vesicle movement, including the finding that anterograde APP movement powered by kinesin-1 displays the characteristics of
mechanisms, we developed a high-resolution imaging system to track the movement of amyloid precursor protein (APP) vesicles in Drosophila nerve axons.
Bidirectional axonal transport driven by kinesin and dynein along microtubules is critical to neuronal viability and function. To evaluate axonal transport
mechanisms, we developed a high-resolution imaging system to track the movement of amyloid precursor protein (APP) vesicles in Drosophila nerve axons.
The behavior of these vesicles in humans appears to be part of the pathogenesis of Alzheimer’s Disease (AD). Computational analyses of a large number of
moving vesicles in defined genetic backgrounds enabled us to test models of in vivo motor activity and coordination. We discovered several previously
unknown features of in vivo vesicle movement, including the finding that anterograde APP movement powered by kinesin-1 displays the characteristics of
non-processive motors. This finding is largely incompatible with the properties of kinesin-1 derived from in vitro biological analyses. Our data also suggest
that kinesin-1 and cytoplasmic dynein motors assemble in stable mixtures on APP vesicles and that their direction and velocity are controlled at least in part by
dynein intermediate chain. We also analyzed mutations of the serine-threonine kinase Glycogen Synthase Kinase 3 (GSK-3), which has also been
proposed to be a central player in Alzheimer’s disease and to regulate axonal transport by kinesin-1. We found that endogenous GSK-3 is a required negative
regulator of both kinesin-1-mediated and dynein-mediated axonal transport of APP. We also discovered that GSK-3 regulates embryonic transport of an
unrelated kinesin-1/dynein cargo, lipid droplets. In both cases our data suggest that GSK3 affects kinesin-1 motor number on moving cargoes. Finally, we
probed the role of Presenilin (PS), also a key component in AD in axonal transport of APP. We found that reduction of PS and other gamma-secretase
components or activity caused increased velocities of APP vesicles. Together these data reveal that key contributors to AD development in humans may
have important and substantial effects on axonal transport of APP.
Contrasting Patterns of Polymorphism and Divergence in the Evolution of Gene Expression. Patricia J. Wittkopp1*, Joseph D. Coolon2, Kraig
Stevenson2, C. Joel McManus3, Breenton Graveley1. 1) University of Michigan, Ann Arbor; 2) University of Michigan, Ann Arbor; 3) University of
Connecticut Stem Cell Institute, University of Connecticut Health Center, Farmington.
The regulation of gene expression is essential for proper development of an organism and evolutionary changes in this regulation contribute to phenotypic
evolution. Understanding the genetic and molecular mechanisms responsible for regulatory divergence is therefore key to understanding the process of
evolutionary change. RNA-seq (i.e., using next-generation sequencing to determine the distribution of transcripts in a cDNA library) provides a powerful
way to examine patterns of polymorphism and divergence in gene expression genome-wide as well as to investigate the genetic and molecular mechanisms
underlying expression differences. Using this approach, we are examining regulatory changes that have evolved within and between Drosophila species of the
melanogaster subgroup.
Chromosomal Organization of Drosophila melanogaster Genome. Igor F. Zhimulev1,2,*, Tatiana Yu. Vatolina1, Vladimir N. Babenko1,2,*, Sergey A.
Demakov1, Elena S. Belyaeva1. 1) Inst Chemical Biology & Fundamental Medicine, Novosibirsk, Russian Federation; 2) Department of Molecular and
Cellular Biology. The distribution of the molecular and genetic characteristics (unevenness of gene distribution, replication timing and underreplication, gene silencing,
localization of nucleosome density, chromosome proteins and histone modifications) relatively to cytological structures of interphase polytene chromosomes
(bands and interbands) is considered. Regions of densely condensed chromatin (black bands, comprising more than 50% of genome) are represented by
silent chromatin, lately replicated during S phase and comprising specific set of chromosome proteins characteristic for condensed chromatin, very often but
not always proteins of Pc-G-dependent silencing and SUUR protein. These regions known for a long time as an intercalary heterochromatin demonstrate low
density of genes and long intergenic intervals. Diffused, weakly condensed early replicated bands are moderately active in transcription and demonstrate
high gene density. They contain specific protein set contrasting from those of the black bands. Interbands demonstrate the characteristics of open chromatin,
namely specific sets of proteins and histone modifications (Chriz, RNAPol II, BEAF-32, Trx, CP190, BRE1, WDS and GAF, histone modifications
characteristic for open chromatin (data of modENCODE project), high level of P element insertions and low level of nucleosome and histone H1 densities
(taken from MacAlpine et al., 2010), early replication, DNAasel hypersensitivity, however they are not active in transcription and are predominantly
represented by intergenic and non coding sequences. According to computations, Drosophila genome contains about 4000-5000 such regions in diploid cells,
which fits the number of cytologically observable interbands in polytene chromosomes. So, the polytene and interphase diploid chromosomes have similar
patterns of distribution of functional domains reflecting, probably, the basic principle of interphase chromosome organization.

DNA in eukaryotes is packaged with histone proteins into nucleosomes, the basic unit of chromatin. Chromatin is not static; instead, nucleosomes are dynamic particles that promote or hinder DNA access. New nucleosomes are assembled throughout the genome during DNA replication every cell cycle. In addition, regulatory elements, promoters, and active genes undergo repeated cycles of dis-assembly and re-assembly. Conserved histone variants play key roles in the nucleosome dynamics of active regions. Our recent work has focused on characterizing the machinery and mechanisms that assemble variant nucleosomes. Genetic analysis of these mechanisms delineates the importance of nucleosome dynamics for gene regulation, chromosome function, and the heritability of chromatin states.


When the specific species were selected for the 12 genomes sequencing project, an effort was made to choose species with different degrees of genetic divergence. Choosing species with different ecologies and mating systems to gain insights into the genomic underpinnings of the contrasting evolutionary trajectories of each was another guiding principle. Just as Dobzhansky once said that nothing in biology makes sense outside of evolution, we cannot assume that evolution can be fully understood outside ofthe context of ecology. Genomic data reveal that for Drosophila, one size does not fit all. Phenotypic differences among Drosophila observed in the laboratory, especially differences in life history, reproduction and metabolism, can also be extreme. In nature the lives of females and males of different Drosophila species are even more interesting than the differences seen in laboratory studies. I will discuss the lives of flies in nature, focusing on two components of their biology -- reproduction and diet -- and the implications for genetic and evolutionary studies of observations on laboratory populations.

Autoregulatory and Paracrine Control of Synaptic and Behavioral Plasticity by Octopaminergic Signaling. Vivian Budnik. Neurobiology, University of Massachusetts Medical School, Worcester.

Adrenergic signaling has important roles in synaptic plasticity and metamorphicity. However, the underlying mechanisms remain poorly understood. Here we examined the role of octopamine, the invertebrate counterpart of adrenaline and noradrenaline, in synaptic and behavioral plasticity in Drosophila. We show that an increase in locomotor speed induced by food deprivation was accompanied by an activity and octopamine-dependent extension of octopaminergic arbors, and that the formation and maintenance of these arbors required electrical activity. We found that octopaminergic arbor growth was controlled by a cAMP and CREB-dependent positive feedback mechanism that required Oef2R octopamine autoreceptors. Importantly, this autoregulation was necessary for the locomotor response. In addition, octopamine neurons regulated the expansion of excitatory glutamatergic neuromuscular arbors, through Oef2Rs on glutamatergic motorneurons. These studies provide a mechanism of global regulation of excitatory synapses, presumably to maintain synaptic and behavioral plasticity in a dynamic range.


One of the largest surprises of the past decade has been the elucidation of diverse post-transcriptional regulatory pathways mediated by non-coding RNAs <30 nucleotides in length, which guide Argonauta (AGO) complexes to target genes. Three major classes of small RNAs include: (1) miRNAs, ~22 nt RNAs that enter AGO proteins to regulate many endogenous genes, (2) siRNAs, ~21 nt RNAs that populate “Slicer”-type AGO2 proteins to regulate selfish genetic elements and some endogenous genes, and (3) piRNAs, ~24-30 nt RNAs that reside in “PIWI”-type AGO proteins to mediate normal germline development and silence transposable elements. Collectively, these mediate conserved strategies of gene regulation critical for normal development, physiology, and germline integrity. Moreover, their study has provided powerful experimental tools, insights into disease, and new opportunities for therapy. My laboratory has studied canonical and atypical biogenesis mechanisms for all three classes of small RNAs, and recently characterized both Drosha-independent and Dicer-independent mechanisms of miRNA biogenesis. We are exploring further about these mechanisms using both genetic and biochemical approaches. I will discuss recent progress on understanding non-canonical miRNA biogenesis. We are also interested in the function of small RNA genes, and have focused much of our efforts on the nervous system. We have sought to characterize phenotype caused by loss of miRNA pathway components and individual miRNAs usually standard approaches, such as homozygous animals or mitotic clones. These methods can be complicated by developmental consequences of miRNA loss. We developed a positively-marked conditional knockout system that permits excision of FLP-out rescue constructs, and works in post-mitotic cells such as neurons. We are exploring behavioral phenotypes of deleting miRNA pathway components and specific miRNAs in the nervous system or in specific neural subpopulations.


Social insects display not only complex collective organization, but also sophisticated individual problem-solving strategies. We show, for example, that humble bees learn when to learn, and that ants integrate many criteria in their decisions about nest quality. The evolution of such behaviors, and why they are present in some species but not others, can only be understood in the context of the species ecology. For example, learning is adaptive only at

The Mechanics of Shape Change in the Drosophila Embryo. Eric F. Wieschaus1,2*, Adam C. Martin1, Bing He1, Matthias Kasschueb2. 1) HHMI, Department of Molecular Biology, Princeton University, New Jersey; 2) Lewis-Sigler Institute for Integrative Genomic, Princeton University, New Jersey.

With the first three hours of development, the Drosophila embryo establishes a precise pattern of transcription factors that divides the blastoderm into groups of cells destined to form different organs and tissues in the adult. Along the dorsal ventral axis, the first and perhaps most important of these cell fate decisions is the establishment of mesoderm controlled by expression of the Twist and Snail transcription factors. The immediate response of these cell fates decisions is the formation of the ventral furrow or reorganization of the cytoskeleton, adhesion and motor activities to achieve distinct shape. In my talk I will discuss the relationship between the initial transcription profiles and a novel pulsating reorganization of the Actin/Myosin cytoskeleton in the apical region of cells that will make the ventral furrow. We show that the resultant contractile pulses drive cell shape changes in the entire mesodermal primordium. The individual contractions appear to be unpolarized but they result in polarized wedge-like constrictions because global tension in the sheet is polarized along the AP axis. We analyze the force distributions in the mesodermal primordia using a combination of genetics and RNAi to lower adhesive strengths between cells, and laser dissections to locally disrupt the cytoskeleton. We investigate the role of cell membranes in constraining cell shape change and characterize gastrulation movements in mutant embryos blocked in cellularization.
1 Control of the mitotic cleavage plane by local tissue topology. William T Gibson1,2,4,5, James H Veldhuis1, Boris Y Rubinstein2, Heather N Cartwright2, Norbert Perrimon5, G Wayne Brodland2, Radhika Nagpal1, Matthew C Gibson2,6. 1) Program in Biophysics, Harvard University, Cambridge, MA 02138, USA; 2) Stowers Institute for Medical Research, Kansas City, MO 64110, USA; 3) Department of Civil and Environmental Engineering, University of Waterloo, Waterloo, ON N2L 3G1, Canada; 4) Department of Genetics, Howard Hughes Medical Institute, Harvard Medical School, Boston, MA 02115, USA; 5) School of Engineering & Applied Sciences, Harvard University, Cambridge, MA 02138, USA; 6) Department of Anatomy and Cell Biology, Kansas University Medical Center, Kansas City 64110, KS.

It has long been observed that, as a default mechanism, cell shape strongly influences the orientation of the mitotic cleavage plane (e.g. Hofmeister, 1863). However, we still understand very little about the complex interaction between cell shape and cleavage plane orientation in monolayer cell sheets, where cell geometries emerge from a variety of factors, including cell packing, growth, and cell division itself. Here, using mechanical modeling, we predict that the polygonal shapes of individual cells can bias the long axis orientations of their adjacent mitotic neighbors. Confirming these predictions, monolayer cell sheets in both plants and animals exhibit a robust, stereotyped correlation between local cell topology and cleavage plane orientation in vivo. Analytically, we show that this effect derives from fundamental packing constraints. Our results suggest that as a default mechanism, local epithelial topology may be a key determinant of cleavage plane orientation. We proposed that this effect may be a widespread property of cell layers in plants and animals.


In Drosophila embryo, the switch from maternally-controlled to zygotically-controlled divisions (the midblastula transition) is evidenced by a broad range of changes, none more dramatic than the transition from nuclear divisions in a common cytoplasm (syncytium) to a cellularized epithelium just prior to gastrulation. The syncytial divisions occur synchronously and form metaphase furrows, a specialized form of cytokinesis. Rather than bisecting the spindle at anaphase and telophase like traditional cleavage furrows, metaphase furrows encompass the spindle during prophase and metaphase. While quite a bit is known about the composition and regulation of metaphase furrows, very little is known about why traditional cleavage furrows do not form after each syncytial nuclear division. We address this issue by demonstrating that, with one exception, all of the central spindle proteins of conventional cytokinesis (RacGAP50C, Aurora B, Polo, Pavartariu, Foccetto and INCENP) localize both to the site of the metaphase furrow and the central spindle of the syncytial divisions. The exception is Pebble/RhoGEF, which is not transcribed until cellularization. Instead, the syncytial cycles are supplied with RhoGEF2, a maternal-specific RhoGEF. RhoGEF2 localizes to the site of metaphase furrow formation but not the central spindle. Sequence analysis demonstrates that RhoGEF2 lacks the specific domains that localize Pebble/RhoGEF to the central spindle and thus cannot locally activate Rho kinase to initiate a cleavage furrow that bisects the spindle. This is supported by our observation that by driving actin polymerization (which bypasses the need for RhoGEF) in the embryo results in the ectopic formation of cleavage furrows bisecting the spindle prior to cellularization. Our observations describe a simple yet elegant mechanism of separating dividing nuclei while maintaining a syncytium, based largely on the properties of RhoGEF.


The conserved insulin/Target-of-Rapamycin (TOR) pathway regulates cell growth in eukaryotes. In Drosophila, this pathway links nutrition to cell and organismal growth. Although upstream signaling components of the insulin/TOR pathway are well studied, less is known about downstream mechanisms that control cellular metabolism and growth. We, and others, have previously shown that insulin/TOR controls metabolic gene expression. We identified the DNA binding motif for the transcription factor DREF as enriched in promoters of a subset of genes down-regulated upon loss of insulin/TOR signaling. Here we examine a role for DREF in insulin/TOR-mediated growth. We show that homozygous DREF mutants are lethal and exhibit strong larval growth arrest. This effect is likely due to reduced cellular growth, since both mutant and DREF RNAi cells and clones have reduced size in larval imaginal discs and endocycling tissues. Interestingly, these cell growth defects become exacerbated when larvae are cultured in conditions where insulin/TOR activity is reduced, such as in limited nutrition or feeding with the TOR inhibitor Rapamycin. We also find that loss of DREF inhibits TOR-induced cell growth. Moreover, loss of TOR signaling reduces DREF mRNA expression in Drosophila larvae and S2 cell culture. This suggests that DREF may be a target of the insulin/TOR pathway. Our data also indicates that silencing DREF in the larval fat body delays organismal growth and decreases final size. This phenocopies loss of TOR signaling in the fat body and is consistent with a role for DREF in non-autonomous cell growth. One important TOR-regulated process required for growth is the control of ribosome synthesis. We find that DREF is required for the transcription of the ribosome biogenesis (Ribo) genes suggesting one possible mechanism by which DREF could regulate growth.

4 Wbp2, a novel regulator of the Hippo organ size-control pathway. Kieran F. Harvey1, Xiaomeng Zhang1, Claire C. Milton1, Carole L.C. Poon1, Wanjin Hong2. 1) Cancer Cell Biology, Peter MacCallum Cancer Ctre, Melbourne, VIC, Australia; 2) Institute of Molecular and Cell Biology, Singapore.

The Salvador-Warts-Hippo (Hippo) pathway is a key controller of tissue growth in both flies and mammals, and deregulation of pathway activity contributes to tumour formation. The Hippo pathway regulates cell proliferation and apoptosis by restricting activity of the Yorkie transcriptional co-activator protein. The proteins that function together with Yorkie to drive transcription and tissue growth are beginning to be revealed and include the Scalloped, Teashirt and Homothorax transcription factors. Here we define Wbp2 as a promoter of Yorkie-dependent growth of Drosophila melanogaster tissues. Mammalian WBP2 was previously identified as a protein that interacted with the mammalian Yorkie homologue, Yes-associated protein. WBP2 has been shown to enhance steroid hormone-dependent transcription in cultured cells but its in vivo function has remained obscure. We show that D. melanogaster Wbp2 interacts with Yorkie in a WW domain- and PY motif-dependent fashion and that Wbp2 can enhance Yorkie’s transcriptional co-activator properties. In vivo, Wbp2 is rate-limiting for overgrowth of tissues that possess hyperactivated Yorkie protein, thus defining a role for Wbp2 as a downstream component of the Hippo organ size- control pathway.

Both intra- and inter-cellular oncogenic alterations play important roles in malignant transformation of tumors. However, the underlying mechanisms of how each oncogenic mutation cooperates with other mutations to progress toward malignancy remain elusive. Using a Drosophila model system, we studied the genetic pathway of epithelial tumor growth mediated through cell-cell communications. Clones of cells expressing oncogenic RasV12 in Drosophila imaginal epithelia lead to the formation of benign tumors. Using the MARCM technique, we introduced additional mutations in RasV12-expressing clones and isolated a series of mutants that cause non-cell autonomous overgrowth of their surrounding wild-type tissue. The non-cell autonomous overgrowth was not simply caused by a compensatory proliferation triggered by dying cells. We therefore investigated the underlying mechanism of this phenomenon by performing a dominant modifier screen using a series of chromosomal deficiency lines and isolated several mutations that suppressed or enhanced the overgrowth phenotype. Genetic analyses of non-cell autonomous tumor growth mediated by these genes will be presented.

Dynamics and Biomechanics of Histoblast Expansion. Sofia Menezes-Cabral1,2, Carla Prat-Rojo1, Philippe-Alexandre Pouille1, Javier Buceta1, Enrique Martin-Blanco1. 1) IBMB-CSIC, PCB, Barcelona, Spain; 2) Doctoral Programme in Biomedicine and Experimental Biology (PDBEB), CNC, Universidade Coimbra, Coimbra, Portugal; 3) Co.S.Mo. Lab, PCB, Barcelona, Spain.

Tissue remodeling in development and disease involves coordinated invasion of neighboring territories and/or replacement of entire cell populations. Cell guidance, transitions from passive to migratory epithelium, cell growth and death, and extracellular matrix remodeling all impinge on epithelial spreading. Significantly, we are still far from having a clear understanding of how these complex processes are regulated. In order to address and better understand these issues, we use as our model system the expansion of histoblasts (Drosophila abdominal epithelial founder cells), which by active migration, invade and replace the surrounding epithelium of larval epidermal cells (LECs). In previous work, we have identified signaling elements controlling the different division/growth transitions that histoblasts undergo and also the important role Decapentaplegic (Dpp) plays in this system. As a complementary approach to understand the biomechanics of epithelium tissue dynamics and, particularly, how is histoblast expansion process coordinated, we have started to use analytic and quantitative methodologies to extract quantitative parameters from in vivo time-lapse movies. We aim to characterize the wild-type system and answer a series of fundamental questions regarding dynamics of the expansion, cell division (synchrony, division axis orientation), cell apical constriction and delamination from the epithelium. Taking advantage of two different approaches/tools (watershed based segmentation and PIV analysis of the movies), we are now able to get information about the general behavior and dynamics of the nest during expansion, and also data for each individual histoblast within the nest. This will provide us with an unbiased parametric framework that will allow future analysis of mutant conditions. Moreover, hydrodynamic modeling of the process provides insight of the active forces developed in time and space.


The role of the conserved Salvador-Warts-Hippo (SWH) tumor suppressor pathway in growth control is a topic of great interest, yet the mechanism by which pathway activity is controlled upstream of the core SWH components remains elusive. Here we report the identification of a novel SWH pathway member, Tao-1. Like Hippo, Tao-1 is a member of the sterile-20 kinase family, but it has not previously been implicated in proliferation control. Loss of Tao-1 function results in the hallmark SWH characteristics of overgrowth of imaginal discs, an expansion of the apical domain of epithelial cells, and activation of Yorkie transcriptional targets. Consistent with a role in SWH signaling, these effects are dependent upon Yorkie. Tao-1 also displays genetic interactions with previously characterized members of the SWH pathway. In S2 cells, we find that Tao-1 can promote the phosphorylation of both core SWH pathway regulators and the transcriptional co-activator Yorkie via a PPxY:WW-domain interaction. Myopic interacts with previously characterized members of the SWH pathway.

A Screen for Conditional Tumor Suppressor Genes identifies Myopic as an Endosomal regulator of Yorkie nuclear signal outputs. Melissa Gilbert1, Marla Tipping2, Alexey Veraka3, Kenneth H. Moberg1. 1) Department of Cell Biology, Emory University School of Medicine, Atlanta, GA; 2) Department of Biology, University of Massachusetts, Boston MA.

Recent studies in Drosophila melanogaster have uncovered growth suppressor genes whose mammalian homologs have clear roles in tumorigenesis. In view of the widely accepted notion that most initiating oncogenic lesions in mammals lead to apoptosis, we designed and implemented a tissue-specific FLP/FRT mosaic screen in the Drosophila eye for mutations that require a block in apoptosis to unleash a growth advantage. A chromosome carrying a small deletion (H99) that prevents all developmentally programmed cell death was mutagenized and subject to mitotic recombination to generate clones of mutant tissue in the eye. In this manner, we are able to identify rare mutations that confer a growth advantage upon mutant cells in the context of a block in apoptosis.

We identified alleles of myopic, the Drosophila homolog of the candidate tumor-suppressor and endosomal regulator His-domain-protein-tyrosine-phosphatase (HD-PTP), as mutations that require a synergistic block in apoptosis to promote tissue overgrowth. We find that Myopic restricts signaling by the Salvador/Warts/Hippo (SWH) tumor suppressor pathway and binds the transcriptional co-activator Yorkie via a PPxY:WW-domain interaction. Myopic co-localizes with Yorkie at endosomes, and loss of Myopic alters Yorkie endosomal association and elevates Yorkie protein levels. Analysis of myopic gain and loss-of-function activity indicates that it controls expression of pro-growth Yorkie targets but not the anti-apoptotic gene diap1. These data establish Myopic as a novel Yorkie-regulator and implicate Myopic-dependent control of Yorkie endosomal complexes as a regulatory step in determining nuclear outputs of the Salvador/Warts/Hippo pathway.
The regulation and maintenance of adult cardiac function, and provide a model for the Let-7 miR family in vertebrates. We are also in the process of investigating the functions of other cardiac-expressed miRs in the Drosophila heart model.

Contribute to the let-7 phenotype by reducing their function in a let-7 mutant background. These studies are expected to demonstrate the importance of let-7 in several mRNAs of genes that have previously been found to be critical for heart function are up-regulated in let-7 hearts, including the Calcium channel of let-7, as well as for potentially (indirect) effectors, including ion channel genes that may account for the rhythm anomalies. Indeed, we observed that piRNA-mediated adaptation to transposon invasion in Drosophila.

We showed previously that piRNA-mediated adaptation to transposon invasion in Drosophila, maternally deposited piRNA clusters, and that these new insertions are the source of piRNAs and are inherited with high fidelity by the offspring of the dysgenic females, however, a subset of mobilized resident transposons do show biased insertion into into piRNA clusters, and that these new insertions are the source of piRNAs and are inherited with high fidelity by the offspring of the dysgenic females, consistent with a role in silencing. P-element transposon invasion thus triggers genome-wide transposon mobilization, and adaptation to transposon invasion is linked to both de novo piRNA processing and genetic modification of the germline.

MicroRNAs (miRs) are emerging as major contributors to cardiovascular diseases, but only few specific miRs have been characterized in the heart, in part because of redundancy that complicates analysis. We are using the genetic power and low genetic redundancy of the fly, coupled with sophisticated optical method for analyzing and quantifying heart contraction parameters, to investigate the cardiac roles of miRs. Heart-specific miR expression profiling revealed that let-7, the only let-7 family miR in Drosophila, as highly expressed in the adult heart. Investigating the requirement for let-7 in cardiac physiology, we found that let-7 mutants display a slower, highly arrhythmic heartbeat, including a high incidence of non-beating events (asystoles), as well as a significantly dilated heart phenotype. To further characterize the role of let-7 in the heart, we performed heart-specific qRT-PCR for direct candidate targets of let-7, as well as for potentially (indirect) effectors, including ion channel genes that may account for the rhythm anomalies. Indeed, we observed that several mRNAs of genes that have previously been found to be critical for heart function are up-regulated in let-7 hearts, including the Calcium channel cacophony, the K-ATP channel subunit dSur and the Ks channel KCNQ. We are currently testing whether these potential let-7 targets and effectors contribute to the let-7 phenotype by reducing their function in a let-7 mutant background. These studies are expected to demonstrate the importance of let-7 in the regulation and maintenance of adult cardiac function, and provide a model for the Let-7 miR family in vertebrates. We are also in the process of investigating the functions of other cardiac-expressed miRs in the Drosophila heart model.
13 miRNA-mediated feedback inhibition of JAK/STAT morphogen signaling establishes a cell fate threshold. Wan H. Yoon, Denise J. Montell. Department of Biological Chemistry Center for Cell Dynamics Johns Hopkins University School of Medicine 855 North Wolfe St., Suite 450 Baltimore, MD 21205, USA.

An essential regulatory mechanism in embryonic development is the patterning of cell identities by morphogens. Although by definition morphogens are graded, all-or-none responses can occur over short distances, even between neighboring cells. The mechanisms by which graded signals are converted into all-or-none responses are incompletely understood, however. In the Drosophila ovary, the secreted morphogen Unpaired (Upd) specifies the migratory border cell population by sustained, high level activation of the JAK/STAT pathway and specifies a non-migratory population of squamous anterior follicle cells by lower level and transient JAK/STAT activity. Here we identify miR-279 as a key component of a feedback inhibitory pathway that terminates the response to Upd in cells that receive a low level of the JAK/STAT activation. We found that miR-279 directly repressed STAT expression at the post-transcriptional level. Loss of miR-279 perturbed interpretation of the Upd gradient by anterior follicle cells and resulted in cell specification and migration defects similar to ectopic activation of JAK/STAT signaling or loss of Apoptic (APT), a known feedback inhibitor of STAT. APT is essential for miR-279 expression in the anterior follicle cells that do not migrate. Within forming border cells by contrast, another STAT target, Ken and Barbrie (Ken), down-regulates miR-279. Thus, miR-279, APT, and Ken cooperate to form a regulatory circuit that sharpens the cellular response of migratory and non-migratory follicle cells to the Upd gradient.

14 The Drosophila pan gu kinase complex regulates RNP stability via ubiquitin-dependent proteinolysis. James E. Wilhelm, Brian Sato, Risa Broyer. Cell and Developmental Biology, UC San Diego, La Jolla, CA.

While mRNA is considered to be quite labile, maternal mRNA is highly stable with a half-life greater than two weeks in the oocytes of some organisms. This stability is thought to be due to the protective effects of the protein subunits of maternal RNA-protein (RNP) complexes. Unfortunately, this extreme mRNA stability, while an asset when the oocyte is stockpiling transcripts to drive early embryogenesis, poses a potential problem for the maternal to zygotic transition when many maternal messages are degraded to pave the way for the control of development by zygotic transcription. Previous studies have focused on defining the pathway for maternal mRNA degradation:activation of the protein kinase, Pan gu, triggers translation of the RNA binding protein, Smaug, which in turn recruits the CCR4/Twin deadenylase causing message destruction. However, these studies also demonstrated that that there is an additional branch pan gu dependent branch in the pathway that is required for mRNA degradation. We have found that in addition to regulating mRNA stability, pan gu is required for the specific degradation of three subunits of the maternal RNP complex - the translational repressor, Cup, the RNA helicase, Mec31B, and the LSm domain protein, Tral. The degradation of these three subunits occurs independently of mRNA degradation demonstrating that pan gu regulates two distinct pathways that degrade maternal RNPs - a pathway that regulates mRNA stability and a pathway that regulates the stability of the proteins that protect maternal messages. Furthermore, Cup, Tral, and Mec31B are degraded by ubiquitin-mediated proteinolysis and are degraded throughout the early embryo except for a small pool of protein that is associated with polar granule material at the posterior - the site where messages such as nanos are protected from degradation. These results suggest that the regulation of maternal protein stability may contribute to the spatial regulation mRNA stability in the early embryo.


Intracellular mRNA localization is a conserved mechanism for generating asymmetry in eukaryotic cells. Localization of oskar (osk) mRNA to the posterior pole of the Drosophila oocyte during mid-oogenesis and its subsequent translation is essential to initiate the formation of germ plasm, a specialized cytoplasmic marker necessary for germline development. The germ plasm is also essential for development of the anterior-posterior body axis through its role in posterior recruitment of the abdominal determinant nanos (nos) during late oogenesis. Localized mRNAs are recognized by complexes of proteins that bind to cis-acting localization signals and mediate mRNA trafficking and anchoring. We identified two factors, Lost and Rump, that purify biochemically with the nos localization signal and are important for the recruitment of nos to the germ plasm. Surprisingly, although Lost and Rump were isolated as localization factors for nos mRNA, they act together to direct a second phase of osk localization that occurs concomitant with nos localization during late oogenesis. Through an analysis of rump and lost null mutants combined with live imaging of osk mRNA, we have determined that localization of osk during mid-oogenesis is not sufficient for osk-dependent germ plasm function in either anterior-posterior body axis or pole cell formation. Rather, the continued accumulation of osk mRNA results in an amplification of the germ plasm that is essential for abdominal and germine development. We propose that Rump and Lost are part of a core localization complex that promotes utilization of the late localization pathway by multiple mRNAs.

16 Drosophila HPat interacts with the fragile X mental retardation protein and the miRNA pathway to regulate synaptic structure. Sarala J Pradhan1, Leslie M Rozeboom1, Ravijot Dhatt1, Mani Ramaswami2, Scott A Barbee1. 1) Department of Biological Sciences and Eleanor Roosevelt Institute, University of Denver, Denver, CO 80208, USA; 2) Smurfit Institute of Genetics and TCIIN, Lloyd Building, Trinity College Dublin, Dublin-2, Ireland.

The local synthesis of new proteins is required to consolidate and maintain long-term changes in synaptic efficacy and synapse structure. Synaptic mRNA translation is regulated by sequence motifs within mRNAs that act in concert with specific mRNA binding proteins and microRNAs (miRNAs). Together, the composition of these ribonucleoprotein (RNP) particles determines whether mRNAs are transported to the synapse, locally translated, or targeted for storage and/or degradation. We have previously shown that RNPs in Drosophila neurons share a highly conserved mRNA regulation machinery with cytoplasmic RNA processing bodies (P-bodies). P-bodies are sites of both general and miRNA-mediated translational regulation. This study focuses on the function of HPat, a highly conserved P-body protein, involved in P-body assembly, translation repression, and mRNA degradation. We have found that HPat is expressed in the Drosophila larval central nervous system (CNS) and co-localizes to puncta with the Fragile X Mental Retardation Protein (FMRP) in neurites of primary cultures of larval motor neurons. Furthermore, our studies indicate that HPat is a dominant modifier of FMRP function at the larval neuromuscular junction (NMJ) and functions on the presynaptic side of the NMJ to regulate synapse size via the miRNA pathway. Together, our results suggest that HPat has essential functions in the control of FMRP- and miRNA-mediated plasticity processes in neurons in vivo.

Neural stem cells or neuroblasts (NBs) are responsible for producing the diversity of neural and glial lineages that populate the Drosophila central nervous system. Extensive studies of the ventral nerve cord have revealed mechanisms involved in NB formation and specification; however, comparatively little is known about how these same processes occur in the developing brain. Here we investigate the embryonic primordium of the dorsomedial brain, a neurogenic region of the procephalic neuroectoderm that comprises a system of small identifiable neuroepithelial placodes, which maintain a constricted apical connection throughout the period of NB delamination. Here, we present an analysis of cell fate specification in one such placode that uniquely expresses Castor and dChtX1 in a group of about 8 cells. We show that the placode produces at least three distinct NB fates: (1) a single Type I neurosecretory (NSC) NB that produces the brain insulin-producing cells (IPCB); (2) a single Type II Posterior ASENSive Negative NB; and (3) multiple non-NSC Type I NBs. The IPC NB is stereotypically the first NB to delaminate and is the only NB to produce NSC lineages from the placode. Leading to the specification of the IPC NB, the placode exhibits several coordinated activities: It undergoes a synchronous round of mitosis before neurogenesis; and it activates EGFR and Notch in its placode cohorts, independent of the signaling state of neighboring placodes. Loss of Delta/Notch signaling around the time of IPC NB formation causes all placode cells to delaminate en masse and develop entirely as NSC/IPC NBs. Loss of Spitz/EGFR signaling causes cell death of all placode cells, except for the primary fated IPC NB, whose lineage survives normally. In summary, our results suggest that this placode, which may represent the general properties of dorsomedial placodes, begins from an equipotential state whose default fate is the IPC NB. Following an initial state of IPC NB competence, the remaining placode cells switch to a non-NSC fate potential state(s) that requires EGFR signaling for cell survival.

The bHLH Repressor Deadpan is Differentially Required for Maintaining the Self-renewal of Two Distinct Types of Neural Stem Cells in Drosophila. Sijun Zhu1, Jill Wildonger1, Suzanne Barashow1, Susan Younger1, Yaling Huang2, Tzumin Lee1, Lily Jan1, Yuh Nung Jan1. 1) Dept of Physiology, Howard Hughes Medical Institute, University of California San Francisco, San Francisco, CA; 2) Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA.

Neural stem cells (NSCs) are able to self-renew while giving rise to neurons and glia that comprise a functional nervous system. However, how NSC self-renewal is maintained is largely unknown. Using the Drosophila NSCs called neuroblasts (NBs) in the developing larval brain as a model, we demonstrate that the conserved bHLH repressor Deadpan (Dpn) is required to maintain NB self-renewal. The loss of dpm function leads to premature loss of NBs and precocious accumulation of nuclear Prospero (Pros) in NBs. The loss of NBs in dpm mutants can be partially rescued by lowering Pros expression. Conversely, dpm over-expression increases the number of NBs and causes NBs to persist into adulthood, which never occurs in wild type animals. Notably, type II NBs, which generate transit amplifying intermediate progenitors similar to vertebrate NSCs, are more severely affected by changing Dpn levels than type I NBs. Furthermore, over-expressing certain proneural genes while lowering Dpn expression drastically reduces the type II, but not the type I, NBs. Thus, Dpn is both necessary and sufficient for maintaining NB self-renewal. While the mechanistic details in maintaining self-renewal differ between these two NB types, our study reveals that Dpm maintains NB self-renewal at least in part by regulating the expression of Pros and/or antagonizing the activity of proneural proteins.


Emerging evidence in both vertebrates and invertebrates is redefining glia as active and mobile players in synapse formation, maturation and function. We use Drosophila melanogaster, a simple and genetically tractable model system, to understand the molecular mechanisms by which glia communicate with neurons at glutamatergic neuromuscular junctions (NMJs). Wnt/Wg signaling plays a pivotal role during synapse development and plasticity, including the coordinated development of the molecular architecture of the synapse. While previous studies demonstrated that Wg is secreted by motor neurons, we here provide evidence here that a significant amount of Wg at the NMJ is additionally provided by glia. We have previously shown that a specific subtype of glia, subperineuronal peripheral glia cells (SPGs), establish dynamic transient interactions with synaptic boutons of the NMJ. Here we report that loss of glia function in mutants of reverse polarity (repo) results in a dramatic disruption of synapse growth and glutamate receptor (GluR) clustering, a phenotype that is also observed in wg mutants. Interestingly, we found decreased synaptic Wg levels in both repo mutants and larvae expressing repo RNAi in SPGs. In contrast, gain of Repo function in SPGs increased synaptic Wg levels, suggesting that glia could also secrete Wg to influence synapse development and function. This model was supported by the observation that when Wg::GFP was expressed in SPGs, the GFP label was observed throughout the NMJ. Furthermore, RNAi mediated knock-down of Wg in SPGs decreased GluR clustering, although it did not affect overall synaptic growth. In summary, our results suggest that Wg expression is regulated by Repo in SPGs, and that glial-derived Wg, together with motor neuron-derived Wg, orchestrate different aspects of synapse development.


Here, we show that the Drosophila homolog of Chd7, kismet, is required for proper axonal pruning, guidance, and extension in the developing fly central nervous system. In addition to defects in neuroanatomy, flies with reduced kismet expression show defects in memory and motor function. Mutations in Chd7 have been associated with CHARGE Syndrome (CS, OMIM #214800), a rare autosomal dominant disorder. We suggest that these analyses of kismet function in the fly CNS allow for a better understanding of the role of kismet in neural development, and Chd7 in CS pathogenesis.

Selective Disruption of Dscam1 Homophilic Interaction Reveals Its Essential Role in Neuronal Self-Avoidance. Wei Wu1, David Baker2, Larry Zipursky1. 1) Department of Biological Chemistry, HHMI/UCLA, Los Angeles, CA; 2) Department of Biochemistry, HHMI/University of Washington, Seattle, WA.

Drosophila Dscam1, a homologue of the human Down syndrome cell adhesion molecule (DSCAM), is an immunoglobulin (Ig) superfamily protein, which plays a crucial role in forming precise neuronal connections in the Drosophila brain. Through alternative splicing, Dscam1 potentially gives rise to 19,008 different extracellular domains linked to one of two alternative transmembrane segments. Isoforms exhibit exquisite isoform-specific homophilic binding. Dscam1 is required for promoting branch segregation of both dendritic and axonal processes in vivo. Expression studies showed that each individual neuron expresses multiple Dscam1 isoforms chosen in a largely random fashion. These studies led to a model that Dscam1 diversity gives individual neurons a unique identity, and through homophilic repulsion, Dscam1 promotes self-avoidance function. Here we critically assess the role of Dscam1 homophilic
interaction in vivo. Using X-ray crystal structures as a guide, we generated Dscam1 mutant isoforms that have lost the ability to bind homophilically. To ensure that these mutations altered specificity rather than more general structural features of the ectodomain, mutations were designed to not only lose homophilic binding, but to simultaneously acquire heterophilic binding specificity. This change in specificity from homophilic to heterophilic binding was demonstrated in biochemical experiments and in vivo. Using homologous recombination, we introduced the homophilic binding deficient isoforms into the endogenous Dscam1 locus. We showed that Dscam1 homophilic interaction is required for both axons and dendrites to distinguish between self and non-self processes. Since vertebrate DSCAMs also exhibit homophilic interaction and promote self-avoidance, we propose that homophilic repulsion is a general property of Dscam proteins and provides the molecular basis for Dscam mediated self-avoidance.

22 Kataninp60-Like1 regulates dendritic outgrowth of Drosophila larval class IV sensory neurons and the nocifensive response by promoting microtubule growth. Andrea Stewart1, Dan Tracey2, Nina Tang Sherwood1. 1) Biology, Duke Univ, Durham, NC; 2) Anesthesiology, Cell Biology and Neurobiology, Duke Univ, Durham, NC.

Dendritic arbors of multi-dendritic arborization (da) neurons show remarkable diversity in branch morphology and function, but the molecules and mechanisms that confer these characteristics are not well understood. Class IV arbors are the most highly branched of the four da sensory neuron classes and are known to mediate larval nocifensive signals. We show that the putative microtubule-severing protein, Katanin p60-like 1 (Kat-60L1, CG1193) is required for the proper outgrowth of class IV da arbors during larval development and for normal nocifensive responses. In kat-60L1 mutants, terminal branch number and branch length are reduced and larvae exhibit reduced sensitivity toward noxious heat and mechanical stimuli compared to wild-type controls. Expressing kat-60L1 RNAi specifically in class IV neurons recapitulates the mutant phenotypes, while expression of a Venus-tagged UAS-kat-60L1 transgene in this neuronal subset completely restores mutant phenotypes. Kat-60L1 therefore functions within class IV neurons to sculpt the complex dendritic arbor. To address whether this function is mediated through Kat-60L1 regulation of the microtubule network, we used live imaging of GFP-tagged EB1, a plus-end tracking protein, to visualize and measure microtubule dynamics in real time. kat-60L1 mutant larvae exhibit a 2-fold reduction in the number of EB1-GFP comets in class IV dendrites compared to wild-type controls but no change in the speed of growing microtubules. The specific reduction in growing microtubule number suggests that Kat-60L1 promotes new microtubule growth in class IV neurons via microtubule severing. These findings suggest a novel role for Kat-60L1 and provide insight into how microtubule-severing proteins contribute to dendritic morphology and nocifensive function through the regulation of neuronal microtubules.

23 dEHBP1 regulates endocytosis and active zone assembly at the developing neuromuscular junction. Nikos Giagtzoglou1, Yong Qi Lin1, Claire Haueter1, Hugo Bellen1,2,3,4. 1) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX. 77030; 2) Program in Developmental Biology, Baylor College of Medicine, Houston, TX. 77030; 3) Department of Human and Molecular Genetics, Baylor College of Medicine, Houston, TX. 77030; 4) Department of Neuroscience, Baylor College of Medicine, Houston, TX. 77030.

dEHBP1 regulates intracellular trafficking of various components of Notch signaling pathway and loss of dEHBP1 results in defects in patterning and cell fate acquisition. However, homozygote escapers of weak loss of function mutations of dEHBP1, that do not affect Notch signaling, display uncoordinated movement, suggesting that dEHBP1 may affect neuronal function. We examined synapses at the larval neuromuscular junction (NMJ) of dEHBP1 mutant animals and observed that abnormal vesicular structures appear within synaptic terminals of dEHBP1 mutants, similarly to other endocytic mutants. Accordingly, electrophysiological studies at the larval NMJ of dEHBP1 mutants reveal that under conditions of intense stimulation, neurotransmission cannot be maintained. Importantly, Eps15, a protein that is involved in scaffolding of the endocytic network, is mislocalized. Furthermore, in dEHBP1−/− synaptic terminals, the number of the active zones are decreased and key components of active zones, such as Bruchpilot, are mislocalized. These results indicate that dEHBP1 is simultaneously a novel, critical component of the endocytic network throughout the synaptic vesicle cycle and regulator of active zone assembly. To understand the mode of action of dEHBP1, we are assessing the interplay of dEHBP1 with other endocytic proteins such as Eps15, at the biochemical, cellular and electrophysiological level. In addition, we are also exploring the functional relationship of dEHBP1 with key players of AZ development, such as Rab3 GTPase. Thus, dEHBP1 controls structural and functional aspects of synapse development and function. Further analysis of dEHBP1 may elucidate fundamental mechanisms of structural and functional synaptic plasticity.

24 Rotund regulates odorant receptor choice by specifying sensillar subtypes. QINGYUN LI1, TAL SOO HA1, DEAN P. SMITH1, PELIN C. VOLKAN1,2. 1) Biology, Duke University, Durham, NC; 2) Duke Institute for Brain Sciences, Durham, NC.

In fly olfactory system, a given class of ORNs express roughly only one out of 60 OR genes in the genome. Recent analysis of OR cis-regulatory elements revealed a hierarchical model for OR choice. However, molecular components of the regulatory network are largely unknown. In flies, ORNs are housed in clusters of up to four cells within olfactory sensilla covering the surfaces of two sensory organs, the antenna and maxillary palp. These sensilla are classified into three types: basiconica, trichoidea, coeloconica, and each of them can be further divided into subtypes. For example, trichoidea consists of 4 subtypes, which house ORNs that express stereotypical subsets of OR genes: at1 (Or67d), at2 (Or23a, Or83c), at3 (Or19a, Or2a, Or43a), and at4 (Or47b, Or88a, Or65a). In addition, different sensillar subtypes and thus the ORN classes they house are restricted to different zones on the sensory organs. From a previous electrophysiological screen, we recovered mutants in rotund, which lacked responsiveness to fly pheromone cVA, normally detected by Or67d ORNs in at1 sensilla. Here, we show evidence that it is due to the sensory conversions of at1 and at3 ORNs to at4 sensillar subtype ORNs. Further analysis suggests that the kruppel like zinc-finger transcription factor rotund is expressed in a third of ORN classes (including at1, at3), and broadly regulates OR gene expression by specifying sensillar subtypes within three main types of antennal sensilla. Mutations in rotund cause conversions of sensillar subtypes, associated with zonal expansions of some OR genes at the expense of others. Our results point to the presence of default sensillar fates which have to be repressed in order to execute others. Rotund is the first example of such a regulatory molecule that leads to the diversification of sensillar subtypes. Our findings fill an important gap in our understanding of the developmental events during the sensory specification of individual classes of ORNs.
The assumption that distinct base substitutions are the result of independent events forms a lynchpin for the study of molecular evolution and population genetics. Bloomington, IN.

Details of individual presentations can be located in the online Schedule of Events at drosophila-conf.org.

PLATFORM: Evolution and Quantitative Genetics
Details of individual presentations can be located in the online Schedule of Events at drosophila-conf.org.

The Drosophila Genetic Reference Panel is a community resource of 192 inbred Drosophila melanogaster lines with measured quantitative traits. 40 lines have been sequenced to a minimum of 12X coverage using both 454 and Illumina sequencing platforms. The remaining 152 lines are being sequenced using the Illumina platform. Alignments have been generated using BWA, and polymorphisms identified using a newly developed method, accounting for the exact population structure of this data. We find approximately 500,000 SNPs, and 50,000 indels per inbred line, relative to the reference sequence. Extensive quality control genotyping has ensured both sequence and strain sample integrity, matching and true homozygosity for all of the lines. Contaminated and heterozygous lines have been replaced to ensure no confounding effects on association studies. Test whole genome association experiments on the released Freeze 1, (162 lines) lines have identified between 20-100 polymorphism associated with complex traits with p-values less than 10-5 Most of these genes are novel, and many have pleiotropic effects on multiple traits. Thus, we believe this tool will provide a new understanding of Drosophila genetics, complementary to the large body of work based on artificially induced mutational screens. We also present multiple methods for the validation of associations. As a community resource we are working to facilitate the reference panels use in as many setting as possible. The fly stocks are currently available from the Bloomington stock center. We are also preparing a website for genome wide associations allowing input of phenotypes measured on the lines, and outputting putative polymorphisms and associated p-values. We hope this web tool will allow any Drosophila geneticist with the ability to work on flies to utilize the reference panel - even to the level of high school students counting leg bristles.

26 Widespread multi-nucleotide mutation events in Drosophila melanogaster and other organisms. Daniel R. Schrider1,2, Jonathan N. Hourmozdi1,2, Matthew W. Hahn1,2, 1) Department of Biology, Indiana University, Bloomington, IN; 2) School of Informatics and Computing, Indiana University, Bloomington, IN.

The assumption that distinct base substitutions are the result of independent events forms a lynchpin for the study of molecular evolution and population genetics. This assumption allows inferences of the time since divergence between sequences, estimates of effective population size and mutation rates, and detection of signatures of natural selection. However, mutation events involving multiple substitutions have been observed in variety of taxa.

In order to determine whether such multi-nucleotide mutations (MNMs) are present in Drosophila, we examined single nucleotide polymorphisms in 37 D. melanogaster genomes. In this data set, we found tens of thousands of groups of neighboring substitutions that occurred on the same lineage and exhibit only two haplotypes, while few of these events would be expected if all mutations were independent. We also sequenced 8 D. melanogaster mutation accumulation (MA) lines and found that several MNMs had arisen during the experiment. Examinations of previous MA studies in D. melanogaster, C. elegans, S. cerevisiae, and A. thaliana all found MNMs when none would be expected if mutations were independent. We also examined two human genomes and found tens of thousands of MNMs.

Our results strongly suggest that these multi-nucleotide mutation events occur either simultaneously or in rapid succession. In either case, these events represent a sizeable percentage of mutations (>2%) across a wide variety of species, and can confound analyses that assume mutations are independent. MNMs also provide alternative explanations for events that seem puzzling under the assumption of independent mutations: for example, they provide a mechanism for compensatory mutations to occur simultaneously (or nearly simultaneously), thereby avoiding any low-fitness intermediate states.


The Neutral Theory of molecular evolution has provided a powerful context for the development of null models of evolutionary change. The McDonald-Kreitman (MK) test compares the levels of divergence between species to the levels of polymorphism within species for synonymous and non-synonymous nucleotide positions. Here we develop an MK format for phenotypic change of specific mitochondrial DNA variants in controlled nuclear genetic backgrounds. We use the levels of synonymous divergence and polymorphism in complete mitochondrial genomes as the neutral contrast, and compare these to levels of divergence and polymorphism for several fitness and life history traits in strains of D. melanogaster carrying mtDNAs from D. melanogaster and D. simulans introgressed using clean balancer chromosome replacement. For virtually all phenotypes (fecundity, development time, desiccation, starvation, chill coma recovery, hypoxia stress, mitochondrial enzyme complex activity) there was no detectable effect of species-level divergence of mtDNA. In contrast, most of the phenotypes showed statistically significant variation among individual strains of mtDNA within each species (Dmnel or Dsim). When scaled against neutral divergence and polymorphism, the data revealed that there is between 10 and 30 times more phenotypic polymorphism among mtDNAs within species versus divergence between Dmnel and Dsim mtDNA. These data show that the ~100 amino acid differences between Dmnel and Dsim mtDNA are neutral substitutions, while standing variation in mtDNA is consistent with segregation of mildly deleterious phenotypic variation. This study underscores the role of purifying selection in the evolution of Drosophila mtDNA and points to the generality of segregating mtDNA polymorphisms as a source of disease alleles.
Ultra-fine scale recombination heterogeneity in *D. melanogaster*. Nadia Singh¹, Eric Stone¹, Charles Aquadro², Andrew Clark². 1) Genetics, North Carolina State University, Raleigh, NC; 2) Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Heterogeneity across the genome in recombination intensity has a major impact on the extent to which natural selection affects linked sites. In *Drosophila*, inference of recombination intensity across the genome, measured as cM per Mbp, has relied on the local slope of the relationship between the physical and genetic maps. However, the resolution of these methods is limited by the genetic map, and apart from a few targeted regions like the *rosy* locus, considerable uncertainty remains about the scale at which local recombination intensities vary. Here we assess the magnitude of recombination intensity variation in *D. melanogaster*, and determine the physical scale at which it occurs. We generated a strain of *D. melanogaster* carrying mutations in *garnet* and *scalloped*; these X-linked genes are separated by ~2 Mb and ~7 cM. We screened hundreds of thousands of flies to identify over 6700 males containing a single recombination event in this region. Using a combination of sequence-capture and multiplex next-generation sequencing technologies, we sequenced this 2 Mbp region to a total of ~6400-fold coverage in pools of our recombinant males. After applying the Joint Genotype for Inbred Lines (JGIL) to these data, we identified over 6000 high-confidence SNPs differentiating our parental lines. We developed a novel joint restricted maximum likelihood estimator to infer the allele frequency of each SNP in each pool of recombinant males, and translated the changes in allele frequency across the 2 Mbp interval to infer local recombination intensities. Preliminary analysis indicates heterogeneity in recombination intensity spanning two orders of magnitude. This recombination map of maximal resolution reveals the subtleties of recombination intensity fluctuation in *D. melanogaster* in unprecedented detail, which in turn helps inform our understanding of the appropriate physical scale to consider when considering the interplay between selection and linkage.

29 Intraspecific variation in recombination rates in *Drosophila melanogaster* based on ultra-dense whole-genome genetic maps. Josep M. Comeron¹,², Ramesh Ratnappan³, Samuel S. Bailin¹. 1) Department of Biology, University of Iowa, Iowa City, IA; 2) Roy J. Carver Center for Genomics, University of Iowa.

Recombination rates vary significantly across genomes, not only differentiating regions of suppressed and non-suppressed recombination but at a much smaller scale. The resolution of most genetic maps in *D. melanogaster*, while appropriate for detecting general tendencies associated with recombining regions, are however too coarse to enable accurate analysis of evolutionary patterns at the scale of single/few genes. Additionally, population genetic models often assume that for a given genomic region the recombination rate is invariant among individuals within species even though intra-specific differences in crossover frequencies have been documented in many organisms at a cursory scale. As a part of an integrative study to investigate intra- and inter-specific variation in recombination rates in *Drosophila*, we have generated ultra-dense genetic maps in *D. melanogaster* to capture within and between population variation using direct estimates of crossing over frequencies. To this end, we have genotyped thousands of RAILs (Recombinant Advanced Intercross Lines) using single-fly libraries and Illumina genomic sequencing. Here we present a collection of whole-genome, high-density genetic maps based on more than 25,000 crossing over events with a median distance between SNP markers of less than 3Kb. We describe intra-specific variability in recombination landscapes across the genome and discuss evolutionary and genomic consequences of fine variation in recombination rates along chromosomes as well as between individuals.

30 Genetic asymmetry at the Dobzhansky-Muller gene *Lhr* is the result of cis-by-trans regulatory divergence of an ancestral hybrid lethal function. Shamoni Maheshwari, Daniel Barbash. Dept Molec Biol & Gen, Cornell Univ, Ithaca, NY.

Reproductive isolation between closely related taxa is in part caused by hybrid incompatibility, the sterility and inviability of interspecies offspring. To explain the evolution of such maladaptive traits in a manner consistent with natural selection, Dobzhansky and Muller proposed a simple two-locus interaction model. Dobzhansky-Muller (D-M) genes are loci that have acquired species-specific divergence and interact epistatically in hybrids to cause incompatibility. F1 hybrid sons of *D. melanogaster* mothers and *D. simulans* fathers die as third instar larvae. However, if hybrid sons have a loss of function mutation in the *D. simulans Lhr* (Lethal hybrid rescue) gene, they survive. In contrast, a mutation in the *D. melanogaster Lhr* ortholog does not rescue hybrid sons, implying that the lethal interaction is a derived state specific to the *D. simulans* lineage. *Lhr* orthologs have also evolved rapidly under positive selection, further supporting the hypothesis that *Lhr* has functionally diverged between *D. melanogaster* and *D. simulans*. Contrary to this expectation, we find using matched *Lhr* transgenes that the lethal activity is shared by both orthologs. We have identified 8 amino-acids within the *Lhr* coding sequence that are critical for its hybrid lethal activity. Remarkably, this region is conserved between the sibling species, which is consistent with the lethal activity being an ancestral function. Examination of the heterochromatic localization patterns of *Lhr* orthologs also fails to reveal any evidence of functional divergence. Instead we have discovered that asymmetric expression of *Lhr* orthologs in hybrids caused by *cis-by-trans* compensatory regulatory evolution is the cause of the asymmetric effects of *Lhr* mutations on hybrid lethality. It is striking that although the *Lhr* coding sequence diverged under positive selection, it is the regulatory divergence of an ancestral function that underlies its D-M properties. Moreover, this is the first observation of *cis-trans* compensatory evolution directly affecting a locus with a major effect on HI.

31 Many small-effect mutations in a transcriptional enhancer caused evolution of *Drosophila* larval morphology. Nicolas Frankel¹, Deniz F. Erezylmaz¹, Alistair P. McGregor², Shu Wang¹, François Payre¹, David L. Stern¹. 1) Howard Hughes Medical Institute and Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544, USA; 2) Institut für Populationsgenetik, Veterinärmedizinische Universität Wien, A-1210 Vienna, Austria; 3) Université de Toulouse et Centre National de la Recherche Scientifique, Centre de Biologie du Développement, UMR5547, Toulouse, F-31062, France; 4) equal contribution.

Morphology evolves often through changes in developmental genes, but the number and effects of the causal mutations remain largely unknown. The differentiation of naked cuticle—rather than trichomes—on larvae of *Drosophila sechellia* evolved through changes in five transcriptional enhancers of *shavenbaby*, a gene encoding a transcription factor that governs trichome morphogenesis. Here we show that the function of one of these enhancers evolved through many nucleotide substitutions that altered both the timing and level of expression of *shavenbaby*. The consequences of these nucleotide substitutions on larval morphology were quantified with a novel functional assay. Each substitution had a small phenotypic effect and the substitutions displayed non-
additive effects to generate a large phenotypic change. These data provide unprecedented resolution of the phenotypic effects of evolved mutations and show how individual nucleotide substitutions can lead to morphological evolution.

32 The genetic basis of rapidly evolving male genital morphology in Drosophila. John P. Maasly, Justin E. Dalton, Sudeep Srivastava, Liang Chen, Michelle N. Arbeiter. 1) Department of Zoology, University of Oklahoma, Norman, OK; 2) Division of Biological Sciences, University of Southern California, Los Angeles, CA.

The external genitalia are some of the most rapidly evolving morphological structures in insects. The posterior lobe of the male genital arch shows striking differences in both size and shape among closely related species of the Drosophila melanogaster species subgroup. Because this structure is involved in copulation, divergence in species-specific thought is thought to have been driven by sexual selection. Here, we dissect the genetic basis of posterior lobe morphology between D. mauritiana and D. sechellia, two island endemic species that last shared a common ancestor roughly 300,000 years ago. We test a large collection of genome-wide homozygous D. mauritiana genetic introgressions, which collectively cover approximately 50% of the genome, for their morphological effects in a D. sechellia genetic background. We find several introgressions that have large effects on posterior lobe morphology and that posterior lobe size and posterior lobe shape can be separated genetically. Using next generation sequencing technology, we perform whole transcriptome analyses of the larval genital imaginal discs of two introgressions that each have large effects on posterior lobe size and shape, respectively, and find that both genotypes possess several genes in the insulin receptor signaling pathway that are expressed at D. mauritiana expression levels. Moreover, each introgression hybrid shows D. mauritiana expression levels in unique sets of these genes, but they also show D. mauritiana expression levels in identical genes, which suggests that the insulin signaling pathway might integrate size and shape genetic inputs to establish differences in overall posterior lobe morphology between D. mauritiana and D. sechellia. We propose a model that describes how the insulin and other growth related signaling pathways might integrate separate genetic inputs for size and shape to specify species-specific posterior lobe morphology.


Previous studies of organisms with well-differentiated XY chromosomes, e.g. Drosophila and mammals, consistently detected excess of genes moving out of the X chromosome and gaining testis-bias expression. Several selective evolutionary mechanisms were shown to be associated with such nonrandom gene traffic, contributing to the evolution of the X chromosome and autosomes. If selective mechanisms impel the gene traffic, they should also exist in the species with ZW chromosomes, namely those species with female heterogamety. However, no previous investigation of gene traffic in the species with female heterogamety has found gene movement with any biased patterns despite the reported bias distribution of sex-expression genes (e.g., chicken). Here, we report the excess of retrogenes moving “out of” the Z in an organism with ZW chromosomes, Bombyx mori, and show that those retrogenes tend to derive ovary-bias expression, different from the retrogenes moving out of the X chromosome in mammals and fruitfly that was found to impact the testis-functions. These properties of the gene traffic in the ZW system, which is symmetrical to the gene traffic in the XY system, suggests a general role of the heterogamery of the sex chromosome in determining of the chromosomal locations of sex-biased genes and their evolution.

34 Selection pressures on sex-biased genes depend on the degree of sexual dimorphism in the tissue and developmental stage in which they are expressed. Richard Meisel, John Malone, Brian Oliver, Andrew Clark. 1) MBG, Cornell Univ, Ithaca, NY; 2) NIDDK, NIH, Bethesda, MD.

Although all tissues within an organism share the same genome, the degree of sexual dimorphism varies wildly across tissues and throughout development. Here we seek evidence for the claim that this is driven by variation in the strength of sex-specific selection pressures. Clearly, sex-limited reproductive tissues in adults are responsible for most sexual dimorphism, while other tissues and other developmental time points are highly similar between males and females. Genes expressed in sex-limited tissues are expected to have sex-biased expression, but some genes have sex-biased expression even in tissues shared by both sexes. We tested whether genes with sex-biased expression in tissues and developmental time points in which the sexes are monomorphic are under different selection pressures than genes expressed in sex-limited tissues. Microarray and RNA-seq measures of expression were obtained from: 1) male and female heads and whole flies from four Drosophila species, 2) male and female larva of D. melanogaster, and 3) larval and adult organs of D. melanogaster. We find that the rapid evolution of sex-biased genes is limited only to those genes expressed in sex-limited tissues. Additionally, previous studies have consistently revealed a paucity of X-linked male-biased genes, suggesting that selection for male traits is more efficacious for autosomal variation. However, we find that genes with male-biased expression in sexually monomorphic tissues do not tend to be underrepresented on the X chromosome. We hypothesize that sex-biased genes expressed in sexually monomorphic tissues or non-sexually-mature juveniles are not under the same sexual (antagonistic) selection pressures acting on genes expressed in sex-limited reproductive tissues.


Drosophila germline stem cells (GSCs) can both self-renew and differentiate to give rise to oocytes or sperm. We have shown that multiple GSC genes are experiencing rapid, adaptive protein evolution in Drosophila melanogaster and the closely related species, D. simulans, suggesting that it is beneficial for these proteins to accumulate amino acid changes. We have focused on one of these adaptively evolving genes, bag of marbles (bam), to understand the functional consequences of this adaptive evolution. The best characterized function of bam is initiating GSC differentiation in ovaries. We have used interspecies complementation to test whether adaptive evolution of bam has caused detectable functional differences. Specifically, we have assayed the ability of a bam ortholog from D. simulans to complement the male and female sterility associated with a bam mutation in D. melanogaster. We have found that the D. simulans bam ortholog can complement male sterility but fails to fully complement the female sterility. The D. simulans ortholog can complement bam’s function in differentiation, but shows stem cell loss, improper number of cells/cyst, and mitotic synchrony defects which are likely due to an increased stability of D. simulans Bam protein. These data suggest that the evolutionary force driving the diversification of bam is focused on the female germline, and we hypothesize this force may be conflict with maternally-inherited bacterial endosymbionts. The endosymbiont Wolbachia pipientis is an
obligate, intracellular bacterium that has been shown to manipulate both the male and female germline in a variety of insects. To determine if any interaction existed between bam and Wolbachia, we tested the ability of Wolbachia to suppress bam hypomorphic mutants and found that the presence of Wolbachia can suppress bam female sterility. We also found suppression of female sterility in flies with D. simulans transgenic bam in our complementation assay. We are currently examining the nature of the interaction between bam and Wolbachia to try and understand the mechanism of suppression.

36 The two faces of sperm evolution: Insights from analysis of six Drosophila sperm proteomes. Timothy Karr1, Elizabeth Washbrough2, Steve Dorus2. 1) Biodesign Inst, PO Box 875001, Arizona State Univ, Tempe, AZ; 2) Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath, UK BA2 7AY.

We report the first molecular evolutionary, functional genomic, bioinformatic and comparative analysis of sperm proteomes in the Drosophila clade. Advances in mass spectrometry technology, high-throughput proteomics and genome annotations have resulted in significant increases in our molecular understanding of sperm composition. Using improved separation and detection methods and an updated genome annotation, a re-analysis of the Drosophila melanogaster sperm proteome (DmSP) has resulted in the identification of 956 sperm proteins. Comparative analysis with our previous proteomic dataset revealed 766 new proteins and an updated sperm proteome containing a total of 1108 proteins, termed the DmSP-II [1]. This expanded dataset includes additional proteins with predicted sperm functions and confirms previous findings concerning the genomic organization of sperm loci. Bioinformatic and protein network analyses demonstrated high quality and reproducibility of proteome coverage across three replicate mass spectrometry runs. The use of whole-cell proteomics in conjunction with characterized phenotypes, functional annotations and pathway information has advanced our systems level understanding of sperm proteome functional networks. We have used the DmSP-II to anchor our ongoing molecular evolution and comparative analysis of the 12 Drosophila genomes and report here the analysis of six Drosophila species representing five members of the melanogaster subgroup and D. pseudoobscura as the outgroup species. Our intraindividual analysis reveals sperm as “chimeric”, containing both a highly conserved core element of tubulins and a newly discovered class of leucyl aminopeptidase and an unexpectedly high level of variability in sperm proteome content. These results will be discussed in the context of sperm function, morphology and evolution. [1] Washbrough, E.R., et al., (2010). The Drosophila melanogaster sperm proteome-II (DmSP-II). J Proteomics 73, 2171-185.

37 Phenomics, Transcriptomics, and Metabolomics of Genotype-by-Diet Interactions underlying Metabolic Syndrome in Drosophila. Laura K. Reed1, Alison Motsinger-Reif2, David Reif2, Greg Gibson4. 1) Dept. of Biol. Sci., University of Alabama, Tuscaloosa, AL; 2) BRC, Dept. of Stat., North Carolina State University, Raleigh, NC; 3) National Center for Computational Toxicology, EPA, Research Triangle Park, NC; 4) School of Biology, Georgia Institute of Technology, Atlanta, GA.

Diseases linked to Metabolic Syndrome (MetS) such as type-2 diabetes and cardiovascular disease are rapidly increasing due to the influences of a modern Westernized-life style, but the genetic, environmental, and physiological mechanisms linking the symptoms of Metabolic-syndrome remain to be elucidated. Large-scale studies to systematically assess how genotype interacts with the environment to cause complex disease are very difficult in humans, but such studies are relatively tractable in genetic models systems such as Drosophila melanogaster. We have shown previously that there is a very substantial contribution of genotype-by-environment interactions to the phenotypic variation observed for MetS-like symptoms in a naturally genetically variable population of D. melanogaster. In this study, we performed metabolic and transcriptomic profiling of the genotype-by-diet interactions underlying gross metabolic phenotypes in 20 isofemale lines across four diets, and integrated the data across physiological levels to develop a systems biology framework for understanding these traits. We demonstrate clear correlations between metabolomic and gene expression profiles and MetS-like symptoms as they vary across diet. Module-based analysis of co-expressed genes and metabolites allows us to identify pathways contributing to gross metabolic variation. We are also able to identify genes previously lacking phenotypic descriptions as interacting with diet to moderate the metabolic phenotypes.

38 Extra genomic copies increase energetic demand. Luke A. Hoekstra, Kristi L. Montooth. Department of Biology, Indiana University, Bloomington, IN 47405, USA.

Changes in gene expression, via gene duplication or regulatory change, represent a major class of adaptive mutations. Mutations that increase gene expression should increase resource allocation to transcription and translation. In order for mutations that increase gene expression to persist, any increase in energetic expenditure must be selectively neutral. Yet despite ample evidence for weak selection in Drosophila, the energetic costs of increased gene expression have been difficult to quantify and thus frequently ignored. Are mutations that increase gene expression really selectively neutral? To quantify the energetic costs of increased gene expression we used lines of Drosophila melanogaster that differ in heat-shock protein (Hsp70) gene copy number and differ by as much as ten-fold in the inducibility of Hsp70 mRNA expression. Using flow-through respirometry, we measured the metabolic rates of larvae with different numbers of Hsp70 gene copies before, during, and after induction of Hsp70 expression. During induction of Hsp70 expression, larvae that express twice the wild-type number of Hsp70 copies experience a substantial increase in metabolic rate. In general, energetic expenditure during induction of Hsp70 increases with Hsp70 gene copy number and larvae with more copies of Hsp70 take longer to return to their resting metabolic rate. Combined with evidence that expressing extra copies of Hsp70 reduces locomotor performance, these results suggest a direct tradeoff between the expression of extra genomic copies and efficient energy use. If metabolic rate is itself a target of natural selection, then the energetic cost of increased gene expression may set upper bounds on the persistence of genetic material available for natural selection.

Cancer is characterized by uncontrolled cell proliferation. Anti-cancer therapy is largely comprised of radiation, surgery and chemotherapy. Despite decades of anti-cancer research, most therapies target only a single process, uncontrolled cell proliferation. Although a single mode of therapy can be effective in treating certain types of cancer, none presents a cure for cancer in general. Multi-modal therapy, the use of more than one anti-cancer agent (e.g., radiation and chemotherapy together), shows much potential for a more effective treatment of cancer. These combination therapies are being assessed in multiple clinical trials (www.clinicaltrials.gov). We addressed the problem of identifying new drugs that will allow multiple combinations to choose from, increasing the likelihood of clinical success. Through our Drosophila screen we have identified a family of molecules that show promise as enhancers of radiation and chemotherapy in HNC and non-small cell lung carcinoma cells. We have now shown that one of them is a radiation sensitizer on a non-small cell lung carcinoma line. Furthermore, when this cell line was used to produce flank xenografts in mice, the drug synergized with radiation to cause tumor regression. We are currently testing the remaining two molecules in a variety of cell lines in combination with both radiation and chemotherapeutic agents. The most effective combinations will be tested in mouse xenografts.

Genetic and pharmacological approaches that overcome immune suppression caused by elevated CO2 (hypercapnia) in Drosophila and mammals. Greg J. Beitel1, I. Taneli Helenius1,2, Thomas Krupinski3, Humberto Trejo3, Khalilah L. Gates4, Peter H. S Sporn2, Jacob I. Szajder2. 1) Dept. of Molecular Biosciences, Northwestern University, Evanston, IL; 2) Dept. of Medicine, Div. of Pulmonary and Critical Care, Northwestern University, Chicago, IL.

Obstructive pulmonary diseases, including COPD and cystic fibrosis, are the fourth leading cause of death in the United States. Patients with these diseases often have elevated CO2 levels (hypercapnia), which are associated with increased bacterial infections and death. We have shown the elevated CO2 suppresses innate immune responses regulated by NF-kB-family proteins in flies and in mammals. Correspondingly, both flies and mice show increased bacterial loads and mortality during infection in hypercapnic environments. To identify compounds that could be used to develop pharmacological treatments for hypercapnic patients, we used Drosophila S2* cells to screen ~9,000 compounds for their ability to prevent suppression of the antimicrobial peptide Diptericin. One compound could increase Diptericin expression by 2.5-fold, and could increase other antimicrobial peptides, such as Metchnikowin, to near normocapnic levels. Importantly, this compound also overcomes hypercapnic suppression of IL-6 expression in human THP-1 macrophages. These results suggest that this compound acts on conserved pathways that regulate responses to hypercapnia. Critically, this compound can improve survival of hypercapnic flies infected with S. aureus. We also find that CO2 has a unique effect on expression of metabolic genes, and that survival of infected hypercapnic flies can be improved by overexpression of key metabolic regulator genes. Together, these results demonstrate the utility of the Drosophila model of hypercapnic immune suppression. Importantly, they are also the first proof-of-principle that in vivo hypercapnic immune suppression can be overcome by therapeutic treatments other than reducing CO2 levels, which is not feasible for many patients with advanced obstructive pulmonary diseases.

Epigenetic regulation of learning & memory by Drosophila EHMT/G9a. Jamie M. Kramer1, Korinna Kochinke1, Merel A. W. Oortveld1, Hendrik Marks2, Daniela Kramer1, Eiko K. de Jong1, Zoltan Asztalos1, J. Timothy Westwood2, Hendrik G. Stunnenberg2, Marla B. Sokolowski4, Krystyna Keleman5, Huqing Zhou1, Hans van Bokhoven1, Annette Schenck1. 1) Department of Human Genetics, Radboud University Nijmegen Medical Centre, The Netherlands; 2) Department of Molecular Biology, Nijmegen Centre for Molecular Life Sciences, The Netherlands; 3) Aktogen Ltd., Department of Genetics, University of Cambridge, Cambridge, UK; 4) Department of Biology, University of Toronto, Canada; 5) Institute of Molecular Pathology, Vienna, Austria.

The epigenetic modification of chromatin structure and its effect on complex neuronal processes like learning and memory is an emerging field in neuroscience. However, little is known about the “writers” of the neuronal epigenome and how they lay down the basis for proper cognition. Here, we have dissected the neuronal function of the Drosophila euchromatin histone methyltransferase (EHMT/G9a), a member of a conserved protein family that methylates histone 3 at lysine 9 (H3K9). EHMT is widely expressed in the nervous system and other tissues, yet EHMT mutant flies are viable. Neurodevelopmental and behavioral analyses identified EHMT as a regulator of peripheral dendrite development, larval locomotor behavior, non-associative learning and courtship memory. To uncover the molecular mechanisms underlying these phenotypes we generated genome-wide H3K9 dimethylation profiles using ChIP-seq technology. Loss of H3K9 dimethylation in EHMT mutants occurs at 5% of the euchromatic genome and is enriched at the 5’ and 3’ ends of distinct classes of genes that control neuronal and behavioral processes that are corrupted in EHMT mutants. Our study identifies Drosophila EHMT as a key regulator of cognition that orchestrates an epigenetic program featuring classic learning and memory genes. Our findings are relevant to the pathophysiological mechanisms underlying Kleefstra Syndrome, a severe form of Intellectual Disability caused by mutations in human EHMT1, and have potential therapeutic implications. Our work provides novel insights into the epigenetic control of cognition in health and disease.

Altered signaling at the muscle synapse causes temperature-sensitive seizures in Drosophila dystrophic muscles. April K Marrone1, Mariya M Kucherenko1, Robert Wiek2, Martin Goepfert2, Halyra R. Scherbata1. 1) Max Planck Institute for biophysical chemistry Am Fassberg 11, 37077, Goettingen, Germany; 2) Department of Cellular Neurobiology, Georg August University, Max Planck Institute for experimental medicine, Hermann Rein 3, 37075 Goettingen, Germany.

Muscular dystrophies (MDs) are associated with malignant hyperthermia (MH) like episodes that pose a risk to human life. Since the reasons for this are unknown, we used a Drosophila model of MD to address this problem. Loss of Dystrophin (Dys) during NMJ development leads to heat sensitive muscular seizures dependent upon neuronal input. Proteins that are involved in neurotransmitter receptor localization affect the phenotype indicating that Dys is involved in neuron-muscle communication. We have determined that this phenotype is only displayed when Dys is down regulated during muscle development and NMJ establishment and is independent from the role of Dys in muscle degeneration. Unexpectedly, this phenotype is not shared by, and depressed by Dys’s binding partner Dysregulocan (Dg). Additional testing implies that Dg’s suppression of this phenotype is due to upstream malfunctioning of the NMJ, supposedly due to the perturbation in Ca-ion homeostasis. Also, the Ca2+ regulator Calmodulin can rescue the Dys phenotype, which implies
that ultimately Ca2+ mis-regulation is responsible for the seizures and supports numerous other studies contending that Ca2+ is mis-regulated in MD patients. Our study provides the first in vivo measurements on live animals that offers an explanation for why MD patients have a higher risk for MH like episodes including seizures, cardiac arrest and sudden death upon exposure to depolarizing muscle relaxants and/or neuromuscular blocking agents.

43 Living without telomeres: complete suppression of tefu/atm developmental phenotype. Sarah R. Oikemus1, Uma Chalasani1, Sze Ham Chan2, Amy Marie Yu2, Mitch McVey2, Michael Brodsky1. 1) Dept PGF&E, Univ Massachusetts, Worcester, Worcester, MA; 2) Dept Genetics, Tufts School of Biomedical Sciences, Boston, MA.

The ATM gene encodes an evolutionarily-conserved kinase required for DNA damage responses and normal telomere function. Patients with ATM gene mutations exhibit symptoms that include cancer predisposition, radiation sensitivity, immune defects, neurodegeneration and premature aging. In a mouse model of ATM, severe organismal phenotypes are only observed when telomeres are shortened to resemble human telomeres. Mutations in the Drosophila atm homolog, telomere fusion (tefu), cause a high frequency of telomere fusions, pervasive spontaneous apoptosis, developmental defects, loss of motor control and shortened lifespan. Here we examine if blocking the cellular response to unprotected telomeres in tefu mutants can ameliorate the organismal defects. Previously, we demonstrated that mutations in p53 partially suppress the spontaneous apoptosis associated with tefu mutants. To determine if fusion of unprotected telomeres contribute to the p53-independent apoptosis, we tested the effect of mutations in genes that block DNA repair by classic end joining (lsg4) and by alternative end joining (mus308). While lsg4 mutations reduce chromosome fusions by 40%, mus308 mutations reduce fusions by 80% and disruption of both pathways completely suppresses fusions. Consistent with a model where aneuploidy due to telomere fusions cause p53-independent apoptosis, blocking both the classic and alternative end joining pathways eliminates all apoptosis in a tefu p53 mutant background. The contribution of telomere fusions and p53-dependent apoptosis to the organismal phenotypes was tested by blocking either p53-dependent apoptosis or fusions. Alone each could partially suppress tefu phenotypes. However, mutations in p53, lsg4 and mus308 completely suppressed defects in external morphology, motor control and premature death, suggesting that blocking cellular pathways activated by unprotected telomeres can dramatically suppress the adverse effects of ATM loss in Drosophila.

44 Manipulating the Kinome in Drosophila Gli to Model Human Glioma. Renee D. Read1, Tim Fenton2, Frank B. Furnari2, Webster K. Cavenece2, John B. Thomas1. 1) Molecular Neurobiology Laboratory, Salk Institute for Biological Studies, La Jolla, CA; 2) Ludwig Institute for Cancer Research, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA.

Gliomas, the most common primary brain tumors, infiltrate the brain, grow rapidly, and are refractory to current therapies. Signature genetic lesions in gliomas include mutation of the EGFR receptor tyrosine kinase and activating mutations in components of the PI-3 kinase (PI3K) pathway. Despite years of study, how these pathways specifically regulate glial pathogenesis is unclear. To identify new genes involved in this disease, we developed a Drosophila glioma model, which builds on observations that Drosophila glia are strikingly similar to their mammalian counterparts. We found that constitutive co-activation of EGFR and PI3K signaling in Drosophila glia gives rise to proliferative, neoplastic, and invasive glial cells that create transplantable tumors. Using lineage analysis and cell-type specific markers, we have found that neoplastic glial cells originate from committed glial progenitor cells, rather than from differentiated glia or multipotent neuroblasts. We used this Drosophila model in a forward genetic screen of the Drosophila kinome to identify new kinases that drive neoplastic transformation and to identify pathways that function in the glial cell types prone to neoplasia. These functional screens have identified about 60 kinases, many of which are essential for glial development, that govern neoplastic glial proliferation and migration. Mammalian orthologs of 21 kinases uncovered by these screens were functionally assessed in mammalian glioma model systems and human tumors. Our results show that 10 of these kinases, several of which are mutant in gliomas, are required for proliferation and/or survival of human glioma cells, including essentially uncharacterized kinases as well as kinases that have been nominally implicated in Tor signaling, cell migration and shape change, ion homeostasis, and p53 regulation. Ongoing studies of these kinases in mammalian and Drosophila systems may reveal promising new therapeutic targets for human glioma.


Constitutively increased intracellular pH (pHi) is a hallmark of mammalian cancer cells. Increased pHi is suggested to be necessary for adaptive behaviors of cancers and reducing pHi has been proposed as a therapeutic approach to limit cancer progression. However, these predictions have not been experimentally confirmed in situ. Using Drosophila models of dysplasia we tested effects of deleting Dnhe2, the Drosophila homolog of the mammalian plasma membrane Na-H exchanger NHE1 that extrudes H+ to generate increased pHi. Dnhe2null flies are semi-lethal (<10% oocyte). Surviving adults appear morphologically normal, although analysis of third instar imaginal discs indicate fewer proliferating cells than wild-type. Expression of mutationally activated Raf in the eye (GMR-ARaf) induced increased pHi and an expanded proliferation of imaginal disc cells compared with wild-type. Adults have externally rough eyes and tangential sections reveal dysplasia with missing or extra photoreceptors and ommatidial orientation defects. We predicted that removing Dnhe2 in a GMR-ARaf background would limit dysplasia; however, tangential sections revealed necrosis with a profound loss of retinal organization and rhabdome structures. We are now testing prediction that in the absence of Dnhe2, metabolic acids generated by ARaf expression cannot be extruded, resulting in constitutive acidosis and cell death. Because NHE1 is overexpressed in mammalian cancers, we also generated Drosophila expressing Dnhe2 in differentiating eye cells (GMR-Dnhe2). The GMR-Dnhe2 phenotype resembles GMR-ARaf, including expanded proliferation of imaginal disc cells and externally rough adult eyes having dysplasia, visible as ommatidial orientation defects. These data suggest that a constitutive increase in pH through overexpression of Dnhe2 causes oncogenic phenotypes, including dysplasia and expanded proliferation. Our data also suggest that loss of Dnhe2 may act as a synthetic lethal in transformed but not normal cells, which would have significant impact for developing therapeutics that selectively kill cancer cells.
46

Genome wide screening for modulators of Upd and HopTuml-mediated JAK/STAT pathway signalling at the Sheffield RNAi Screening Facility (SRSF). Martin P Zeider, Katherine H Fisher, Amy Taylor, Stephen Brown. The Sheffield RNAi Screening Facility & Department of Biomedical Science, The University of Sheffield, Sheffield, S10 2TN, UK.

Gain-of-function mutations in human JAK2 are responsible for the majority of myeloproliferative neoplasms. Consistent with the high levels of evolutionary conservation of this pathway, gain-of-function mutations in the Drosophila JAK homologue, Hopscotch (Hop), also result in constitutive STAT92E activity, the over-proliferation of haemocytes and the development of haematological tumours. We have recently established the Sheffield RNAi Screening Facility (SRSF) as a resource for the wider scientific community to undertake the full spectrum of cell-based, genome-wide RNAi screens. We have used the facilities of the SRSF to undertake proof of concept screens for loci that interact with pathway signalling activated by the Upd ligand and HopTuml, the gain-of-function mutation analogous to the oncogenic human JAK2 V617F mutation. We have compared the hits identified in these two screens and retested candidate interactors using the recently identified gain-of-function STAT92E truncation alleles to generate epistatic data. Using this data we have been able to classify regulators of JAK/STAT signalling that function at the level of the Hop kinase. Data will be presented regarding both screens and the resultant epistasis. Comparisons to the original JAK/STAT RNAi screens will be shown and the resources available to external screening groups at the SRSF will be described.

47


Prostate cancer is the second most common form of cancer and the most frequent cause of cancer deaths in males. Prostate cancer cells are typically secretory and have unusual, but unexplained, metastatic properties, migrating to secondary sites in bone and exploiting BMP signalling to remodel this tissue. The Drosophila accessory gland shares some functional parallels with the prostate, secreting a cocktail of proteases, protease inhibitors and other molecules that capacitate sperm and modulate the behaviour and fertility of the female. We initiated a detailed analysis of the adult accessory gland, focusing on the 30-40 secretory (or secondary) cells that populate the distal tip of the gland. We find that mating induces substantial growth and progressive delamination of these cells from the epithelium. The cells have intrinsic migratory activity and can move proximally along the duct of the gland. Blocking these effects reduces male fertility and inhibits the ability of males to prevent females from remating. We show that several signalling pathways control these events, including the BMP signalling cascade and the pathway regulated by the steroid ecdysone. We propose a model in which BMPs and ecdysone play both synergistic and antagonistic roles in controlling mating-dependent secondary cell behaviour, ensuring that these cells contribute to semen content, while for the most part, remaining attached to the glandular epithelium as flies age. Remarkably, BMP and androgen signalling have both been strongly implicated in prostate cancer and their effects on growth and metastasis appear to mirror the actions of BMPS and ecdysone on secondary cell growth and migration in flies. These parallels suggest that the accessory gland provides a powerful new model to dissect out the cellular events that underlie tumorigenesis in prostate.

48

Rubex-5 loss results in dramatic hemocyte overproliferation and melanotic mass formation: a potential tumor suppressor and model for leukemia. Theresa Reimels, Cathie M. Pfleger. Department of Oncological Sciences, Mount Sinai School of Medicine, New York, NY.

Signaling through Ras is extremely important in regulating growth and differentiation. Excess Ras activity can promote developmental disorders and tumor formation. Recently, our laboratory identified Rubex-5 as a component of Ras signaling that restricts growth in Drosophila. Loss of Rubex-5 increases Ras activity and contributes to a larger body size, eye and wing overgrowth, and an eye-to-antenna fate switch. Rubex-5 deficient larvae show increased size, developmental delays, and larval or pupal lethality. These larvae frequently develop melanotic masses, which are often associated with hematopoietic disorders. Since activated Ras is implicated in human hematological malignancies and Rubex-5 is downregulated in human lymphomas and leukemias, we are examining Rubex-5 as a potential tumor suppressor in the hematopoietic system. We used the UAS/Gal4 system to drive GFP expression specifically in hemocytes and report here that homozygous deletion of Rubex-5 increases the number of larval hemocytes and alters their normal patterning. Removing one Rubex-5 copy also increases the number of a subset of hemocytes involved in the melanization process. Ongoing structure-function studies are determining which catalytic domain is required to suppress these hemocyte phenotypes and whether they are dependent on Ras and/or other signaling pathways. The Drosophila immune system is known to respond to tumors and tissue damage and may result in hemocyte overproliferation and melanotic mass formation. We are currently addressing models in which the hemocyte overproliferation and melanotic mass formation result from (1) misregulation of hematopoeisis, (2) an immune response to aberrant tissue, or (3) a combination of both. These studies may establish Rubex-5 loss-of-function mutations as a cancer model and/or as a model to study the involvement of the immune response in cancer.

49

Mutations in seizureERG and KCNQ cause both electrical and morphological remodeling of the Drosophila heart. Karen Ocorr. Development & Aging, Sanford-Burnham Medical Research Institute, La Jolla, CA.

Congenital mutations and/or blockers of cardiac potassium (K) channels can generate early afterdepolarizations (EADs), leading to ectopic beats and triggering lethal arrhythmias in humans. We previously showed that mutations in the KCNQ K channel also causes cardiac arrhythmias in the fly. Here we use our optical recording methodology to show that mutations in the hERG K channel homolog seizure (sei) also result in increased cardiac arrhythmias that are aggravated with age. Interestingly, in hearts from sei mutants we routinely observed dilation of the posterior two heart chambers and phalloidin staining confirmed the presence of a thinned and disorganized myofibrillar structure in this region (anatomical remodeling). In addition, the altered myofibrillar structure was accompanied by alterations in the extracellular network of collagen IV, simulating aspects of morphological remodeling seen in compromised human hearts. We have now used intracellular recording techniques to examine electrical activity in the fly heart. We find an increase in prolonged action potential (AP) durations in young sei and KCNQ mutants compared to wildtype (wt). In addition, we observed frequent EADs in hearts from young mutant flies, which are not seen in young wt flies. We also observed trains of EADs lasting several seconds in mutant flies (electrical remodeling) suggesting an
arrhythmic potential for this electrical activity. Significantly, we were able to directly correlate this altered electrical activity with cardiac arrhythmia by simultaneous electrical and optical recording. These results suggest that the conservation of many fundamental aspects of heart function between flies and vertebrates may also extend to morphological remodeling in response to ion channel deficiencies. Thus, this system will be useful in elucidating fundamental molecular/cellular links between electrical and morphological remodeling of the heart.

50

Drosophila as a model for amyloid-induced cardiac dysfunction. Girish C Melkani1,2, Rolf Bodmer2, Karen Ocorr3, Sanford I Bernstein1. 1) Department of Biology, Molecular Biology and SDSU Heart Institutes, San Diego State University San Diego, CA 92182; 2) Development and Aging Program, Sanford-Burnham Institute for Medical Research, La Jolla, CA 92037.

Several human diseases are associated with the expression of mutated, misfolded and aggregation-prone amyloid proteins. Huntington’s disease (HD) is caused by mutation in the Huntingtin protein that results in an expanded polyglutamine (PolyQ) repeat and formation of amyloid-like inclusions. The length of the PolyQ-repeat is also important in the progression of disease. Recent evidence indicates that patients with HD demonstrate a greater occurrence of cardiovascular events but very little is known as to how HD leads to cardiac failure. Using the UAS-Gal4 expression system, we expressed short and long UAS-PolyQ in the Drosophila heart using a cardiac specific driver (Hand-Gal4) to explore PolyQ amyloid-induced cardiac dysfunction. Expression of extended PolyQ (UAS-97Q) resulted in severe cardiac defects. However, no such defects were seen upon expression of short PQ (UAS-25Q) under similar conditions. For example, the arrhythmicity index (overall measure of cardiac arrhythmia) of 1 week old PolyQ-97 fly hearts was twice that of the same age flies expressing PolyQ-25. Further, more progressive cardiac dysfunction (six fold increases in arrhythmicity index) was observed after 3 weeks in fly hearts expressing PolyQ-97 compared to same age fly hearts expressing short PolyQ. In addition to severe cardiac defects, expression of extended PolyQ results in dilatation of the cardiac tube. Furthermore, heart period, diastolic and systolic intervals of 1 and 3 week old fly hearts expressing PolyQ-97 were higher compared to the same age flies expressing PolyQ25. Our results show that expression of extended PolyQ causes progressive cardiac dysfunction. We are currently examining structural defects and possible amyloid aggregates in the cardiac tube that arise upon expression of extended PQ. Our study will be an important step in exploring mechanism of amyloid-induced cardiac failure, as cardiac failure is the second most common cause of death in HD patients.

51

Mutation of the planar cell polarity gene, prickle, causes epilepsy from flies to humans. J. Robert Manak1, Salleh Ehaideb1, Levi Stowers2, Hirotaka Tao1, Naoto Uno1, Jeffrey D. Axelrod3, Diane Slusarski1, Alex Basuk1. 1) Dept Biol, Univ Iowa, Iowa City, IA; 2) Dept Pediatrics, Univ Iowa, Iowa City, IA; 3) Div Morphogen, Nat Inst Basic Biol, Okazaki, Japan; 4) Dept Pathol, Stanford Univ Sch of Med, Stanford, CA.

Human epilepsy has a large genetic component, yet very few causative genes have been identified. There are animal models of epilepsy, but no single gene has shown to be associated with epilepsy in both vertebrates and invertebrates, thus limiting our ability to use genetically tractable model organisms to study the disease. In Drosophila melanogaster, prickle participates in the non-canonical WNT signaling/planar cell polarity (PCP) pathway, and prickle mutants display abnormalities in the polarity of structures associated with a variety of tissues. We now show that mutations in Prickle homologues are associated with seizures in humans, mice, and flies. Humans and mice either homozygous or heterozygous for Prickle mutations are seizure-prone. Strikingly, flies homozygous or heterozygous for the pk0980 mutation display pronounced seizures that are strongly responsive to valproic acid, an anti-epileptic medication widely prescribed for epileptic patients. Additionally, through backcrossing the pk0980 flies to an Oregon R background across 5 generations, we show that pk0980 heterozygous flies are epilepsy-prone compared to Oregon R controls, even though the only difference between these two fly lines is that the heterozygotes have a two-fold reduction in expression of a single prickle transcript isoform encoding the largest protein isoform. Finally, a statistically significant enrichment of neuronal migration defects in pk0980 mutant embryos compared to controls, suggesting that the prickle epilepsy may have its basis in a developmental defect. To our knowledge, this is the first demonstration of a mutation in a homologous gene in fly, mouse, and human involved in epilepsy. These studies provide a starting point for dissecting conserved developmental mechanisms underlying human epilepsy.

52

The mauve eye color gene encodes the homolog of the Chediak-Higashi Syndrome gene and has equivalent phenotypes. Mokhlasur Rahman, Adam Haberman, Charles Tracy, Helmut Kramer. Department of Neuroscience, UT Southwestern Medical Center, Dallas, TX.

In a screen for trafficking mutants, we identified a allele of the eye color mutant mauve (mv). The classical mv1 and our mv2 allele contained mutations in CG11814, this was confirmed by a genomic rescue construct which restored wild-type eye color in both mv alleles. The mv gene encodes the homolog of mouse Beige and human CHS1. Mutations inactivating human CHS1 cause Chediak-Higashi Syndrome (CHS) which is characterized by oversized lysosomes and lysosome-related organelles (LROs) such as melanosomes and different secretory granules. Patients rarely survive to adulthood but succumb to complications of recurring infections most likely reflecting defects in phagocytosis. We found that these hallmarks of CHS closely resemble the phenotypes of mv alleles: (i) One example of LROs in Drosophila are pigment granules; their volume in mv2/Df eyes is more than 50 times enlarged compared to wild type. (ii) Staining with antibodies against Mv co-localized with markers for lysosomes. (iii) mv2/Df flies are susceptible to infections with E. coli at doses non-pathogenic to wild type. This is likely due to a defect in phagocytosis as antimicrobial peptides, another arm of innate immunity in flies, are induced at elevated levels compared to wild type. To explore an additional cellular pathway to lysosomes, autophagy was induced by starving larvae. In fatbodies of starved mv2/Df larvae, lysotracker staining indicated reduced formation of autolysosomes. By contrast, initial induction of autophagy, as measured by ATG8–RFP punctae, was not inhibited. Together, these data point to a requirement of Mv for the fusion of lysosomes with autophagosomes. A physiological role for Mv in autophagy was supported by the significantly reduced life span of mv2/Df flies upon starvation. The cell biological role of CHS1 is still poorly understood and we propose that mv mutants constitute an excellent model system for its analysis. Support: NIH grant EY10199.
**PLATFORM: Pattern Formation**
Details of individual presentations can be located in the online Schedule of Events at drosophila-conf.org.

---

53

**Live analysis of the Dorsal nuclear gradient.** Gregory T. Reeves¹, Angelike Stathopoulos². 1) Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC; 2) Division of Biology, California Institute of Technology, Pasadena, CA.

The Dorsal patterning system is one of the most well-studied morphogen systems in biology. A ventral-to-dorsal gradient in nuclear Dorsal is established in the early embryo, and is responsible for patterning the entire dorsal-ventral axis. However, despite its importance in axis specification, no one has quantified a timeseries of nuclear Dorsal in live embryos. In this study, we imaged a Dorsal-GFP fusion protein in live embryos and quantified the Dorsal-GFP gradient from nuclear cycles 10-14. Our results agree with our previous measurements of the Dorsal gradient in fixed tissues as well as other live imaging studies [1-3]. We found the Dorsal levels to be more dynamic than originally predicted, begging the question of how gene expression boundaries are established in the face of ever-changing Dorsal levels. However, using a mechanistic model of mRNA dynamics and gene regulatory interactions, with our complete spatiotemporal measurements of Dorsal-GFP levels as input, we show that boundaries of gene expression can indeed be established by gastrulation. Furthermore, we find that ventro-lateral genes are expressed first, followed by more ventral genes, consistent with in situ hybridization data.


54

**Deubiquitinases and ubiquitin ligases fine-tune interpretation of the Dpp and Dorsal morphogen gradients.** Stuart J. Newfeld, Michael Stinchfield, Ashley Castillo, Estela Arcineiga, Norma Takaesa. Sch Life Sci, Arizona State Univ, Tempe, AZ.

The ability of secreted TGF-beta family members (such as Dpp) to elicit powerful responses dictates that their influence be strictly regulated. A recent study in vertebrates revealed a novel regulatory mechanism - monoubiquitination of the TGF-beta signal transducer Smad4 (homolog of Medea) by Ectoderm inactivated but did not degrade it. It was also shown that USP9X (homolog of the Drosophila Fat Facets deubiquitinase) reverted monoubiquitination and restored Smad4 activity. Here we report that deubiquitination of Medea by Fat Facets is a conserved mechanism for regulating TGF-beta signal transduction. Our data reveals that Fat Facets is an essential feature of the signal transduction system that cells utilize to interpret the Dpp pattern that patterns the embryonic dorsal-ventral axis. For example, maternal faf mutations have the same effect on dpp mutations as maternal mutations in Medea and a maternal Medea allele enhanced faf mutations to generate embryos that resembled ventralized dpp mutants. Our hypothesis is that the loss of the faf deubiquitinase resduces Medea activity below the minimum necessary for Dpp signaling due to excessive ubiquitination. Evidence that a maternal deubiquitinase is needed in dorsal-ventral patterning is derived from studies in Drosophila S2 cells - monoubiquitinated Medea is the predominant isoform. In contrast, the ubiquitination of Medea by Bonus (homolog of Ectoderm) is not involved in Dpp-dependent dorsal-ventral patterning. Instead, embryos derived from bonus germ-line clones phenocopy those of dpp mutants. This similarity suggests that the failure to maternally dampen a signaling protein results in loss of Dpp activity. Numerous studies have shown that maternal hyperactivation of the Dorsal pathway results in dpp repression. Thus maternal Bonus may play a role in modulating the Dorsal pathway. Overall our study reveals that intracellular modulation of signal amplitude by ubiquitination and deubiquitination of signal transducers is a previously unrecognized mechanism for fine-tuning cellular interpretation of developmental signals.

---

55

**Positioning of Bicoid target gene expression boundaries by combinatorial repression mechanisms.** Hongtao Chen, Zhe Xu, Jerry Huang, Stephen Small. New York University, New York, NY10003.

In the early Drosophila embryo, Bicoid (Bcd) forms a long-range gradient along the anterior-posterior (A-P) axis and is required for the formation of all the cephalic and thoracic structures. It is hypothesized that Bcd functions as a morphogen, which sets up concentration thresholds to position its target genes’ expression boundaries into a specific anterior to posterior order. However, this classical view cannot explain why target genes can be activated by Bcd concentrations that are lower than those present at their anterior boundary positions in wild type embryos, or why some target genes make boundaries of gene expression in embryos with flattened Bcd gradients. To better understand the Bcd dependent patterning system, we analyzed 52 known Bcd dependent enhancers, including 30 new enhancers we found recently, and discovered an overrepresented DNA motif that is unique to enhancers driving expression only in the cephalic region. This motif is similar to the binding motif of Runt (Run). Adding Run sites into the hhP2 enhancer progressively represses its expression into more anterior regions. Removing Run and other repressors present in middle of the embryo (Cic and Kr) changes the order of expression boundaries of various Bcd target genes. We suggest that these repressors form a series of antagonistic gradients and work together to position Bcd dependent genes into their wild type A-P order. We conclude that Bcd gradient is a part of a combinatorial regulatory network and that antagonistic repression is the main mechanism responsible for differential boundary positioning.

56

**Spatial diversification of BMP signaling by a patterned receptor across Drosophila species.** Matthew G. Niepielko, Yainna Hernández-Hernández, Nir Yakoby. Biology Department and Center for Computational and Integrative Biology, Rutgers, The State University of New Jersey, Camden, NJ 08102.

Despite extensive studies of cell-to-cell signaling, the mechanisms governing diversification signaling among species remain mostly unknown. The bone morphogenetic protein (BMP) signaling pathway is a major regulator of tissue development across animals, and in Drosophila melanogaster, perturbations in BMP signaling are associated with eggshell structures’ deformations. The Drosophila eggshell is formed by the follicle cells (FCs), a monolayer of epithelial cells surrounding the developing oocyte. Here, we reason that the natural variation in eggshells’ structures found among 16 Drosophila species is an excellent system to explore mechanisms of BMP signaling diversification in tissues. In the FCs of D. melanogaster, early BMP signaling is exclusively along the anterior-posterior axis, and later it acquires dorsal-ventral (DV) polarity. We determined that signaling is indeed diverse in all Drosophila species, and while early BMP signaling had a similar pattern in all species, late signaling varied and was clustered into four unique groups with clear DV polarities. Furthermore, we demonstrated that in most Drosophila species, dynamic patterning of the type I BMP receptor thickveins (tkv) could account for the diversity in late BMP signaling. Interestingly, in species within the virilis-repleta group, tkv was uniformly expressed and could account for only a portion of the BMP signaling pattern specific for this group. Our results support that diversification in BMP signaling across 45 million years of Drosophila evolution is controlled by a receptor-based mechanism.
57  
Wnt signal plays an essential role in the left-right asymmetric development of the embryonic gut in Drosophila. Junpei Kuroda¹, Reo Maeda¹, Yasuhiro Kawakatsu¹, Mitsutoshi Nakamura¹, Naotaka Nakazawa¹, Ayumi Ozaki¹, Akira Ishio¹, Ryo Hatori¹, Shigenori Nonaka², Kenji Matsuno¹. 1) Department of Biological Science and Technology, Tokyo University of Science, Noda, Japan; 2) National Institute for Basic Biology, Okazaki, Japan. 

Many bilaterally symmetric animals develop left-right (L-R) asymmetry in their internal organs. The mechanisms of L-R asymmetric development are well understood in vertebrates, whereas they are still elusive in invertebrates. Therefore, we decided to study the genetic pathway of L-R asymmetric development in Drosophila. To identify the genes required for L-R asymmetric development in Drosophila, we screened EMS (Ethylmethanesulfonate) induced mutations that affect stereotypical L-R asymmetry of the embryonic gut. From this screening, we isolated arrow mutant that affected L-R asymmetry in the anterior midgut. Arrow is an ortholog of a mammalian gene encoding low-density lipoprotein receptor-related protein 5/6, which is a coreceptor in the Wnt signaling pathway. This result suggests that Wnt signaling is essential for the L-R asymmetric development of the anterior midgut. We next identified the ligand and receptor of Wnt signaling required for the L-R asymmetric development of the anterior midgut. Drosophila genome encodes seven Wnt ligands. Among them, we found that embryos homozygous for Wnt4 showed the similar L-R defects to those of arrow. In addition, frizzled2, encoding a receptor of Wnt signaling, is required for the L-R asymmetric development of the anterior midgut. The anterior midgut consists of the visceral muscle and epithelial tube. Our analysis revealed that Wnt signal in the visceral muscle but not the epithelium of the midgut is required for the normal laterality development of this organ. These results suggest that Wnt signal activated by Wnt4 in the visceral muscle play an essential role in the L-R asymmetric development of the anterior midgut.

58  
hedgehog activation of Notch drives proliferation at the AP organizer of the Drosophila wing imaginal disc. David J. Casso¹, Brian Biehs², Thomas Kornberg². 1) Dept Biochem/Biophysics, Univ California, San Francisco, San Francisco, CA; 2) Cardiovascular Research Institute University of California San Francisco, CA. 

Notch has multiple roles in the development of the Drosophila melanogaster wing imaginal disc. It helps specify the Dorsal-Ventral compartment border and it is needed for the wing margin, veins and sensory organs. Here we describe an additional role: stimulating growth in response to Hedgehog. We show that Notch signaling is activated in the cells of the Anterior-Posterior organizer that produce the region between wing veins 3 and 4, and we describe strong genetic interactions between the gene that encodes the Hedgehog pathway activator Smoothened and the Notch pathway genes Notch, presenilin, Suppressor of Hairless, and the Enhancer of split complex. This work thus describes a novel collaboration by the Hedgehog and Notch pathways during development, and it reveals a mechanism for regulating proliferation in the 3-4 intervein region independently of Decapentaplegic.

59  
Scaling properties of Dpp signaling in the growing Drosophila wing imaginal disc. Fisun Hamaratoglu¹, Aitana Morton de Lachapelle², Georgios Pyrowolakis³, Sven Bergmann¹, Markus Affolter¹. 1) Growth and Development, Biozentrum, University of Basel, Basel, Switzerland; 2) Department of Medical Genetics, University of Lausanne & Swiss Institute of Bioinformatics, Lausanne, Switzerland; 3) Department of Developmental Biology, University of Freiburg, Freiburg, Germany. 

The wing of the fruit fly, Drosophila melanogaster, with its simple, two-dimensional structure is a model organ well suited for a systems biology approach. The wing arises from an epithelial sac referred to as the wing imaginal disc which undergoes a phase of massive growth and patterning during larval stages. Decapentaplegic (Dpp), a Drosophila member of the transforming growth factor-β (TGF-β) family has the ability to coordinately regulate patterning and growth and represents the best studied case of a morphogen inducing different fates at different distances from its source of origin. We asked how robust the Dpp morphogen gradient is and concentrated on a feature often associated with robustness- scaling. Here we show that the primary readout of Dpp signaling activity, P-mad levels, scales with the growing tissue. The more downstream targets of the pathway, brinker (brk) and optomotor blind (omb), also scale but to a lesser degree. On an attempt to find a potential mechanism for how scaling might be achieved, we turned our attention to the feedback regulators of the pathway. We present data with one of these regulators, Pentagone (Pent); we find that scaling gets less precise in pent mutant background.
60
Regulation of translesion DNA polymerases by the E3 ubiquitin ligase NOPO during early embryogenesis. Julie A. Merkle, Laura A. Lee. Cell and Developmental Biology, Vanderbilt University, Nashville, TN.

In a screen for cell-cycle regulators, we identified a Drosophila maternal effect-lethal mutant, “no poles” (nopo). Embryos from nopo females undergo mitotic arrest with barrel-shaped, acentrosomal spindles during the rapid S-M cycles of syncytial embryogenesis. Our genetic data indicated that activation of Checkpoint kinase 2 (Chk2, encoded by mnk) causes this arrest, suggesting that NOPO is required for genomic maintenance. mnk nopo-derived embryos exhibit a significantly shorter interphase 11 than wild type, suggesting that nopo embryos enter mitosis with incompletely replicated DNA to elicit a Chk2-mediated arrest. We hypothesize that NOPO regulates timing of S-M transitions to ensure completion of DNA replication prior to mitosis.

nopo and its mammalian homolog, TRIP (TRAF-interacting protein), encode E3 ubiquitin ligases. TRIP was identified as a binding partner of TRAFs in TNF signaling and is required for early embryogenesis in mice. To gain insight into the roles of NOPO/TRIP, we performed a yeast two-hybrid screen for human TRIP interactors. We identified a family of non-canonical DNA polymerases that facilitate replicative bypass of damaged DNA (translesion synthesis) as TRIP interactors. We have also shown that NOPO interacts with Drosophila Y-family DNA polymerases. Furthermore, we observe an enhanced ubiquitylation of Y-family polymerases by TRIP in cultured human cells. In C. elegans, the Y-family polymerase POLH-1 prevents replication fork stalling and promotes cell-cycle progression in the early embryo. We generated a null mutation in DNApol-eta to determine if it has a role in early Drosophila embryogenesis and observe decreased hatch rates and nopo-like spindle defects in DNApol-eta-derived embryos. Mutation of the human homolog, POLH, results in a variant Xeroderma Pigmentosum, a disease characterized by UV sensitivity and skin cancer. We find that DNApol-eta-derived embryos are also UV sensitive. We hypothesize that NOPO ubiquitylates Y-family polymerases during S-phase of early embryogenesis to regulate their activity and promote cell-cycle progression.

61
The translational regulator Caprin is specifically phosphorylated at the mid-blastula transition. Ophelia Papoulas. Sect MCD Biol, Univ Texas, Austin, TX.

The molecular signals driving the developmental shift referred to as the mid-blastula transition (MBT) remain mysterious. At the MBT, embryonic cleavage divisions give way to longer asynchronous divisions and the onset of morphogenetic movements. In the syncytial Drosophila embryo, rapid synchronous nuclear divisions pause at the MBT to permit membrane invagination and formation of a cellular blastoderm. These morphological changes must be coordinated with the ongoing hand-off from maternal to zygotic genetic control. We have found that the translational regulators Fragile X mental retardation protein (FMRP) and Cytoplasmic activation/proliferation-associated protein (Caprin) act at the MBT to modulate levels of cell cycle regulators. FMRP is an RNA-binding protein known to control the local translation of specific mRNAs within neuronal dendrites. Its function in the nervous system has been extensively studied because loss of FMRP in humans causes Fragile X Syndrome, the most common form of heritable mental retardation. Vertebrate Caprin is also an RNA-binding protein implicated in transcript-specific translational repression in neuronal dendrites. We find both these translational regulators are present throughout early embryogenesis but act specifically at the MBT. In both neuronal dendrites and embryos dFMRP and Caprin appear to mediate rapid changes in translation in response to specific signals. The nucleo-cytoplasmic ratio has long been viewed as a key signal triggering events of the MBT, but the molecular nature of the signal is unknown. Through immunoblotting of precisely staged embryos we have found that Caprin (CAPR) becomes phosphorylated specifically at the MBT. We are currently using a candidate genetic screen to identify the kinase responsible for this modification and determine whether it affects CAPR activity. Through these and other studies on the regulation of CAPR activity we hope to arrive at a better understanding of developmental control of local translation, and the specific signaling mechanisms underlying the MBT.

62
Role of RNA polymerase II in controlling the number of nuclear divisions and onset of cellularization. Hung-wei Sung, Jörg Grosshans. Department of Developmental Biochemistry, Göttingen Center for Molecular Biosciences, Göttingen, Niedersachsen, Germany.

In the early embryos, the nuclei proliferate by invariantly 13 synchronous nuclear divisions. Concomitantly with the pause of the cell cycle, the zygotic transcription is activated, maternal RNAs are degraded and cellularization starts. The transition is commonly referred to as mid-blastula transition (MBT). It is not clear how the number of nuclear divisions is so robustly controlled, and how the different processes are coordinated. Previously it has shown, that (1) the extension of interphases and the cell cycle regulators such as grapes, (2) the degradation of maternal RNAs, such as string mRNA, and (3) the expression of zygotic mitotic inhibitors, such as frühstart are involved in the timing of the transition. Here we present the evidence for a direct role of the RNA polymerase II in timing MBT. By screening mutants in germ line clones with morphological defects in early embryogenesis, we identified a novel allele of RNA polymerase, RPII215X161, with a single nucleotide exchange in the 3'-untranslated region of the gene. Half of the mutant embryos (independent of the zygotic genotype) undergo only 12 nuclear divisions and start cellularization precociously. In addition, zygotic genes slam and frühstart are expressed earlier and maternal transcripts of CDC 25 homologs, string and twine are degraded earlier than normal in all embryos. We will discuss conceivable models for how level of RNA polymerase II is involved in the robust timing of MBT.
The role of the mitochondria in apoptosis has been clarified in mammals, but has remained unclear in Drosophila. Elucidation of conserved mitochondrial roles in cell death signaling pathways is required. The purpose of this study was to assess how the mitochondrial fusion/fission machinery might affect cell death induced by Reaper or γ534 irradiation. Strikingly, knockdown of MARF causes a tremendous loss of adult wing tissue and significant apoptosis in the larval fat body. In the follicle cells six genomic regions are amplified during differentiation. Strikingly, mRNA-seq shows that amplification is not the sole mechanism to drive gene expression and that it does not uniformly lead to abundant expression. In addition to revealing the prevalence of differential replication, our results show that the DNA replication and transcriptional programs can be mechanistically uncoupled. Furthermore, given that SuUR is a key component of a newly identified repressive chromatin domain, our results suggest that this domain can be remodeled during development to modulate the replication and transcription programs. As well as uncovering tissue-specific underreplication, our genome-wide analysis has provided the opportunity to probe the mechanism by which SuUR promotes underreplication. Our results clearly show that SUUR does not block origin firing, but rather affects replication fork progression, thus providing new insights into how underreplication is established.

Apoptosis negatively regulates necrosis in Drosophila. Yong Yang, Lin Hou, Meng Yang, Lei Liu. Peking University, Beijing, China. Cell death can generally be classified into three types, including apoptosis, autophagy and necrosis. However, necrosis is poorly defined at the molecular level, and be considered a stochastic event for a long time. To better understand the biology of necrotic cell death, we generated a transgene to express a leaky ion channel (bBNC1G430C), human Brain Na+ Channel 1 Gly 430 to Cys mutation, we named the transgenic fly, “C16” in Drosophila. After heat shock activation of C16, we observed fly lethality. Consistent with calcium overloaded cell injury in worms, the C16 flies could be rescued by loss-of-function in calreticulin, calpain and cathepsin. Using transmission electron microscope, we confirmed the morphology of cell death in the fly brain was necrosis, because of the ruptured cell membrane in the C16 flies. Therefore, the lethality of conditional expression of C16 likely belongs to the calpain-mediated necrosis. While characterized the C16 flies, we surprisingly found that conditions that rescued apoptosis could enhance necrosis, including overexpression of dIAP2 and P35 (a viral protein). Moreover, we found that IAP2 RNAi or overexpression of Drone (an initiator-caspase to activate apoptosis) could rescue the C16 lethality. Because caspase can cleave dIAP2, we speculate that apoptosis may block necrosis through down regulation of dIAP2. Currently, we are testing this hypothesis and working on the function of dIAP2.

The role of mitochondrial dynamics in Drosophila apoptosis. Eltyeb Abdelwahid1, Michael Thomenius2, Christopher Freel2, Sarah Horn2, Ronald Kriese1, Rachel Cannon1, Sujatha Balasundaram1, Sally Kornbluth2, Kristin White1. 1) Cutaneous Biol Research Ctr, Massachusetts General Hosp, Charlestown, MA. USA; 2) Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina 27720 USA. The role of the mitochondria in apoptosis has been clarified in mammals, but has remained unclear in Drosophila. Elucidation of conserved mitochondrial roles in cell death signaling pathways is required. The purpose of this study was to assess how the mitochondrial fusion/fission machinery might affect apoptosis in Drosophila. Both in vivo and in vitro systems are used in this study. Particularly, we used overexpression and knockdown in the fly and in tissue culture cells. Recently, we showed that the mitochondrial fission mediator Drp1 is important for mitochondrial changes in response to Krr expression and ionizing radiation. Inhibition of Dp1 resulted in a significant inhibition of mitochondrial disruption and apoptosis in fly cells. We are now investigating the role of mitochondrial fusion proteins (mitofusins) in Drosophila cell death. Our results indicate that Drosophila MFN (MARF) over-expression can inhibit cell death induced by Reaper or γ irradiation. Strikingly, knockdown of MARF causes a tremendous loss of adult wing tissue and significant apoptosis in the developing wing discs. Interestingly, Reaper can induce mitochondrial fragmentation by binding to and inhibiting the mammalian pro-fusion protein (MFN2) and MARF. We are also undertaking experiments to determine how inhibition of MARF activates apoptosis, by assaying genetic interactions. Future experiments may allow us to characterize how regulation of mitochondrial fragmentation can modulate apoptosis in Drosophila.

Cdk5 and Mekk1 mediate a Pro-Apoptotic Signaling Response to Endoplasmic Reticulum Stress. Min-Ji Kang, Hyung Don Ryoo. Dept Cell Biol, New York Univ Sch Med, New York, NY. Animal tissues respond to various stress conditions by activating signaling pathways that either repair or eliminate the afflicted cells. While the activation of cell death programs can be induced under certain conditions, stress-induced death of vital cells underlies many chronic diseases. Here, we report the identification of a signaling pathway that is activated by endoplasmic reticulum stress (ER) and induces the apoptotic cell death program. Specifically, we identified Cdk5 as a mediator of ER-stress-induced apoptosis through an in vivo RNAi screen in Drosophila. Cdk5 physically interacts with Mekk1, and together, activate the INK pathway for apoptosis. Moreover, inhibition of this pathway can delay the course of age-related retinal degeneration in a Drosophila model of Autosomal Dominant Retinitis Pigmentosa. These findings establish a previously unrecognized branch of ER-stress response signaling relevant to disease.

138
The synaptic vesicle-associated Ca\textsuperscript{2+} channel Flower couples synaptic exo-endocytosis cycle and regulates synaptic growth. Chi-Kuang Yao\textsuperscript{1,2}, Yong Qi Lin\textsuperscript{1,2}, Claire Hauteur\textsuperscript{1,2}, Hugo J. Bellen\textsuperscript{1,2}. 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Howard Hughes Medical Institute, Baylor Col Medicine, Houston, TX.

Synaptic function and growth are two key components for synaptic plasticity that has been implicated in learning and memory. Synaptic function is achieved by synaptic vesicle (SV) exocytosis that, in response to action potentials, elicits a fusion of SV with the presynaptic membrane, leading to the release of neurotransmitters to provoke postsynaptic responses. SVs must then be properly endocytosed to sustain repeated transmission. Hence, a tight coupling of exo- to endocytosis is critical for synaptic function. Yet how this coupling is controlled remains poorly understood. Our previous work (Yao et al., Cell 2009, 138(5):947-60) has shown that the SV-associated Ca\textsuperscript{2+} channel Flower (Fwe) promotes synaptic endocytosis and thereby couples exo- to endocytosis. Intriguingly, loss of fwe also leads to synaptic outgrowth at larval neuromuscular junctions (NMJs), characterized as a increased number of satellite boutons, similar to those observed in many endocytic mutants, including shi, synj, endo, dapi160, and lap. The similarity in phenotype suggests that these endocytic proteins may govern a common cellular machinery underlying SV endocytosis and synaptic growth. However, what these cellular mechanisms are is still unclear. More recently, we have been focusing on understanding the mechanisms underlying Fwe-mediated synaptic growth. Our results indicate that the Ca\textsuperscript{2+} influx triggered by Fwe is required for synaptic endocytosis but not growth, suggesting that Fwe has two distinct cellular functions. Furthermore, synaptic outgrowth associated with loss of fwe is significantly suppressed by inactivating BMP or JNK signaling, indicating that Fwe may negatively regulate these two signaling pathways at NMJs. Most interestingly, our preliminary results suggest that trafficking of lysosomes and mitochondria may play an important role in Fwe-mediated synaptic growth. We are attempting to determine the relationship between these different pathways.

Dopamine neurons modulate pheromone responses in Drosophila courtship learning. Krystyna M. Keleman, Jai Y. Yu, Sebastian Kruettner, Eleftheria Vrontou, Barry J. Dickson. IMP, Vienna, Austria.

Courtship conditioning in Drosophila is a natural form of learning, whereby a male fly learns to discriminate between receptive virgins and unreceptive mated females in order to maximize his mating success. We show that this courtship learning is mediated by olfactory neurons that detect the pheromone cis-vaccenyl acetate (cVA), present on mated but not virgin females. The cVA signal is conveyed via DA1 olfactory projection neurons to the mushroom body (MB). We identify a specific class of dopaminergic neurons that innervates the MB gamma lobe, and is critical for courtship conditioning. Activation of dopamine neurons modulate pheromone responses in Drosophila courtship learning. Functional screening of defined sets of MB extrinsic neurons. Here we show that MB-V2 neurons are essential for retrieval of both short- and long-lasting memory, but not during memory formation or consolidation. We further show that MB-V2 are cholinergic efferent neurons that project from the MB gamma lobe, and is critical for courtship conditioning. Activation of dopaminergic neurons in naïve flies mimics training. Behavioural plasticity triggered by natural training or the activation of dopaminergic neurons requires the D1 dopamine receptor in the MB gamma neurons. These studies define key features of the neural circuit and molecular mechanisms that mediates courtship learning in Drosophila.

Neural Circuit Responsible For Aversive Olfactory Memory Retrieval In Drosophila. Julien Séjourné\textsuperscript{1}, Pierre-Yves Plaçais\textsuperscript{1}, Yoshinori Aso\textsuperscript{2}, Igor Siwanowicz\textsuperscript{2}, Stevanus R. Tedjakumala\textsuperscript{1}, Guillaume Isabel\textsuperscript{1}, Kei Ito\textsuperscript{1}, Paul Tchénio\textsuperscript{1}, Hiromu Tanimoto\textsuperscript{1}, Thomas Preal\textsuperscript{1}. 1) Genes and Dynamics of Memory Systems, Neurobiology Unit, ESPCI, CNRS, PARIS, France; 2) Max-Plack-Institut fur Neurobiologie, Am Klopferspitz 18, D-82152, Martinsried, Germany; 3) Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-0032, Japan.

Aversive olfactory memory is formed in Drosophila mushroom bodies (MB). Memory retrieval requires MB output, but it remains unknown how the memory trace in the MB drives conditioned avoidance of the learned odor. To identify neurons in olfactory memory retrieval, we performed an anatomical and functional screening of defined sets of MB extrinsic neurons. Here we show that MB-V2 neurons are essential for retrieval of both short- and long-lasting memory, but not during memory formation or consolidation. We further show that MB-V2 are cholinergic efferent neurons that project from the vertical lobes of the MB to the middle superiormedial protocerebrum and the lateral horn, a brain region implicated in innate response to odours. Surprisingly, using in vivo calcium imaging we show that MB-V2 neurons have a decreased response to the conditioned odor, suggesting these neurons are inhibited by MB after conditioning. We propose that during memory retrieval, the MB could increase the avoidance to the conditioned odour via a modification of the activity of the MB-V2 neurons.

Functional organization of the neural circuitry for male courtship behavior in Drosophila. Yufeng Pan, Carmen Robinett, Brian Dias, Bruce Baker. HHMI: Janelia Farm Research Campus, Ashburn, VA.

fruitless (fru) and doublesex (dsx) are two terminal genes in the sex hierarchy in Drosophila melanogaster. Here we artificially activate fru\textsuperscript{mH} (male-specific fru) or dsx neurons in males using a temperature sensitive activator dTRPA1, enabling us to dissect two key components in male courtship behavior; courtship behavioral outputs and courtship recognition. Almost all steps of courtship behavior, from courtship song to ejaculation, could be induced through activation of all fru\textsuperscript{mH} or dsx neurons in males, even without FruM function. The system for male recognition still works well when all fru\textsuperscript{mH} or dsx neurons are activated, but breaks down in the absence of FruM. We will also show that activation of fru\textsuperscript{mH} or dsx neurons promotes courtship to specific sensory cues. Taken together, our results indicated that execution of courtship outputs and promotion of courtship to certain sensory cues are mediated through stimulatory regulation by fru\textsuperscript{mH} or dsx neurons, and courtship coordination and recognition are through inhibitory regulation by fru\textsuperscript{mH} neurons. Our data give insights into how courtship programs and mate recognition are genetically programmed and organized into a courtship circuitry that specified by both fru\textsuperscript{mH} and dsx.

The transcription factor Mef2 links the central circadian clock to neuronal remodeling. Anna Sivachenko, Katharine Abruzzi, Michael Rosbash. Dept Biol, Brandeis Univ, Waltham, MA.

The transcription factor Mef2 is a key regulator of muscle development in Drosophila. Its mammalian homologues play diverse roles in development, and are implicated in regulation of synaptic plasticity and neuronal morphology. We identified Mef2 in a screen for transcripts enriched in the pacemaker
neurons that govern circadian behavior in Drosophila. Importantly, our recent genome-wide analysis revealed that dMEF2 itself is a direct target of the master circadian regulator Clock. These findings taken together led us to a hypothesis that MeF2 might serve as a regulator of circadian neuronal remodeling, one of the important mechanisms by which core pacemaker cells transfer the information to downstream systems. Indeed, genetic manipulations of MeF2 levels in the pacemaker cells lead to abnormal morphology and disrupted circadian remodeling in their axonal projections. To elucidate molecular mechanisms underlying MeF2 function, we performed genome-wide ChIP-on-chip analysis of MeF2 target genes in adult Drosophila heads, and found that putative MeF2 targets include many genes with known roles in neurogenesis and synaptic plasticity. Our results indicate that function of MeF2 may provide an important link between the core circadian remodeling of neuronal circuits in the fruit fly.

Remote control of Drosophila behavior using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). Charles D. Nichols1, Jaime Becnel1, Oralee Johnson1, Bangning Yu1, Bryan L. Roth1. 1) Dept Pharm, LSU Hlth Sci Ctr, New Orleans, LA; 2) Dept Pharm, UNC Med School, Chapel Hill, NC.

We are developing a powerful new genetic tool in Drosophila to selectively, rapidly, and reversibly control neuronal function and behaviors utilizing Designer Receptors Exclusively Activated by Designer Drugs (DREADD). Through a process of directed molecular evolution, we have generated a set of mammalian muscarinic acetylcholine G-protein coupled receptors that have lost affinity for their endogenous ligand, acetylcholine, but have gained the ability to be fully activated by an otherwise biologically inert chemical, clozapine-N-oxide (CNO). The Gi/o coupled R1 receptor is believed to be a silencer, whereas the Gs coupled Rs receptor and the Gq coupled Rq receptor are believed to be activators. We have subcloned the mammalian DREADD receptors into the pUAST vector, generated transgenics, and assessed their effectiveness to modulate behaviors in combination with various neuronal GAL4 drivers. First, we determined that CNO fed in food (up to the 10 mM tested) has no observable overt behavioral or developmental effects on flies. Next, utilizing various neuronal GAL4 drivers to selectively express DREADD receptors, we were able to successfully and dramatically alter behaviors that included olfactory learning and memory, courtship and mating, and circadian behaviors by simply feeding flies CNO. An important feature of this system is that, as opposed to the ‘on-off’ regulation of TRP or NaChBac channels and channelrhodopsins, DREADD receptors can be activated dose dependently, allowing for greater control of the magnitude of response by simply altering the drug concentration. Significantly, due to the near ubiquitous expression of GPCRs, this technology is not limited to the study of neuronal tissues. DREADDs can be used to manipulate individual signal transduction pathways both in short term and long term studies to investigate any tissue in the organism with no need for specialized equipment due to convenient systemic treatment with activating ligand.

Expanding the Olfactory Code in Drosophila by in Silico Decoding of Receptor-Odor Chemical Space. Sean M Boyle1, Shane McInally2, Sana Tharada1, Anandasankar Ray1,2. 1) Genetics Genomics, and Bioinformatics, University Of California, Riverside, CA; 2) Department Of Entomology, University of California, Riverside, CA.

Little is known about how a large ensemble of 60 odor receptor proteins in Drosophila detects small volatile molecules with high specificity and sensitivity. Understanding these interactions is particularly challenging due to the extreme diversity in both odorant structures and receptor sequences. Each receptor can detect several odors from a possible odor coupled receptors of hundreds of thousands of volatile compounds. Since the structures of odor receptors are not available for receptor-odor docking, we have analyzed these interactions by identifying the shared structural features amongst known activating odors for each of 24 antennal receptors. We then use these receptor-optimized systems to screen a large library of compounds (>240,000) in silico for novel ligands, including an extensive collection of plant and animal volatiles. Functional validation using electrophysiology supports a very high success rate (>75%) for the screen allowing us to model representation of the >240,000 library by the ensemble of 24 odor receptors in the Drosophila antenna. We identify hundreds of new activators and several inhibitors for each receptor, a small fraction of which evoke patterns of temporal activity that are unusual. Behavioral testing suggests that odors with different temporal responses can elicit different behaviors. In summary this chemoinformatics driven approach allows, for the first time, a systems level view of odor coding in Drosophila one of the premier models for studying olfactory coding and dramatically expands the known olfactory code.

Dietary restriction improves neurotransmission at an adult NMJ in dynactin complex mutant flies. Joel Rawson1,2, Holly Davison1, Tabita Kreko1, Rebekah Mahoney1, Leo Chang1,2, Alex Bokov2, Jonathan Gelfond2, Gregory Macleod1, Benjamin Eaton1,2. 1) Dept. of Physiology, UTHSCSA, San Antonio, TX; 2) Barshop Institute for Aging and Longevity Studies, UTHSCSA, San Antonio, TX.

Mutations in the DCTN1 gene belong to an important class of dominant mutations that are linked to adult-onset neurodegenerative motor disease in humans. The neuronal mechanisms that are affected by aging and influence the pathogenesis of disease in individuals harboring DCTN1 mutations remain unknown. Using the cibarial muscle nine neuromuscular junction (CM9 NMJ) of the adult fly, we find that age-dependent changes in NMJ morphology correlate with age-dependent reductions in proboscis extension in flies expressing dominant negative glued (the Drosophila DCTN1 homologue) in the CM9 motor neuron. Because the CM9 motor neuron has been shown to be necessary and sufficient for proximal proboscis extension the behavioral output is a direct reflection of motor neuron function. Electrophysiological analysis finds that reduced synaptic vesicle release occurs prior to age-dependent changes in synapse morphology at mutant CM9 NMJs. These data provide evidence that reduced presynaptic function is an early event during the pathogenesis of neurodegeneration in dynactin complex mutants, and that the ameliorating effects of DR on motor function are the result of improved synaptic vesicle release. Importantly, a similar effect of diet on neurotransmission was also observed in control flies demonstrating a generalized effect of diet on neurotransmission at the adult NMJ. These observations have important implications for the effects of diet and obesity on nervous system function.
The CCCTC-binding Factor (CTCF) of Drosophila Contributes to the Regulation of the Ribosomal DNA and Nucleolar Stability. Paola A. Guerrero, Keith Maggert. Dept Biol, Texas A & M Univ, College Station, TX. 77843.

The 35S rDNA gene clusters on the X and Y chromosomes are repeats of approximately 150 to 225 copies. Each are transcribed as a single unit by RNA Polymerase I and modified into the 18S, 5.8S, 2S and 28S ribosomal rRNAs. Reduction in the array copy number results in a bobbed phenotype, due to a decrease in protein production. In some copies within the arrays, R1 and R2 retrotransposable elements are inserted in a conserved region of the 28S gene which represses the transcription of a functional rRNA. Inserted arrays are transcribed at very low levels, but it is not clear how they are identified for repression. Similarly, a subset of uninserted arrays are silenced, and the epigenetic mechanism controlling how this decision is made it is also unknown. The CCCTC binding factor (CTCF) is a boundary element binding protein and a transcriptional regulator found in the nucleolus of differentiated mammalian cells, whose localization requires poly (ADP-ribosyl)ation. We investigated whether CTCF might be involved in the regulation of rDNA expression in Drosophila. Our data show that CTCF is found at the nucleolus of both polytene and diploid nuclei, and we have identified binding sites in the 28S gene, R1 and R2 elements. ChIP data indicate that CTCF binds only to the site in the R1 retrotransposon. Reduction of CTCF or members of the poly(ADP-ribosyl)ation pathway by RNAi in S2 cells causes an increase in the transcription of the 35S rDNA gene, R1, and R2 elements. In flies, CTCF and PARG mutant alleles show disrupted nucleoli and increased rDNA transcription. Mutant alleles of CTCF suppress variegation of a P-element inserted in a 35S rDNA array, but not of elements inserted elsewhere in the genome. Consistent with a role for CTCF in rRNA regulation, we found that during oogenesis CTCF is recruited to the nucleolus of nurse cells at early stages when the demand of ribosomes is low and it leaves this compartment in later stages when the cell increases rDNA transcription. We conclude from these studies that CTCF acts as a regulation of rDNA transcription by RNA polymerase I.

Spatial and temporal dynamics of homologous recombination repair in Drosophila heterochromatin. Irene Chiolo1, Aki Minoda1, Serafin U. Colmenares1, Aris Polyzos1, Silvain V. Costes1, Gary H. Karpen1,2. 1) Genome Biology, LBNL, Berkeley, CA; 2) MCB, UC Berkeley, Berkeley, CA.

Double-strand breaks (DSBs) in heterochromatid repetitive DNAs pose unique threats to genome integrity, but information about how such lesions are processed and repaired is sparse. We have investigated how Drosophila cells respond to DSBs in heterochromatin. We observed striking expansion and dynamic protrusions of the heterochromatin domain in response to irradiation (IR). We also discovered that heterochromatic DSBs are repaired by homologous recombination (HR), but with notable differences from euchromatic HR repair. Proteins that respond to early HR steps (resection), are rapidly recruited to DSBs within heterochromatin. In contrast, Rad51, involved in strand invasion, only associates with DSBs that relocalize outside the domain. Such relocalization is coordinated with heterochromatin expansion and relies on checkpoint activation. Finally, we the Drosophila Smc5/6 complex belongs to heterochromatin, and is required to exclude Rad51 from the domain and prevent abnormal recombination. We speculate that the spatial and temporal control of DSB repair in heterochromatin safeguards genome stability by reducing the frequency of aberrant exchanges between repeats.


The invadolysin gene encodes a novel, conserved metalloprotease of the M8 family which is essential for viability in Drosophila melanogaster. Phenotypes include late larval lethality, mitotic chromosome and spindle defects, accumulation of nuclear envelope proteins, and germ cell migration defects (McHugh, et al. JCB, 2004). We have recently shown that Invadolysin localises to the surface of lipid droplets, and strikingly, mutants have a diminished triglyceride:protein ratio (Cobbe, et al. JCS, 2009). To begin to understand Invadolysin’s mechanism of action, a second site non-complementation screen was carried out in order to identify genetic interactors of invadolysin. Herein we present that invadolysin interacts genetically with non-stop, a histone deubiquitinating enzyme, to play a role in chromatin modification in Drosophila. The invadolysin and nonstop mutants exhibit similar chromosome phenotypes, both resulting in abnormal chromosome condensation and accumulation of ubiquitinated histone H2B. Mitotic chromosome condensation is regulated by several signaling activities including cyclin-dependant kinases and ubiquitin-mediated proteinolysis. We propose that the increased level of ubH2B leads to the hypercondensed chromosome phenotype in both invadolysin and nonstop mutants. In addition, homozygous mutant larvae (for both invadolysin and nonstop) exhibit extreme larval longevity and abnormally-structured salivary gland polytene chromosomes. Moreover, both mutants accumulate histone H3 tri-methylated on lysine 4 and show an increased frequency of apoptotic cells in neuroblasts. Overexpression of the H2B ubiquitin ligase (bre1) results in a similar chromosome condensation phenotype and an increase in apoptotic cells in larval brains.
Loss of Suppressor of Hairy-wing protein affects nuclear organization and gene expression during Drosophila oogenesis. Alexey A. Soshnev1, Ryan M. Baxley1, Kyle A. Nilson1, Kai Tan2, Bing He2, Pamela K. Geyer1,3. 1) Molec & Cellular Biol, Univ Iowa, Iowa City, IA; 2) Internal Medicine, Univ Iowa, Iowa City, IA; 3) Biochemistry Dept, Univ Iowa, Iowa City, IA.

Cell differentiation is accompanied by dramatic changes in chromatin architecture that influence gene expression. One example is the differentiation of nurse cells (NCs) during Drosophila oogenesis. NCs are the synthetically active cells within the 16-cell germline cyst that support the oocyte maturation. NCs undergo genome amplification by endoreplication associated with changes in chromosome architecture from a condensed polytene to a dispersed polytide state. The loss of the Suppressor of Hairy wing [Su(Hw)] protein causes female sterility and blocks changes in NC chromosome structure. Analyses of su(Hw) mutant phenotypes reveal that female fertility does not require NC chromosome dispersal. Chromosome decondensation is blocked in the fertile su(Hw)/mutant that encodes a protein with a defective zinc finger 10. These data indicate a novel function for this domain in promoting changes in chromosome structure. Gene expression changes between multiple su(Hw) wild type and mutant backgrounds were defined using microarray analyses of dissected ovaries. Su(Hw) binding sites were mapped using ChIP followed by high throughput sequencing. Coupling transcriptional analyses with germline histone H3.3A is necessary for normal repression of Notch target genes in Notch-inactive cells, specifically neurons. His3.3A null flies exhibit a variety of cause of death in His3.3A mutants is overactive Notch signaling. We are able to rescue His3.3A phenotypes using a Notch loss-of-function allele and also by activation of Suppressor of Hairless (Su(H)). Su(H) functions in the Notch pathway as a molecular switch: in Notch-active cells, Su(H) activates repression of Notch target genes, and in Notch-inactive cells, Su(H) represses transcription of Notch target genes. We propose that activation of Su(H) by histone H3.3A is necessary for normal repression of Notch target genes in Notch-inactive cells, specifically neurons. His3.3A null flies exhibit a variety of phenotypes, including partial lethality at the first instar larval stage, larval posterior paralysis, and defects in glial cell migration during embryogenesis. Only 12% of His3.3A null mutants survive to adulthood, but viability is increased to 78% by a Notch loss-of-function allele. This result shows that the primary cause of death in His3.3A mutants is overactive Notch signaling. We are able to rescue His3.3A phenotypes using a Notch loss-of-function allele and also by ectopically expressing Su(H) in neurons. Using Western analysis and immunohistochemistry, we found that total Su(H) protein levels are reduced throughout embryogenesis in His3.3A null mutants. We used quantitative PCR to demonstrate that Su(H) mRNA levels are also reduced throughout embryogenesis. Our data suggest that H3.3A directly or indirectly activates transcription of Su(H), and Su(H) is then needed to repress Notch signaling in neurons in order for proper glial migration to occur.
The role of Pleiohomeotic and Pleiohomeotic-like in Polycomb repression. Yuri B. Schwartz1,2, Tatyana G. Kahn1,2, Per Stenberg1, Vincenzo Pirrotta2. 1) Dept Mol Biol, Umeå University, Sweden; 2) Dept Mol Biol & Biochem, Rutgers University, NJ.

Polycomb group (PcG) proteins constitute the most appreciated system of epigenetic control of cellular differentiation. Despite recent advances the mechanisms of PcG targeting to chromatin remain poorly understood. Using a genomic approach we have examined the role of Drosophila sequence-specific DNA binding proteins Pleiohomeotic (PHO) and Pleiohomeotic-like (PHOL) in the recruitment of PcG to Polycomb Response Elements (PREs). We find that PHO/PHOL containing complex PhoRC associates with a large fraction of PREs but is dispensable at some. The dissection of the interrelation between the components of PhoRC revealed unexpectedly that its efficient binding to PREs is dependent on the MBT-domain containing component dSFMBT, which is recruited to PREs independently of PHO or PHOL. The two proteins recognize the same DNA sequence and associate with the same set of genomic regions. Surprisingly, the optimal PHO/PHOL binding motif is rarely found at PREs. Instead, PREs bind PHO in preference to PHOL because of its higher affinity for dSFMBT. In contrast, the strongest binding of PHOL is to promoters of transcriptionally active genes that harbor optimal DNA binding sites. Yin Yang-1 (YY1) is an ortholog of PHO and has the same sequence specificity. It is thus commonly assumed to be involved in PcG regulation in mammals. The findings in Drosophila prompted us to revisit this concept. Surprisingly, we find that YY1 binding sites never coincide with PcG binding, implying that YY1 is not involved in PcG regulation in various types of human cells.

Autoregulation of the large noncoding roX1 RNA gene to target chromatin modifications of the Drosophila male X chromosome. Chiat Koo Lim, Richard Kelley. Department of Developmental Biology, Baylor College of Medicine, Houston, TX.

Aberrant changes to chromatin structure changes gene expression pattern and can lead to diseases like cancer. Noncoding RNAs (ncRNA) play critical roles in modulating chromatin architecture but how they are regulated and bring about such changes remain unclear. Dosage compensation in Drosophila is a good example where chromatin architecture is regulated by the interplay of ncRNA and chromatin remodeling proteins. This process doubles the expression level of the X-linked genes in the male’s single X to match those of the female’s two. The objective of this study is to understand how roX1, one of the two ncRNA in the complex, is regulated such that its expression is maintained through adulthood. Expression of roX1 begins in both sexes during cellular blastoderm but is restricted to males after germband retraction. One study argues that MSL2, the only MSL protein expressed exclusively in males, is solely responsible for this while the other postulates that all of the MSL protein subunits, but not roX RNA are required. We observed that roX1 expression fails when we induced MSL2 expression late in development, if roX2, the other ncRNA was also deleted. In other words, many cells lack DC late in development even though a full set of MSL proteins was present. We propose instead that maintenance of roX1 transcription depends on a roX RNA-containing, fully functional MSL complex. To test our hypothesis, we co-expressed a shorter, but functional mini roX1 RNA transgene late in development and ask if it can switch on the expression of the endogenous roX1+ gene that had failed to turn on. Using an in situ hybridization probe that is specific to the endogenous gene, we found that the mini roX1 transgenic RNA, together with the MSL proteins, was capable of turning on and sustaining the expression of roX1 expression in all the male cells. This is the first instance where a large ncRNA is found to positively autoregulate its own expression.
Asymmetric MBC function and F-actin formation in myoblast fusion. Claude Shelton IV, Shruti Haralalka, Heather Cartwright, Susan Abmayr. 1) Stowers Institute for Medical Research, Kansas City, Missouri; 2) Dept. of Anatomy and Cell Biology, University of Kansas School of Medicine, Kansas City, Kansas.

Gene analysis of *Drosophila* myoblast fusion has shown that genes associated with actin polymerization play a critical role. Consistent with these findings, accumulations of F-actin are found at contact sites between fusion competent myoblasts (FCMs) and Founder cells (FOs) or the growing myotube just prior to fusion. Current models suggest actin foci are symmetric, evenly distributed between FCMs and FOs/myotubes. However, whereas some genes associated with F-actin formation appear to be symmetrically distributed, others are exclusive to FOs/myotubes or FCMs. We have utilized classic *Drosophila* genetics, *in vivo* live imaging, and primary myoblast culture for the study of F-actin morphology and protein localization during myoblast fusion. We focused primarily on MBC, one part of a bipartite Rac GEF, activated Rac1, and F-actin. Functional studies show that MBC is essential in FCMs for formation of multinucleate myotubes, where it activates Rac1. MBC is not required in Founder cells. Our *ex vivo* studies of MBC localization corroborate these functional observations, and show that MBC and active-Rac are highly enriched in FCMs at sites of SNS/Kirre adhesion. *Ex vivo* analysis also revealed that, like MBC and active Rac1, F-actin foci are highly asymmetric at points of cell-contact and localize preferentially in FCMs. Furthermore, live imaging of the fusion process *in vivo* clearly shows that F-actin foci are present within the FCMs. Interestingly, *in vivo* and *ex vivo* analysis of mbc mutants revealed that F-actin foci form improperly in its absence. Whereas F-actin accumulations in wild type FCMs are very dense and often appear as single, large foci, those in mbc mutants are dispersed and composed of numerous smaller F-actin formations. Taken together, these results suggest revision of current models of symmetric MBC, Rac1 and F-actin in myoblast fusion to one where many aspects of processes leading to fusion are asymmetric and occur preferentially in the FCM.

The *Drosophila* homolog of *Arfaptin2* (*Darf2*) is a novel dynactin complex associated protein required for normal synapse growth, but not axonal transport. Leo Y. Chang, Yimin Wu, Benjamin A. Eaton. Physiology, UT Health Science Center at San Antonio, San Antonio, TX.

Mutations in the human DCTN1 gene, which encodes a crucial component of the dynactin complex, are linked to progressive motor neuron disease, similar to ALS. Determining the mechanisms underlying the pathogenesis in DCTN1 mutations is confounded by the pleiotropy of dynein function within the motorneuron including axonal transport, synapse morphology, and neuronal cell signaling. To elucidate the mechanisms responsible for the *Drosophila* neurodegeneration in DCTN1 mutants, we have performed an unbiased forward genetic screen to identify genetic modifiers of a dominant mutation in the *Drosophila* DCTN1 homolog *glued*. This screen isolated the *Drosophila* homolog of *Arfaptin2* (*Darf2*), a BAR domain containing protein that binds membranes and has been implicated in coordinating cytoskeletal rearrangements during membrane trafficking. In support of the genetic interaction of *Darf2* with the dynactin complex, co-immunoprecipitation and gradient fraction analysis find that Darf2 is associated with the dynactin complex. Importantly, *Darf2* RNAi in S2 cells results in a substantial reduction of Glued associated with light membrane fractions, suggesting that Darf2 may be functioning to tether the dynactin complex to membranes. Finally, analysis of *Darf2* mutants finds that Darf2 binding to membranes is required for normal synapse growth, but not dynacin-dependent axonal transport or synapse stability. These data suggest that membrane-binding of Darf2 is required for dynactin complex dependent synapse growth within motorneurons.

Identification of Mist, a GPCR involved in epithelial morphogenesis downstream of Folded gastrulation (Fog). Alyssa J. Manning, Kim Peters, Stephen L. Rogers. Biology Department, UNC-Chapel Hill, Chapel Hill, NC.

The ability to perceive and respond to information from the environment is a fundamental property of living cells. For each extracellular signal, cells must have an appropriate receptor to transduce the signal to the interior of the cell, a cytoplasmic system to relay this information within the cell, and effector molecules that produce an appropriate cellular response. Morphogenesis of the early *Drosophila* embryo offers a powerful system to study the mechanisms and principles of signal transduction and we have been using the folded gastrulation (Fog)-concertina (cta) signaling pathway as a model. During gastrulation, Fog causes epithelial sheet remodeling by inducing the presumptive mesodermal cells to constrict apically and internalize. The same pathway is used reiteratively throughout *Drosophila* development to induce actin-based cell shape changes. Our lab has developed a cell culture based assay to study the components of this pathway. S2R+ cells, but not S2 cells, respond to exogenously added Fog protein by forming an acto-myoosin contractile ring. RNAi-mediated depletion of any of the known Fog-cta pathway components blocks the ability of S2R+ cells to contract in response to Fog. We conducted a targeted RNAi screen to identify the *Drosophila* G-protein coupled receptors (GPCRs) involved in this pathway and identified a single orphan GPCR, mist, which is necessary for Fog-induced contractility in S2R+ cells. Moreover, we found that this receptor is sufficient to confer Fog-responsiveness to normally unresponsive S2 cells. Mist is expressed in a stripe along the ventral side of the embryo prior to gastrulation corresponding to the presumptive mesoderm and remains in these cells until after internalization. It is also concentrated in epithelial furrows in larval imaginal discs. Our data suggest that mist is a key signaling molecule that participates in epithelial remodeling events throughout *Drosophila* development.
Control of Gastrulation and Epithelial Cell Polarity by the E3 Ubiquitin Ligase Neuralized. Soline Chanet, Véronique Mayau, François Schweisguth. Institut Pasteur - CNRS URA 2578, Paris, France.

Efficient gastrulation of the mesoderm is driven by the apical constriction of the ventral epithelial cells and the disassembly/reassembly of Adherens Junctions. Both processes are under the control of the transcriptional repressor Snail. Known targets of Snail include genes of the Bearded family that encode inhibitors of the E3 ubiquitin ligase Neuralized. Here, we have investigated the potential role of the Bearded and Neuralized genes in Drosophila gastrulation. Using genomic engineering approaches, we have generated embryos that are deleted for all 8 Bearded family genes. Zygotic mutant embryos are lethal and display a cuticular phenotype associated with strong polarity defects. Consistent with this, we find that epithelial cell polarity is disrupted in stage 8 mutant embryos. These polarity defects are suppressed in the absence of the zygotic activity of Neuralized. This indicates that these inhibitors are required to prevent endogenous Neuralized to block the formation of the embryonic epithelium. So far, the only known function of Neuralized is in Notch signaling. In this context, Neuralized is required for Delta signaling activity. Since loss of Delta failed to suppress the Bearded phenotype, we propose that Neuralized has a novel Delta-independent activity in the control of epithelial cell polarity. Snail is known to repress the expression of the Bearded genes in the mesoderm whereas Twist positively regulates the expression of Neuralized in this tissue. Hence, cells of the mesoderm have high Neuralized activity. We have examined gastrulation in embryos with with strong of Neuralized activity and found that loss of Neuralized results in a specific gastrulation defect associated with defects in the disassembly/reassembly of Adherens Junctions. Together, our data indicate that Neuralized and Bearded family genes have a novel function in the control of gastrulation and epithelial cell polarity.

Regulation of Somatic Myosin Activity by Protein Phosphatase 1beta Controls Drosophila Oocyte Polarization. Yi Sun, Yan Yan, Natalie Denef, Trudi Schupbach. 1) Department of Molecular Biology, Princeton University, Princeton, NJ; 2) Howard Hughes Medical Institute.

The Drosophila body axes are established in the oocyte during oogenesis. Oocyte polarization is initiated by Gurken, which signals from the germline through the epidermal growth factor receptor (Egfr) to the posterior follicle cells (PFCs). In response the PFCs generate an unidentified polarizing signal that regulates oocyte polarity. We have identified a loss-of-function mutation of flapwing encoding the catalytic subunit of protein phosphatase 1beta (PP1beta) that disrupts oocyte polarization. We show that PP1beta, by regulating myosin activity, controls the generation of the polarizing signal. Excessive myosin activity induced by loss of PP1beta function, by a constitutively active Rho kinase construct, or by expressing a mutant myosin binding subunit in the PFCs causes oocyte mispolarization. Strikingly, PP1beta acts specifically in the PFCs, but not the anterior or lateral follicle cells, to regulate Notch signaling. This effect may be due to altered endocytosis of Notch and other transmembrane proteins that we observe in the mutant PFCs. We show that the sensitivity of mutant PFCs to defective Notch signaling is caused by the integrated activation of JAK/STAT and Egfr signaling. Interestingly, our results also demonstrate a role of PP1beta in generating the polarizing signal independently of Notch, indicating a direct involvement of somatic myosin activity in axis formation.

Role of a Kinesin-3 in Dendrite Morphogenesis. Ann Y.N. Goldstein, Xuyen M. Ho, Thomas L. Schwarz. 1) Children's Hospital, Boston, F.M. Kirby Neurobiology Center, Boston, MA; 2) Harvard Medical School, Department of Neurobiology, Boston, MA.

The kinesin-3 motor, Immaculate connections (Imac)/Unc-104, is a plus end directed motor critical for the transport of synaptic vesicle precursors. We have previously reported that Imac has a broader function at the synapse, including development of synaptic boutons and active zones. To look for functions in dendrites that may be independent of presynaptic requirements, we have turned to sensory neurons. We have found that Imac is present in both the dendrites and axon of these cells. Through the use of MARCM and RNAi, we have now uncovered dendritic functions for Imac. Clonal analysis of dendritic arborization (da) neurons revealed that Imac is necessary both for the viability of neurons and the elaboration and maintenance of Class IV dendritic branches. Although Class IV cells still grew throughout larval life, loss of Imac prevented the development of dense branching. Using RNAi, we were able to preserve cell viability and demonstrate that the morphological phenotype was not caused by cell death. With this approach, we have further accessed the function for Imac in dendritic development. The morphology of the ddaC neuron is dependent on the expression of appropriate levels of the transcription factors Cut and Knot/Collier. With Imac RNAi, Class IV neurons had decreased Knot and increased levels of Cut compared to controls. This suggests that Imac is necessary to maintain the normal transcriptional code for the growth and elaboration of this dendrite. Because the polarity of microtubules in Drosophila dendrites are primarily minus end out, we are presently testing the hypothesis that Imac mediates the retrograde transport of cargo required in the post-embryonic development of Class IV neurons.
Anisotropic apical membrane growth mediated by Src42 and dDaam is required for tracheal tube elongation. Kevin S. Nelson¹, Zia Khan², Mona Singh², Matthias Kaschube², Greg J. Beitel¹. ¹) Molecular Biosciences, Northwestern University, Evanston, IL; ²) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ.

The function of many organs, including the lung, kidney, and vascular system, depends upon the formation of appropriately sized tubes. However, the molecular mechanisms of tube size control are poorly understood. In the Drosophila tracheal system, work by multiple labs has defined a size-control pathway in which a lumenal apical extracellular matrix (aECM) restricts tube length. Here we identify Src42, a conserved tyrosine kinase, as the first intracellular component that acts instructively to regulate tube length. Loss-of-function Src42 mutations result in tracheal tubes that are too short, while overexpression of Src42 results in tracheal tubes that are too long. Src42 acts downstream or independently of the aECM pathway since Src42 overexpression causes long trachea without perturbing the aECM. Moreover, Src42 mutations can suppress the long tracheal phenotype of all tested aECM mutants. The tracheal size function of Src42 is regulated by the Diaphanous (Dia)-related formin, dDaam. Like Src42, and in contrast to dia mutations, dDaam mutations cause short tracheal tubes and can suppress the overelongation of aECM mutant trachea. Src42 and dDaam co-immunoprecipitate from embryo lysates, consistent with results from mammalian cells indicating that Src and Daam function as a complex. Quantitative 3-D image analysis of Src42 mutant trachea reveals novel insights into the cellular mechanisms required for tube elongation. Src42 mutations dramatically alter the anisotropy of apical membrane growth in tracheal cells, producing tubes that are shorter in length but larger in diameter than WT tubes. These results show that Src42 is required for controlling the direction of apical membrane growth, a previously unreported function of Src42.

Subapical targeting of Zona Pellucida proteins. Francois PAYRE¹,², Helene CHANUT¹,², Ines GONZALEZ¹,², Laurence DUBOIS¹,², Yvan LATAPIE¹,², Serge PLAZA¹,². ¹) Centre Biologie du Developpement, University of Toulouse, UPS, Toulouse, France; ²) CNRS, UMR5547, Toulouse, France.

The morphological differentiation of epidermal cells leads to the production of finely shaped apical extensions, the trichomes. It requires a precise scaffold of Zona Pellucida (ZP) proteins that locally modify the apical extracellular matrix, to sculpt the shape of trichomes. We have recently shown that 8 ZP proteins are localized in, and define, distinct apical regions, along the growing trichome. ZP proteins thus reveal a sub-compartmentalization of the apical domain. How ZP proteins are addressed to sub-apical regions of the plasma membrane remained to be elucidated. We have undertaken exhaustive genetic screening to unravel the cellular mechanisms underlying ZP protein distribution. We identify several trafficking complexes that are required for the targeting of ZP proteins to sub-apical domains. Within a given complex, however, the inactivation of different members impairs the distribution of only a subset a ZP proteins, showing an overlooked functional diversification of trafficking machineries.

References:
Fernandes et al, Dev Cell, 2010
91 Tissue elongation requires oscillating contractions of a basal actomyosin network. Li He, Xiaobo Wang, Ho Lam Tang, Denise Montell. Biological Chemistry, Johns Hopkins Univ, Baltimore, MD.

Understanding how molecular dynamics lead to cellular behaviors that ultimately sculpt organs and tissues is a major challenge not only in basic developmental biology but also in tissue engineering and regenerative medicine. Here we use live imaging to show that the basal surfaces of Drosophila follicle cells undergo a series of directional, oscillating contractions driven by periodic myosin accumulation on a polarized actin network. Inhibition of the actomyosin contractions or their coupling to extracellular matrix (ECM) blocked elongation of the whole tissue, whereas enhancement of the contractions exaggerated it. Myosin accumulated in a periodic manner prior to each contraction and was regulated by the small GTPase Rho, its downstream kinase ROCK and cytosolic calcium. Disrupting the link between the actin cytoskeleton and the ECM decreased, while enhancing cell-ECM adhesion increased, the amplitude and period of the contractions. In contrast, disrupting cell-cell adhesions resulted in loss of the actin network. Our findings reveal a novel mechanism controlling organ shape and a new model for the study of the effects of oscillatory actomyosin activity within a coherent cell sheet.

92 Nuclear hormone receptor Hr39 functions as a master regulator of female reproductive gland formation. Jianjun Sun, Allan Spradling. Department of Embryology, Carnegie Institution for Science, Howard Hughes Medical Institution, Baltimore, MD.

Glands associated with the female reproductive tract mediate many aspects of sperm survival and function in diverse organisms. In the Drosophila female reproductive tract, the glandular spermathecae store sperm and along with the parovaria produce a secretion that is critical for sperm function and sperm competition. For the first time, we identify the spermathecal and parovariolar precursors in the female genital discs as cells marked by the runt-domain transcription factor Lozenge. We also demonstrate that the SF-1/LRH-1-related nuclear hormone receptor Hr39 functions locally for spermathecal precursor specification, proliferation, and survival during early pupation, in part by regulating lozenge expression. Later, in mid-pupation, the zinc-finger transcription factor Hindsight acts as a critical factor for spermathecal and parovariolar gland cell differentiation. Ectopic expression of Hr39 is sufficient to generate a spermatheca-like structure in the male reproductive tract, implicating Hr39 as a master regulatory gene for sexual differentiation of spermathecae. The evolutionary conserved function of SF-1/LRH-1 family members in reproductive tract formation will also be discussed. These studies suggest that a genetic program for specifying female reproductive tract glands has been conserved between insects and mammals.

93 Lis-1 and asun cooperate to regulate dynein localization during Drosophila spermatogenesis. Poojitha Sitaram, Michael A. Anderson, Laura A. Lee. 1) Department of Cell and Developmental Biology, Vanderbilt University Medical Center, Nashville, TN; 2) Department of Biological Chemistry, Center for Cell Dynamics, Johns Hopkins School of Medicine, Baltimore, MD.

Mutations in human LIS1 cause a brain malformation disorder known as Lissencephaly. LIS1 is essential for nuclear migration mediated by the dynein microtubule motor complex and for the cell cycle functions of dynein. LIS1 protein has been shown to directly bind dynein although the precise role of LIS1 in regulating dynein function remains unclear. Previous work from our lab and others has suggested that dynein plays a critical role in nucleus-centrosome coupling and cell cycle progression in Drosophila spermatogenesis. To assess the role of the Drosophila homolog of LIS1 (Lis-1) in these processes, we have characterized the male-sterile phenotype of flies homozygous for a hypomorphic allele of Lis-1 (Lis-1(1-11702)). Lis-1 males have germ line defects previously associated with decreased dynein function and some new phenotypes. Centrosomes of Lis-1 spermatocytes fail to break their association with the cell cortex to migrate to the nuclear surface during late G2. The Nebenkerne of Lis-1 spermatids exhibit abnormal morphology and loss of attachments to the nucleus and basal body. These data suggest additional roles for dynein in regulating centrosomes and mitochondria during spermatogenesis. Lis-1 co-localizes with dynein during spermatogenesis, and dynein fails to localize to the nuclear surface of Lis-1 germ cells. We previously identified asun (asun) as a critical regulator of dynein localization during Drosophila spermatogenesis. Lis-1 is a strong dominant enhancer of asun in the male germ line, suggesting that these genes cooperate in the regulation of dynein during spermatogenesis. Our preliminary data indicate that asun germ line cells show reduced perinuclear localization of Lis-1, whereas ASUN localization is normal in Lis-1 testes. We present a model in which ASUN and LIS-1 act sequentially to recruit dynein to the nuclear surface, an essential step to ensure proper positioning of centrosomes at meiotic entry and fidelity of meiotic divisions.

94 PAPI, a Novel TUDOR-Domain Protein, Complexes with AGO3, ME31B and TRAL in the Nuage to Silence Transposition. Li Liu, Hongying Qi, Jianquan Wang, Haifan Lin. Yale Stem Cell Center and Department of Cell Biology, Yale University, New Haven, CT.

The nuage is a perinuclear structure that remains functionally elusive. Recently, the nuage in Drosophila was shown to contain two of the three PIWI proteins-- AUBERGINE and ARGONAUTE3 (AGO3)--that are essential for germline development. The PIWI proteins bind to PIWI-interacting RNAs (piRNAs) and function in epigenetic regulation and transposon control. Here we report a novel nuage component, PAPI (Partner of PIWIs), that contains a TUDOR domain and interacts with all three PIWI proteins via symmetrically dimethylated arginine residues in their N-terminal domain. In adult ovaries, PAPI is mainly cytoplasmic and enriched in the nuage, where it partially colocalizes with AGO3. The localization of PAPI to the nuage does not require the arginine methyltransferase dPRMT5 or AGO3. However, AGO3 is largely delocalized from the nuage and becomes destabilized in the absence of PAPI or dPRMT5, indicating that PAPI recruits PIWI proteins to the nuage to assemble piRNA pathway components. Expectedly, papi deficiency leads to transposon activation, phenocopying piRNA mutants. This further suggests that PAPI is involved in the piRNA pathway for transposon silencing. Moreover, AGO3 and PAPI associate with the P body components TRAL-ME31B complex in the nuage and transposon activation is observed in tral mutant ovaries. This suggests a physical and functional interaction in the nuage between the piRNA pathway components and the mRNA-degrading P-body components in transposon silencing. Overall, our study reveals a function of the nuage in safeguarding the germline genome against deleterious retrotransposition via the piRNA pathway.
95 Global tissue rotation polarizes a fibrillar Collagen IV matrix to control elongation of the *Drosophila* egg. Saori L. Haigo, David Bilder. Department of Molecular & Cell Biology, University of California, Berkeley, Berkeley, CA.

The development of the ellipsoid *Drosophila* egg is an elegant case of tissue elongation. Polarized cell behaviors including cell intercalation, cell shape changes and cell division are associated with the elongation of a variety of tissues during metazoan development, but the mechanisms of *Drosophila* egg elongation are not known. Using live imaging of developing egg chambers (follicles), we show that the follicle unexpectedly undergoes repeated rounds of circumferential rotation, relative to the surrounding extracellular matrix, during the elongation phase of oogenesis. Follicle epithelia mutant for elongation are not known. Using live imaging of developing egg chambers (follicles), we show that the follicle unexpectedly undergoes repeated rounds of rotation phase but become misoriented in non-rotating ‘round egg’ mutants. Furthermore, acute degradation of Collagen IV rounds previously elongated follicles, suggesting that follicle rotation polarizes a fibrillar matrix that constrains the growing egg in a ‘molecular corset’, generating its ellipsoid shape. Our work identifies global tissue rotation as a novel morphogenetic behavior that controls tissue shape and we suggest that epithelial rotation may underlie other instances of anisotropic morphogenesis.

96 Investigating the role for the Ste20-like kinase Misshapen in egg chamber elongation and integrin regulation. Lindsay K. Lewellyn, Sally Horne-Badovinac. University of Chicago, Chicago, IL.

Within the Drosophila ovary, each egg chamber elongates along its anterior-posterior (A-P) axis to transform these initially spherical structures into highly elongated eggs. During this process, the follicle cell epithelium displays an unusual planar polarity at its basal surface that is independent of the Frizzled planar cell polarity pathway. This polarization coincides with a newly described motility in this tissue, in which the follicle cells undergo a directed migration that causes the entire egg chamber to rotate around its A-P axis as it lengthens (Haigo and Bilder). Both follicle cell planar polarity and motility appear to be required for elongation morphogenesis, as mutations that disrupt these processes produce spherical eggs; however, little is known about the cellular and molecular mechanisms underlying this phenomenon. Through a forward genetic screen, we have identified the Ste20-like kinase Misshapen (Msn) as a key regulator of egg chamber elongation. Interestingly, there is a position-specific effect to the loss of Msn within the follicle cell epithelium. *msn* mutant clones in medial egg chamber regions disrupt the planar arrangement of basal actin filaments and ECM molecules, as expected for a round egg mutant. In contrast, mutant clones at the egg chamber termini have no effect on follicle cell planar polarity, and instead lead to a novel phenotype, in which wild-type cells at the clone border detach from their mutant neighbors and invade the germ cells. These disparate phenotypes can be explained by our finding that mutant follicle cells show increased integrin levels at their basal surface and adhere more tightly to the ECM. Our data suggest that Msn promotes follicle cell planar polarity and migration by negatively regulating integrins, and highlight the importance of precisely regulating adhesion levels across an epithelium during collective cell behaviors.

97 Transcriptional control in the oocyte requires the cohesin-associated protein dPds5, which participates in insulator body formation under ATM and dChk2 surveillance. Vitor J. Barbosa1, Patricia Silva1, Raquel Santos1, Ruth Lehmann1. 1) Instituto Gulbenkian de Ciencia, Oeiras, Portugal; 2) Helen and Martin S. Kimmel Center for Biology and Medicine at the Skirball Institute of Biomolecular Medicine. NYU Langone Medical Center. New York, NY.

The organization meiotic chromatin into high-order and dynamic domains that participate in gene regulation is a conserved feature of oocytes. Consequently, gamete transcriptional control has been the focus of increasing interest. The somatic nucleus achieves this control partly by forming insulator bodies, which can be visualized by co-localization of insulator proteins with the “promiscuous” component CP190. The cohesin complex is also necessary for insulator body formation by mechanisms that remain largely unknown. The mutant cohiba alleles of the cohesin-associated protein dPds5 activate a DNA damage response dependent on ATM and dChk2. We genetically show that dPds5 is required upon meiotic double strand break formation but is not monitored by the Drosophila ATR gene mei-41, suggesting that in addition to chromatid cohesion/repair other dPds5 functions must be exclusively surveilled by ATM. We provide evidence for a dPds5 function in transcription. The nuclear localization of the Poly-A-binding protein PABP2 is specifically affected in the oocyte of dPds5 germ line mutant clones. Fully functional, tagged dPds5 transgenes suggest a function unique to the oocyte. dPds5-GFP, -Venus and -Myc appear in transient nuclear foci from stage 6 onwards. CP190 co-localizes with these foci and physically interacts with dPds5-Myc in ovary extracts. Moreover, CP190 accumulates in filaments and overgrown foci in dPds5 mutant clones. Together our results suggest that dPds5 restricts CP190 during insulator body formation on meiotic chromatin. We propose that ATM monitors this process of transcriptional control. We discuss the oocyte-specificity of such process, the involvement of other insulator body components and the future approaches to identify oocyte-specific transcripts.
Sexual Compatibility Between the Germline and Soma in Drosophila. Tina L. Tootle, Andrew Spracklen, Tiffany Fagan. Anatomy and Cell Biology, University of Iowa, Carver College of Medicine, Iowa City, IA.

Somatic signals are known to alter germ cell development, but the somatic genes that mediate this process are not well understood. Using genetic screens, we identified several genetic loci that alter the specification of germ cells. The homologs of the yeast genes, checkpoint regulator homologue (CHES-1-like) and Checkpoint suppressor homologue (CHES-1-like), two genes which encode forkhead family transcription factors, mediate all three of these processes during Drosophila cardiogenesis. jumu was identified in a genomewide gene expression screen undertaken to find new genes expressed in the cardiac mesoderm (CM). CHES-1-like, which was examined because of its homology to jumu, is also expressed in the CM. Both genes were initially found to have roles in cardiogenesis in RNAi-based assays. Loss-of-function mutations in either gene exhibit localized changes, both increase and reduction, in cardioblast number as well as misaligned and incorrectly positioned heart cells. We show that these phenotypes are a consequence of both genes playing integral roles both in asymmetric cell division to produce yet a third type of cardiac cell. Finally, we demonstrate that jumu's role in both types of cell division is mediated via a genetic interaction with polo, another gene identified in our gene expression screen, and one which encodes a kinase involved in multiple steps of mitosis.

The development of a complex organ requires both the proper differentiation and production of appropriate numbers of each of its constituent cell types, as well as the correct positioning of these cells within the organ. We show that jumeau (jumu) and Checkpoint suppressor homologue (CHES-1-like), two genes which encode forkhead family transcription factors, mediate all three of these processes during Drosophila cardiogenesis. jumu was identified in a genomewide gene expression screen undertaken to find new genes expressed in the cardiac mesoderm (CM). CHES-1-like, which was examined because of its homology to jumu, is also expressed in the CM. Both genes were initially found to have roles in cardiogenesis in RNAi-based assays. Loss-of-function mutations in either gene exhibit localized changes, both increase and reduction, in cardioblast number as well as misaligned and incorrectly positioned heart cells. We show that these phenotypes are a consequence of both genes playing integral roles both in asymmetric cell division to produce yet a third type of cardiac cell. Finally, we demonstrate that jumu's role in both types of cell division is mediated via a genetic interaction with polo, another gene identified in our gene expression screen, and one which encodes a kinase involved in multiple steps of mitosis.

Two Forkhead Transcription Factors Mediate both Symmetric and Asymmetric Cell Division during Drosophila Cardiogenesis. Shaad M. Ahmad1, Terese Tansey1, Neal Jeffries1, Stephen S. Gisselbrecht1, Alan M. Michelson1. 1) National Heart, Lung, and Blood Institute, Bethesda, MD; 2) Division of Genetics, Dept. of Medicine, Brigham & Women's Hospital, Boston, MA.

The JAK/STAT pathway in the development of asymmetry within the Drosophila embryonic hindgut. Richard E Wells, David I Strutt, Martin P Zeidler. MRC CDBG, Dept of Biomedical Science, University of Sheffield, S10 2TN, UK.

Breaking symmetry during development is essential for the formation of left / right asymmetry. Surprisingly, our understanding of these processes within Drosophila greatly lags behind vertebrate systems. During Drosophila embryogenesis the hindgut forms a left right curve and represents an early model for asymmetric organogenesis. Previously, only mutants in Myo31D-F and single minded have been shown to cause hindgut inversions. However, these hindguts appear to be otherwise morphologically normal and a mechanistic understanding of the process by which this curvature forms is still lacking. Here we present a novel role for the Drosophila JAK/STAT pathway in the establishment and execution of hindgut handedness. All cells within the hindgut are competent to receive the JAK/STAT signal however only the anterior cells of the small intestine release the ligands Upd and Upd2. We confirm previous results showing that polarised pathway activation is required for correct hindgut elongation and show an asymmetry in the location of lateral border cells. Our studies have identified additional novel aspects of the JAK/STAT hindgut phenotype, namely, the inversion of handedness and a reduction in the magnitude of curvature. At the molecular level we have examined a number of factors asymmetrically enriched around the hindgut curve and, using a pathway reporter, show an asymmetry in JAK/STAT signalling. This asymmetry is absolutely required for the asymmetrical, subcellular relocation and lateralisation of the transmembrane protein FasIII, a homophilic cell adhesion factor usual localised in septate junctions. We go on to show that loss or overexpression of FasIII is sufficient to modulate the curvature of tissues more generally using the folds within the larval wing disc as a model system. We suggest that JAK/STAT pathway signalling modulates the subcellular localisation of FasIII and suggest that this novel interaction creates an asymmetry in local cell adhesion effecting the cell movements required for hindgut elongation, curvature and asymmetry.

99

100

101

Sexual Compatibility Between the Germline and Soma in Drosophila. Sheryl M Murray, Mark Van Doren. Johns Hopkins University, Baltimore, MD.

For the propagation of a species, correct development of egg and sperm must occur. The decision to make sperm vs. egg is based on the germline’s sexual identity, which is regulated by a combination of somatic influence and germline autonomous cues. In Drosophila, when somatic sexual identity opposes that of the germline, gametes fail to form, indicating that soma and germline cannot cooperate in gametogenesis when their sexual identities do not match. We investigate the nature of this sex-based soma-germline incompatibility throughout gonad development.

To create soma-germline incompatibility, we mutated the female somatic sexual identity pathway with tra or tra2 mutations (which do not affect intrinsic germ cell [GC] sex) resulting in a somatically male fly with XX GCs. Such "pseudomales" are severely GC-depleted by adulthood but form normal-looking gonads in embryogenesis, implying that later aspects of gonad development are impaired. Due to a decrease in germline stem cell (GSC) number, we investigated interactions between XX GSCs and the male niche and found that while physical attachment seems normal, signaling between the niche and GSCs is perturbed. Specifically, a transcription factor (Stat92E) fails to be highly upregulated in XX GSCs compared to XY GSCs. The Jak-Stat pathway is also known to have a masculinizing effect on GCs during early gonadogenesis. Thus, XX GCs may be less receptive to somatic masculinization signals than
XY GCs. Consistent with this logic, early ectopic expression of the Jak-Stat pathway in XX GCs was able to rescue GC-depletion and differentiation defects in pseudomales, though at a low frequency. Interestingly, XY pseudofemales also exhibit GC loss and XY GCs retain male character in a female soma. Future analysis will reveal whether other defects in pseudofemale germline-soma interactions are similar to those in pseudomales. Such studies may have implications in other animal systems where sex reversal or aneuploidy results in germline defects, including mice and humans.

102 **Shaping cells and organs through the opposing effects of fat body-secreted Collagen IV and Perlecan.** Jose C. Pastor-Pareja, Tian Xu, Dept Genetics, Yale School of Medicine-HHMI, New Haven, CT.

Basement membranes (BMs) are resilient polymer structures that underlie epithelia and surround organs in all animals. It is not known whether BMs and the assembly of their highly conserved components play active morphogenetic roles. To study in vivo the biogenesis and assembly of Collagen IV, the main constituent of BMs in all animals, we developed a GFP-trap RNAi method in Drosophila. With this method, we found that Collagen IV is synthesized in large amounts by the larval fat body, secreted to the hemolymph as a heterotrimer and continuously incorporated into BMs. We further found that incorporation of Collagen IV determines the shape of larval organs, including imaginal discs. We show that loss of Collagen IV causes cells of imaginal discs to expand basally and lose their highly columnar shape, thus flattening the tissue. A release of mechanical tension underlies this phenotype, rather than a signaling or indirect role of Collagen IV, since collagenase treatment phenocopies the loss of Collagen IV in a matter of seconds. Finally, we found that presence of Collagen IV in a BM is required for deposition of the heparan sulfate proteoglycan Perlecan, but not for deposition of Laminin or Nidogen. Surprisingly, loss of Perlecan results in a phenotype opposite to the loss of Collagen IV: increased BM tension and tissue hyperconstriction. Thus, Collagen IV determines organ shape through two mechanisms: first, by basally constricting cells, and, second, through incorporation of Perlecan, which counters constriction by the Collagen IV network.

103 **Proteases in the seminal fluid are necessary for multiple post-mating processes.** Brooke A. LaFlamme, Mariana F. Wolfner. Molec Biol & Gen, Cornell Univ, Ithaca, NY.

Proteases are an important class of proteins in the seminal fluid of animals. In flies, proteases and protease inhibitors account for over 18% of protein species in the seminal fluid. However, physiological roles for these proteases have yet to be uncovered. One of the few proteases that has been studied in the fly seminal fluid is the astacin family member CG11864. Astacin metalloproteases are involved in a variety of developmental and adult processes in animals. CG11864 is transferred to Drosophila females during mating along with sperm and other seminal fluid proteins (Sfps). Using RNAi and, recently, a new null mutation in CG11864 we found that this protease is necessary, within mated females, for the proteolytic processing of at least two other Sfps: the ovulation hormone ovulin and the sperm storage protein Acp36DE. This pathway begins in the male, where CG11864 and its substrates are synthesized together, but is only completed within the female. Thus, we focused our work on understanding the regulation of CG11864 proteolytic activity, which must have both male and female components. Like other astacin proteases, CG11864 is synthesized in a larger form consistent with a predicted inactive zymogen that must be cleaved to form the active protease. This cleavage occurs in the male, after CG11864 has left its tissue of synthesis but prior to its entering the female. We identified the upstream Sfp that is required for cleavage of CG11864; as expected this Sfp is also essential for cleavage of ovulin and Acp36DE within the female. The upstream protein is a trypsin-like serine protease that is also cleaved in transit to the female. In addition, the serine protease is also necessary for separate, CG11864-independent, post-mating events. Specifically, it is an essential member of the pathway that localizes Sex Peptide to sperm, and thereby allows for extended egg-laying and decreased receptivity by mated females. We will present these new results, as well as results of our current tests for the functional relevance of this processing pathway in ovulation regulation and sperm storage.

104 **Phosphoinositides regulate nuclear shaping and chromatin remodeling during Drosophila sperm development.** Lacramioara Fabian1, Ho-Chun Wei1, Kishan Bellamkonda1, Julie A. Brill2. 1) Developmental and Stem Cell Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada.

Spermatogenesis is essential for male fertility and is highly conserved across species. Nuclear shaping and chromatin compaction are critical processes for the formation of functional sperm. During late stages of sperm development, spermatid nuclei undergo dramatic morphogenesis, becoming needle-shaped. This phase involves chromatin condensation and remodeling, in which somatic histones that package the DNA in early spermatids are replaced first by transition proteins and then by protamines, which further remodel and package the chromatin into the long, thin sperm nucleus. Previous studies have focused on the role of protamines, small basic molecules, in chromatin condensation. However, the signals that promote correct packing of chromatin by protamines are currently unknown. Here, we show that phosphoinositide levels are critical for shaping the sperm head and for chromatin condensation during Drosophila melanogaster spermiogenesis. Spermatids in which levels of phosphatidylinositol 4,5-bisphosphate (PIP2) have been reduced show profound defects in nuclear shaping. Protamines are incorporated into the nucleus and colocalize with nuclear DNA, but they do so at earlier stages, when histones have not yet been completely degraded. In addition, transition proteins are missing in these spermatids. By immunofluorescence and immunoelectron microscopy, we show that PIP2 is present in regions adjacent to and inside the nucleus during late stages of spermatid differentiation. Thus, PIP2 is well positioned to regulate interactions between nuclear membranes and factors that control nuclear shaping during sperm development. Our data strongly suggest that spatially restricted PIPs, PIP regulators and PIP-binding proteins control sperm development by regulating cellular processes involved in chromatin remodeling and nuclear shaping.

150
Expression in two patches corresponding to the dorsal appendage forming cells. We have shown that the EGFR pathway down-regulates GALACTOSIDASE expression uniformly and then down-regulated in the dorsal domain during mid-oogenesis. Concurrently, a synthetic enhancers in transgenic flies test ‘grammar’ hypotheses.

We have also identified a fragment in brLE that when removed leads to ectopic /g533- (br), a transcription factor necessary for hunchback expression in the space between the two BR patches. Through genetic experiments, we found that this region in Drosophila embryos was used to identify all transcripts by RNA-seq that are maternally provided and localize to germ cells, as well as transcripts that are expressed at the onset of zygotic transcription in the germline. Analysis of expression levels reveals 94 genes that are zygotically transcribed as early as embryonic stage 8-9, and 121 genes that are transcribed by stage 12-13, in Drosophila germ cells. These genes were used to search for transcription factor binding motifs in order to identify the transcriptional regulatory pathways that help specify germ cells. In addition, small RNA sequence information, as well as expression levels of long non-coding RNAs and transposons are helping us gain a complete picture of the processes that regulate germline gene expression.

Two Non-overlapping Enhancers Regulate broad Expression in Response to EGFR Signaling. Lily S. Cheung Chang1, Alisa Fuchs2, Giorgos Pyrowolakis2, Stanislav Y. Shvartsman1. 1) Chemical and Biological Engineering, Princeton University, Princeton, NJ, USA; 2) Institute for Biology I, Albert Ludwigs University of Freiburg, Freiburg, Germany.

During Drosophila oogenesis, the Epidermal Growth Factor Receptor (EGFR) pathway controls gene expression in the follicular epithelium that forms the eggshell. In particular, the dorso-ventral gradient of EGFR signaling determines the boundaries of expression of broad (br), a transcription factor necessary for the formation of the dorsal appendages in the future eggshell. The inductive signals that control BR expression are well characterized, but the regulatory DNA sequences that interpret these signals remained unknown. We have identified two non-overlapping enhancers in the br locus capable of driving LacZ expression in transgenic flies. Together they recapitulate the dynamic expression pattern of BR. The pattern of BR is first driven by an Early Enhancer (brEE) that is expressed uniformly and then down-regulated in the dorsal domain during mid-oogenesis. Concurrently, a Late Enhancer (brLE) takes over and drives expression in two patches corresponding to the dorsal appendage forming cells. We have shown that the EGFR pathway down-regulates brEE and activates the expression of brLE through its effect of the transcription factor mirror. We have also identified a fragment in brLE that when removed leads to ectopic β-Galactosidase expression in the space between the two BR patches. Through genetic experiments, we found that this region in brLE is required for the regulation by the transcription factor pointed. Further dissection of these enhancers will allow the characterization of the relative contributions of the spatial and temporal cues responsible for patterning the follicular epithelium.

Synthetic enhancers in transgenic flies test ‘grammar’ hypotheses. Richard W. Lusk1, Holli A. Weld2, Michael B. Eisen1,2. 1) Department of Molecular & Cell Biology, UC Berkeley, Berkeley, CA; 2) Howard Hughes Medical Institute, UC Berkeley, Berkeley, CA.

The clustering of transcription factor binding sites in developmental enhancers and the apparent preferential conservation of clustered sites have been widely interpreted as proof that spatially constrained physical interactions between transcription factors are required for regulatory function. However, in a paper we published last year, we called this evidence into question, showing that selection on the composition of enhancers alone, and not their internal structure, leads to the accumulation of clustered and overlapping sites with evolutionary dynamics that suggest they are preferentially conserved. Here we follow up on this work by asking if synthetic enhancers created to mimic the binding site composition of a target enhancer while scrambling the spatial arrangement of its binding sites reproduce the expression pattern of the original. We used two complementary computational models to create scrambled versions of the even-skipped stripe 2 enhancer. In the first, we computationally evolved the D. melanogaster version of the enhancer according to selective constraints on binding site composition derived from orthologous and compositionally similar enhancer sequences. This model allowed us to make stepwise changes to the sequence, limiting any perceived variation in expression to small groups of binding site turnover events. In the second model, we scrambled
the sequence according to a Markov chain, allowing us to maintain the composition of binding sites that may as yet be uncharacterized. Sequences from each of these models were synthesized and used to create transgenic flies. The expression patterns observed in these flies shed light on the nature of spatial constraint in Drosophila developmental enhancers.

109 The regulatory specificity of a homeodomain protein is determined by unique DNA binding sequences. B.W. Busser1, L. Shokri2, S.A. Jaeger2, S.S. Gisselbrecht1, A. Singhania1, M. Berger2, B. Zhou3, M.L. Bulyk2, A.M. Michelson1. 1) National Heart, Lung and Blood Institute, Bethesda, MD; 2) Division of Genetics, Brigham & Women’s Hospital, Boston, MA.

Homeodomain (HD) transcription factors (TFs) have highly specific biological activities, yet they paradoxically bind to very similar DNA sequences. To investigate how HD TFs regulate specific gene expression patterns, we used an integrated approach combining genome-wide expression profiling, high-throughput determination of individual HD binding specificities, computational analysis of genome-wide binding site distributions, and cis and trans tests of target gene regulation. We first showed that muscle founder cell (FC) genes are differentially responsive to overexpression of the muscle HD TFs, Slouch (Slou) and Muscle segment homeobox (Msh). We next used protein binding microarrays to define the specific sequences that are bound by Msh, Slou and other HD TFs that are expressed in the embryonic mesoderm. These studies revealed that the majority of binding sites are shared by all HD TFs, but each TF also binds a number of unique sequences (“HD-preferred sites”). The latter finding led us to hypothesize that these preferred binding sequences might confer HD regulatory specificity. Consistent with this possibility, a computational scan showed that Slou-preferred binding sites are overrepresented along with motifs for a core set of FC TFs in the noncoding regions of Slou-responsive FC genes. To directly test the role of HD-preferred binding sites, we identified and mutated single Slou-preferred sites in known enhancers from two Slou-responsive FC genes. These mutations caused either inactivation or de-repression of the reporter in particular Slou-expressing FCs, results which precisely correlated with the effects of slou loss-of-function on each of the endogenous genes. Thus, distinct HD binding preferences confer regulatory specificity, thereby mediating discrete biological effects. The present findings establish a previously unrecognized mechanism by which cell type-specific HD selectors determine the unique genetic programs of single embryonic cells.

110 RB and CAP-D3 co-regulate transcription in Drosophila and human cells with important consequences for innate immunity. Michelle S. Longworth1,2, Jim Walker1, Endre Anderssen1, Nam-Sung Moon1, Andrew Gladden1, A. Singhania1, M. Berger2, B. Zhou3, M.L. Bulyk2, A.M. Michelson1. 1) National Heart, Lung and Blood Institute, Bethesda, MD; 2) Division of Genetics, Brigham & Women’s Hospital, Boston, MA.

RB proteins are well known for their ability to repress transcription of genes bound by E2F transcription factors. Previously, we discovered a novel, E2F independent interaction of RB with the Condensin II protein, CAP-D3. This interaction is conserved in both Drosophila and human cells. Microarray results from Drosophila mutants which are significantly reduced in their expression of the RB homolog RBF1 or dCAP-D3 demonstrate that RBF1 and dCAP-D3 share a significant number of transcriptional targets. Interestingly, the majority of these co-regulated targets are not E2F regulated genes. In adult flies, RBF1 and dCAP-D3 co-regulate clusters of genes including many antimicrobial peptides (AMPs) involved in innate immunity. ChIP for dCAP-D3 and RBF1 in adult fat body confirms that AMPs are direct targets, with binding of dCAP-D3 and RBF1 increasing in response to infection. In the absence of this transcriptional regulation, RBF1 and dCAP-D3 mutant flies are more susceptible to microbial infection. Finally, we show that human AMPs are co-activated by RB and CAP-D3 in human cells. These data suggest that RB and CAP-D3 transcriptional co-regulation of gene clusters is conserved and possibly important for human disease as well.

111 Forward genetic analysis of a stage- and tissue-specific hormonal response during Drosophila metamorphosis. Robert Ihry, Arash Bashirullah. Division of Pharmaceutical Sciences & Graduate Program in Cellular and Molecular Biology, University of Wisconsin-Madison, Madison, WI.

Sequential pulses of the steroid hormone ecdysone trigger diverse biological processes during metamorphosis. It is unclear, however, how systemic pulses of ecdysone coordinate both stage- and tissue-specific biological responses during development. Here we use a forward genetic approach to tackle this problem. Twelve hours after puparium formation, the prepupal pulse of ecdysone triggers the morphogenesis of future adult structures like head, wings and legs, as well as the destruction of obsolete larval tissues like larval salivary glands and larval abdominal muscles. We generated a new collection of 900 EMS-induced lethal mutations on the third chromosome, which arrest exclusively during metamorphosis. This collection was screened for mutant animals that disrupt the destruction of larval salivary glands but not the morphogenesis of adult structures, thereby selecting for genes that mediate the specificity of ecdysone-induced responses. We identified 30 complementation groups that block salivary gland cell death in otherwise normal looking pupae. These mutations were mapped to small genomic regions using meiotic recombination and complementation tests with molecularly defined deficiencies. So far, we have mapped ten of these complementation groups to genes. Each of these genes are required for the stage- and tissue-specific expression of death activators reaper and hid in salivary glands, which explains the block in gland destruction. We will present our initial molecular and phenotypic analysis indicating that these genes are themselves transcriptional targets of ecdysone and are required for different aspects of the prepupal ecdysone response. We propose the genes identified are components of the ecdysone induced transcriptional hierarchy and act together to provide specific molecular readouts to systemic pulses of ecdysone.
However, the sufficiency of the proposed networks have not been tested in realistic embryo geometries. To directly assess the relationship between geometry that inhibit Pol II binding at the core promoter. Thus, Polycomb mutants convert genes from one state of repression to another. Silent genes lacking Pol II bound boundaries between gap genes and spots of ectopic gene expression localization that are not buffered out by diffusion along the dorsal-ventral axis. We extended the network to consider Bcd averaging and identified a GRN with better quantitative and qualitative fit along both the anterior-posterior and dorsal-ventral axis.

Non-canonical compensation of zygotic X transcription in early Drosophila melanogaster development revealed through single-embryo RNA-Seq. When Drosophila melanogaster embryos initiate zygotic transcription around mitotic cycle 10, the dose-sensitive expression of specialized genes on the X chromosome triggers a sex-determination cascade that, among other things, compensates for differences in sex chromosome dose by hypertranscribing the single X chromosome in males. However, there is more than an hour delay between the onset of zygotic transcription and the establishment of canonical dosage compensation, sometime after mitotic cycle 14. During this time, zygotic transcription drives segmentation, cellularization, and other important developmental events. Since many of the genes involved in these processes are on the X chromosome, we wondered whether they are transcribed at higher levels in females and whether this might lead to sex-specific early embryonic patterning. To investigate this possibility, we developed methods to precisely stage, sex, and characterize the transcriptome of individual embryos. We measured genome-wide mRNA abundance in male and female embryos at eight timepoints, spanning mitotic cycle 10 through late cycle 14, using polymorphisms between parental lines to distinguish maternal and zygotic transcription.

Dissecting the role of the SAGA histone acetyltransferase in tissue-specific gene regulation. The histone acetyltransferase complex SAGA is a multisubunit, multifunctional protein complex involved in transcriptional regulation. SAGA has been well characterized in yeast as a transcriptional coactivator that is targeted to chromatin through interactions with transcription factors. In Drosophila, the SAGA complex is involved in the regulation of a subset of genes that varies between tissues, requiring tissue-specific recruitment of the SAGA complex to these gene loci. However, the mechanism of differential recruitment of SAGA between tissues is unknown. We hypothesize that SAGA interacts with tissue-specific factors which allows for differential tissue-specific recruitment of the SAGA complex. Previous studies in our laboratory determined that SAGA physically interacts with several proteins in the muscle, but not in neuronal tissues. We have focused our studies on several of these putative SAGA-interacting proteins, including MLF (myeloid leukemia factor) and Meso18E. Additionally, muscle-specific transcription factors, including Mef2, are also being examined. Affinity purifications and MudPIT (multidimensional protein identification technology) mass spectrometry analysis indicate that MLF and MEF2 both interact with SAGA in Drosophila S2 cells. Further studies will be performed to determine if these proteins are involved in targeting SAGA to
Evolutionary dynamics of CTCF binding in the Drosophila genome. Xiaochun Ni1,2, Yong Zhang1, Kevin White1,2. 1) Department of Ecology & Evolution, University of Chicago, Chicago, IL; 2) Joint institute for genomics and systems biology, University of Chicago and Argonne National Laboratory, Chicago, IL.

Evolution of gene expression is a driving force for phenotypic diversity. Insulators define regulatory domain boundaries; therefore the evolution of insulators has the potential to drive the evolution of gene expression. Here we show that the genome-wide binding of CTCF, a highly conserved insulator protein, is highly dynamic and has adaptively evolved in recently diverged species. By means of ChIP-sequencing, we generated high quality genome-wide maps of CTCF binding in four closely related Drosophila species. We found that between species binding divergence increased linearly with evolutionary distance, and they are diverging rapidly at the rate of 2.19% per million years (Myr). At least 30 new CTCF binding sites have originated in the Drosophila melanogaster genome since its split from Drosophila simulans. Comparative gene expression profiling revealed that expression divergence of genes adjacent to CTCF binding site was significantly associated with the gain and loss of CTCF binding. As the binding preference of CTCF protein is conserved, this binding evolution is likely driven by target sequence evolution in the genomic regions that define insulators. Using genome sequence data from 37 different strains of Drosophila melanogaster, we detected signatures of selection in both newly gained and evolutionarily conserved binding sites while the younger species show a significantly stronger positive selection signature in recent evolution. Our data indicate that binding of Drosophila CTCF protein has evolved under natural selection and shaped the evolution of gene expression. Our study provides the first insights into the evolution of the global architecture of an insulator complex, the first definitive estimates of binding evolution based on direct measurement across a broad phylogenetic space, and the first evidence for adaptive evolution of genome wide protein-DNA interactions.


We are working towards a new paradigm for how to capture patterns of gene expression in dynamically developing biological system such as Drosophila embryogenesis. Our ultimate goal is to monitor gene activity throughout the entire developing embryo with sufficient resolution to distinguish individual cells and follow them across time. This approach, when applied systematically genome-wide, will enable in silico analysis of the dynamic interplay between gene regulatory and morphogenetic events. To get there we have developed three sets of techniques. First, in order to generate live fluorescent gene expression reporters for arbitrary genes in the genome we established the FlyFos system (http://transgeneome.mpi-cbg.de) that enables, in combination with high-throughput liquid culture recombineering, construction of fluorescently tagged transgenes that recapitulate wild-type expression patterns. Second, to image the dynamic morphogenetic and gene expression changes we employ multi-view Selective Plane Illumination Microscopy (SPIM) that allows us to capture the entire embryo volume throughout embryonic development. We are currently developing several SPIM systems optimized for imaging Drosophila embryo with high frame-rate aiming to parallelize the acquisition of the FlyFos reporters. In toto recording of gene expression patterns generate massive amounts of image data that have to be processed, registered to a common embryo atlas space and analyzed by state-of-the-art segmentation and tracking algorithms to automatically extract and compare developmental lineages. This a huge challenge requiring input from computer vision field and we are in the process of consolidating the currently available algorithmic solutions under the open source Fiji platform (http://fiji.sc). Our vision is to provide these resources as a functional package to the Drosophila research community so that every individual lab can aspire to record gene expression patterns in toto and we can collectively work towards quantitative description of the gene regulatory networks in embryonic development.

A Regulatory Matrix Controlling Selective Odorant Receptor Expression in Drosophila. Mattias Alenius1, Alexander Schleifer2, Liza Alkhori1, Shadi Jaffar1. 1) Department of Clinical and Experimental Medicine, Linkoping university, Linkoping, Sweden; 2) Research Institute of Molecular Pathology, Vienna, Austria.

The mechanism that specifies olfactory sensory neurons to express only one odorant receptor from a large repertoire is critical for odor discrimination but poorly understood. We will present results from two systematic RNAi screens for odorant receptor regulators in Drosophila and the following systematic analysis, which revealed seven transcription factors that in different combinations were necessary for all 32 tested odorant receptors. Contrary to the view that restricted transcription factors are necessary to generate unique neuron classes, none of the identified factors were restricted to one lineage, neuron-class or -type, arguing for a second level of regulation. Moreover, we demonstrate that the same transcription factor can both repress and activate OR expression. Comprehensive bioinformatics and promoter analyses further uncovered a common promoter structure for the receptors with repressive and activating modules that determine the factors function. Thus, the data suggests that combinatorial activation and repression specific for each receptor and neuron class, rather than restricted expression of activating factors, is necessary to specify a large number of related neuron classes and for correct odorant receptor expression.
The pathways of the pathways; how JAK/STAT, JNK and P38 pathways interact in Drosophila hemocyte activation. Jesper Kronhamn, Ines Anderl, Jens-Ola Ekström, Anna Karin Kronhamn, Michael Williams, Dan Hultmark. 1) Umeå University, Umeå, Sweden; 2) University of Tampere, Tampere, Finland; 3) University of Aberdeen, Aberdeen, UK.

When a Drosophila larva is infected by a parasitoid wasp (e.g. Leptopilina boulardi) an immune response is activated to encapsulate the foreign intruder. Characteristic for this response is a release of sessile hemocytes, lymph gland burst and lamellocyte formation. Many genes have been reported to be activated during an immune response, but the relationships between the involved pathways are still elusive. We have analyzed the epistatic relationships between the important JAK/STAT, JNK and P38 pathways to understand how the cellular immune response is activated. Our strategy was based on over- activating one gene exclusively in the hemocytes, simultaneously silencing a second gene, and then analyzing the phenotype. Such analysis has generated a detailed map of the interactions of these pathways, including two receptors for JNK. We also evaluated the importance of each pathway by silencing individual genes and testing the effect after infecting Drosophila larvae with Leptopilina boulardi. Each pathway had its own characteristics when silenced during wasp egg encapsulation. In addition we analyzed the expression profile of the hemocytes after over- activating the different pathways and after infecting larvae with Leptopilina. Our data clearly indicate the existence of feedback loops. Taken together, we have found the epistatic relationship, the relative importance and feed back mechanisms of JAK/STAT, JNK and P38 pathways in hemocytes during wasp infection.

Multiple requirements of EGFR pathway in maintaining gut homeostasis upon bacterial infection. Nicolas Buchon, Nichole Broderick, Bruno Lemaitre. SV-GHI-UPLEM, EPFL, Lausanne, Switzerland.

Gut homeostasis is central to whole organism health and its disruption is associated with a broad range of pathologies. Following damage, complex physiological events are required in the gut to maintain proper homeostasis. Upon infection, we observed that the gut of adult Drosophila undergoes a dynamic morphogenetic process, which coordinates the synthesis of new enterocytes, their proper cellular morphogenesis, and the delamination of damaged enterocytes. We show that the EGFR pathway is strongly activated in both intestinal stem cells (ISCs) and enterocytes upon infection, with a timing that parallels these events. We demonstrate that a canonical EGFR pathway is required in ISCs to promote their proliferation in response to infection. This activation is dependent on the induction of the EGFR ligands, Spitz, Keren and Vein, the latter being induced in the surrounding visceral muscles partially under the control of the JAK/STAT pathway. Additionally, the JAK/STAT and EGFR pathways synergize in ISCs to promote proliferation. Interestingly, the EGFR pathway also contributes to gut morphogenesis through its activity in enterocytes. Flies with reduced EGFR activity in enterocytes have a characteristic long and thin gut that results from the flattening of enterocytes. The active delamination observed upon infection is also dependent on EGFR activity in enterocytes. Thus, one signaling pathway, the EGFR pathway, is key to controlling the steps essential for epithelium renewal, by coordinating the proliferation of ISCs, enterocyte morphogenesis, and delamination of damaged cells.

Drosophila melanogaster as a model organism to study host immune responses to Pseudomonas aeruginosa biofilm infections. Heidi Mulcahy, Christopher D. Sibley, Michael G. Surette, Shawn Lewenza. 1) Department of Microbiology & Infectious Diseases; 2) Department of Biochemistry & Molecular Biology, University of Calgary, Canada.

Pseudomonas aeruginosa is an opportunistic pathogen capable of causing both acute and chronic infections in susceptible hosts. Chronic P. aeruginosa infections are caused by bacterial biofilms, exemplified by Cystic Fibrosis airway infections. Biofilms are highly structured, multicellular, microbial communities encased in an extracellular matrix that enable long-term survival in the host. The aim of this research was to develop an animal model in which to study biofilm infections in vivo. MCherry-labeled P. aeruginosa PAO1 cells were visualized during chronic infection of Drosophila melanogaster. At 24h postinfection, P. aeruginosa biofilms localized to and were visualized in dissected Drosophila crops. These biofilms had a characteristic structure, resembling honeycomb-shaped aggregates of cells and stained positively for DNA and exopolysaccharide, two major characteristics of biofilms in vitro. Mutant strains, defective or enhanced for biofilm formation in vitro retained their defective or hyperbiofilm phenotype in the crop. The non-biofilm mutant was significantly more virulent than PAO1, while the hyperbiofilm strain demonstrated significantly less virulence than PAO1 as indicated by survival of infected flies at day 14. To monitor the fly innate immune response to biofilm and non-biofilm infections qRT-PCR was used to assess the expression of the antimicrobial peptides genes (AMP), diptericin, cecropin A1, and drosomycin in the fly 24h postinfection. Biofilm formation in the crop correlated with induction of AMP gene expression. In summary an inability to form biofilms in vivo, coupled with repression of AMP gene expression, resulted in increased fly death. These results provide novel insights into host-pathogen interactions during a chronic P. aeruginosa infection and highlight the use of Drosophila as an animal model that permits the study of P. aeruginosa biofilms in vivo.

Immune defense is a very complex trait, affected by multiple trade-offs and genetic pleiotropies. We focus here on a single interaction, that between defense and reproduction, as a means to determine the consequences of trade-offs on the function and evolution of defense. We demonstrate that female Drosophila melanogaster suffer reduced immune defense after mating, an observation that is consistent with a trade-off between reproduction and immunity. Using a series of reproductive mutant males, we show that failure to transfer either sperm or sex-peptide (a seminal fluid protein) results in a less intense reduction in female immune defense. We have also found that mated females are significantly less able to induce expression of multiple antimicrobial peptide (AMP) genes in response to infection compared to virgin females. We are currently measuring the role that baseline AMP expression and phenoloxidase activity play in post-mating immune defense. To begin to understand how this trade-off may be evolving, we assayed for genetic variation among females and males for this trait. Our data demonstrate that, while females are highly genetically variable for the degree of immunosuppression they experience after mating, males are not significantly variable in the level of immunosuppression they elicit in their mates. We also fail to detect a genetic interaction between males and females. Therefore, while male ejaculate is necessary to signal post-mating immunosuppression in females, variability in the degree of immune depression is strictly determined by female genotype. Taken together, these data suggest that the ongoing evolution of this trait is likely to involve a trade-off between female reproductive traits and the humoral immune response. We are currently investigating whether reproduction and immunity are involved in an evolutionary trade-off by determining whether post-mating immunosuppression affects the fitness of females.

Priming caused by S. pneumoniae infection causes changes in gene expression in Drosophila melanogaster. Junaid Ziauddin, David Schneider. Microbiology & Immunology, Stanford Univ SOM, Stanford, CA.

We found Drosophila melanogaster can raise a stronger specific immune response to Streptococcus pneumoniae, Beauveria bassiana or Serratia marcescens when the flies have been previously exposed to sublethal doses of each microbe. We call this phenomenon priming. Since flies lack B and T cells, to some it seems theoretically impossible that flies could have an adapting immune response. Data trumps theory. Still, we need to find a mechanistic explanation for this phenomenon, which has now been seen in a variety of insects. We injected Drosophila with a sublethal dose of dead S. pneumoniae, waited for 3 days, re-injected the flies, and measured fly survival, bacterial growth rates and gene expression. We performed a microarray analysis to identify genes modulated by priming during a S. pneumoniae infection. There are many changes in primed flies and we found multiple classes of genes that are modulated following priming: some whose expression rise in only early infection, some whose expression increase at later time points, and some whose expression are reduced post-infection. We followed 34 transcripts at a high level of resolution using QRT-PCR of tight timelines of infected primed and naive flies. We conclude that environment changes the immune response of the fly and this includes past exposure to microbes. We hypothesize that these changes are evolved to be adaptive, where the fly’s immunity changes in a fashion that allows it to respond more effectively to that infection in subsequent exposures. Most of these genes have not been implicated in immunity previously and we will discuss how the altered expression of these genes could lead to altered immunity.


Caspases are cysteinyl aspartate proteases with an important role in programmed cell death. Recently, it has become clear that capsases play additional roles in non-apoptotic processes that include embryonic development, monocyte differentiation, T and B cell proliferation, and NF-κB activation. Mammalian caspase-8 is an important pro-apoptotic molecule with a recently uncovered additional role in NF-κB activation. Dredd is considered the caspase-8 homolog and is essential for an appropriate immune response mediated by the Immune deficiency (IMD) pathway. The IMD pathway responds to gram-negative bacterial challenges and activates c-Jun N-terminal (JNK), caspase, and NF-κB modules that drive an appropriate immune response. In IMD signaling it is widely assumed that Dredd cleaves and activates Relish (Rel, NF-κB p105 homolog). More recently, studies in S2 cells suggested that Dredd might perform an additional non-apoptotic role further upstream in IMD signaling. Specifically, RNAi studies hinted that Dredd might be required for activation of the IMD/dJNK module. However, detailed analysis of the involvement of Dredd in earlier IMD signaling events are still outstanding. We demonstrate for the first time that Dredd is required for the activation of dJNK signaling in vivo. We show that Dredd is an element of a proximal signaling complex required for the transduction of a phosho-relay to Rel and dJNK. Combined, our data reveal a new role for Dredd in IMD signaling. Importantly, the findings substantiate the importance of non-apoptotic roles of caspases.

The hostile take-over of insect intracellular niches by Wolbachia pipientis. Harriet L. Harris1,2, Jennifer A. Biliske2, Lesley J. Brennan2. 1) Dept Biol, Concordia Univ Col, Edmonton, AB, Canada; 2) Dept Biological Sciences, University of Alberta, Edmonton, AB Canada.

The alphaproteobacteria, Wolbachia pipientis are obligate endosymbionts found in all major insect orders including dipterans. They have a major impact on sex ratios, immunity, fecundity, and lifespan in infected hosts. Drosophila species are no exception; many harbour Wolbachia strains that cause cytoplasmic incompatibility or male-killing. In Drosophila melanogaster, Wolbachia infection provides protection against RNA viruses. In spite of the unique lifestyle and pleiotropic behaviour of these bacteria, little is known about molecular interactions between Wolbachia and host cells. We initiated a proteomic screen that detected an up-regulation of antioxidant proteins in Wolbachia- infected mosquito cells (Brennan et al, PLoS One 3(5) 2008), and subsequently found that Wolbachia infection is associated with an increase in reactive oxygen (RO) in mosquito cells in culture, and in Drosophila ovaries and testes. Our hypothesis is that stable Wolbachia symbiosis depends upon maintaining intracellular redox homeostasis. To test this, we have treated cells and whole insects with exogenous antioxidants and antioxidant inhibitors, and followed the intensity of the bacterial infection using quantitative qPCR. We demonstrate that the number of Wolbachia in insect cells in culture and whole flies is a function of antioxidant/RO balance. The consequence of an imbalance between intracellular RO and antioxidant levels for the insect host will be discussed.
Coordination of Triacylglycerol and Cholesterol Homeostasis by the Drosophila DHR96 Nuclear Receptor. Matt Sieber, Carl Thummel. Dept Human Genetics, University of Utah, Salt Lake City, UT.

DHR96 is the single Drosophila ortholog of the CAR/PXR/LXR subclass of nuclear receptors. Like its vertebrate counterparts, DHR96 is expressed in several key metabolic tissues, including the midgut and fat body. Previous work from our lab has identified important roles for DHR96 in the coordinate regulation of triacylglycerol (TAG) metabolism and cholesterol homeostasis. We have recently found that a direct target of DHR96 regulation, CG5932, is a candidate for mediating these effects on lipid metabolism. CG5932 encodes an intestinal lipase that is significantly down-regulated in the DHR96 mutant. RNAi-mediated silencing of CG5932 leads to decreased levels of TAG and an increase in total cholesterol, phenotypes that are similar to those seen in the DHR96 mutant. In vitro enzymatic activity studies show that CG5932 exhibits a high degree of both cholesterol esterase and TAG lipase activity, similar to its mammalian homolog, LipA. Furthermore, RNAi-mediated silencing of CG5932 leads to decreased intestinal cholesterol esterase and TAG lipase enzymatic activity. CG5932 protein is highly expressed in the anterior outer epithelial cells of the proventriculus region of the midgut, where it localizes to large acidic vesicles. These vesicles appear to be released by the epithelial cells and move into the intestinal lumen. Finally, expressing CG5932 in the intestine of the DHR96 mutant is sufficient to rescue the lean phenotype and partially rescue the elevated cholesterol levels in DHR96 mutants. We propose that DHR96 normally induces CG5932 expression under feeding conditions. The CG5932 lipase, in turn, promotes the breakdown of both dietary TAG, to facilitate lipid uptake, and stored cholesterol esters, to maintain proper cholesterol levels. Current work is focused on defining the mechanisms by which DHR96 and CG5932 exert their roles in coordinating TAG and cholesterol homeostasis.

TSPO, a mitochondrial membrane potential modulator, plays an essential role in Drosophila aging. Ran Lin1,2, Douglas C. Wallace1. 1) Children's Hospital of Philadelphia (CHOP), Philadelphia, PA; 2) Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Peking University Health Science Center, Beijing, China.

The translocator protein 18kDa (TSPO), formerly named peripheral benzodiazepine receptor (PBR), is a mitochondrial protein widely expressed in peripheral tissues and central nervous system (CNS), playing an essential role in various mitochondria-related cell functions. We have been analyzing the Drosophila TSPO to obtain insight into its function in humans. The TSPO has been inactivated by a P-element insertion on 2nd chromosome in the TSPO coding region. Homozygous deficient flies are viable. Mitochondrial respiration analysis on young TSPO -/- revealed an increased state IV rate while state III (ADP-stimulated) rate remained unchanged. This implied an increased mitochondrial proton leak, which was supported by a reduced inner membrane potential revealed by TMRM uptake. In older TSPO -/- flies, mitochondrial respiration OXPHOS complex activity progressively declined. Aberrations were also observed in heme metabolism. TSPO -/- flies have a modulated lifespan, and the TSPO ligands, PK11195 and Ro5-4864, can both modulate lifespan of wild type flies. In wild type flies, TSPO expression on both RNA and protein levels increases with age. Therefore, TSPO function is required for normal mitochondrial function, and it modulates mitochondrial function with age.

Stem Cell Response to Nutrient Availability in Drosophila. Lei Wang, Catherine McLeod, Leanne Jones. Laboratory of Genetics, The Salk Institute, La Jolla, CA.

Throughout life, adult stem cells play essential roles in maintaining tissue and organ function by providing a reservoir of cells for homeostasis and regeneration. Therefore, a decline in stem cell number or activity or disruption in their differentiation potential could contribute to compromised organ and tissue function, a characteristic of aging. Drosophila has emerged as an ideal system for studying stem cell behavior during aging, as it has a short lifespan, tissues that are maintained by adult stem cells, and conserved pathways known to regulate longevity. We have established a system in Drosophila to characterize factors that regulate the response of stem cells to chronic metabolic changes, including fluctuations in nutrient availability. Male flies fed a diet lacking protein (protein starvation) exhibited a decline in the average number of germline stem cells (mGSCs) in the testes, and the remaining GSCs proliferate more slowly. Strikingly, GSCs are rapidly replaced upon re-feeding, indicating that stem cells are competent to respond quickly to changes in nutrient availability. A similar decline and recovery in Drosophila intestinal stem cells (ISCs) were also observed in response to protein deprivation, suggesting that a small pool of active stem cells is maintained in both germline and somatic tissues, which is able to respond quickly once favorable conditions resume. We further demonstrated that the response of mGSCs to protein starvation was mediated through the insulin/IGF signaling pathway. Clonal analysis indicated that the Drosophila insulin receptor (dInR) is required cell-autonomously for the maintenance of mGSCs, and activation of insulin signaling in mGSCs and surrounding support cells was sufficient to suppress loss of GSCs upon starvation. Our data indicate that stem cells can directly sense changes of the systemic environment to coordinate their behavior with the overall nutritional status of the animal, providing a mechanism for maintaining tissue homeostasis when an organism is under metabolic stress.
Characterization of a Hormone Dependent Module Regulating Energy Balance in Drosophila. Biao Wang1,2, Noel Moya1, Sherry Niessen3, Heather Hoover1, John Yates III1, Wolfgan Fischer1, John Thomas2, Marc Montminy1. 1) PBL-M, Salk Inst Biological, La Jolla, CA; 2) MNL-T, Salk Inst Biological, La Jolla, CA; 3) The Center for Physiological Proteomics, The Scripps Research Institute, La Jolla CA.

Under fasting conditions, metazoans maintain energy balance by shifting from glucose to fat burning. In the fasted state, SIRT1 promotes catabolic gene expression by deacetylating the forkhead transcription factor FOXO in response to stress and nutrient deprivation. SIRT1 activity, however, does not appear to be regulated directly by hormonal signals, suggesting the involvement of additional effectors in this process. We have identified a hormone-dependent module, consisting of the Ser/Thr kinase SIK3 and the class IIa deacetylase HDAC4, which regulates FOXO activity in Drosophila. During feeding, HDAC4 is phosphorylated and sequestered in the cytoplasm by SIK3, whose activity is upregulated in response to insulin. SIK3 is inactivated during fasting, leading to the de-phosphorylation and nuclear translocation of HDAC4, and to increases in FOXO deacetylation. SIK3 mutant flies are starvation-sensitive, reflecting FOXO-dependent increase in lipolysis that deplete triglyceride stores; reducing HDAC4 expression restored lipid accumulation. Our results reveal a hormone-regulated pathway that functions in parallel with the nutrient-sensing SIRT1 pathway to maintain energy balance.

PGC-1/spargel is a Modifier of Diet-Induced Fat Accumulation and Associated Heart Defects. Soda Diop, Sean Oldham, Rolf Bodmer. Development and Aging Program, Sanford Burnham Institute, La Jolla, CA.

We recently developed a Drosophila model for High Fat Diet-induced (HFD) obesity and heart dysfunction associated with excessive cardiac fat accumulation (Birse et al. 2010). We showed that reducing nutrient-sensitive TOR signaling can prevent the adverse metabolic and cardiac effects of a HFD. To identify other modifiers of obesity-associated heart dysfunction, we conducted a screen of genes that are implicated in various aspects of lipid metabolism. Here, we report that we identified as a candidate the fly homologs of the PPAR-γ Coactivator-1, PGC-1/spargel). In mammals, PGC-1α & -1β and PGC-1-related Coactivator (PRC) genes are involved in numerous metabolic functions including mitochondrial biogenesis and function, muscle fiber switch and thermogenesis (Zechner et al. 2010). In Drosophila, there is only one PGC-1 gene identified, and therefore this may reduce potential redundancies and the functional complexities found in mammals (Tiefenbock et al. 2010). We studied several loss-of-function mutants (P-elements insertions in the 5’ end of PGC-1) that exhibit reduced levels of PGC-1 in the adult heart (by qPCR). We also used PGC- overexpression and RNAi knockdown to study tissue-specific effects of PGC-1 manipulation. Flies with reduced PGC-1 function show exacerbated obesity in response to a HFD, whereas overexpression protects them from fat accumulation in whole flies as well as muscle tissue only. Heart function is also compromised in PGC-1 knockdown to study tissue-specific effects of PGC-1 manipulation. Flies with reduced PGC-1 function show exacerbated obesity in response to a HFD, whereas overexpression protects them from fat accumulation in whole flies as well as muscle tissue only. Heart function is also compromised in PGC-1 mutants. The increased obesity in PGC-1 reduced flies is also correlated to an increase in heart defects in response to a HFD. We conclude that PGC-1 plays an important role in HFD-induced obesity and heart dysfunction. Cell Metabolism 12, 533-544, 2010 The EMBO Journal, 29, 171-183, 2010 Zechner et al, 2010 (in press Cell Metabolism).

tRNA Met Synthesis Controls Growth and Development in Drosophila. Elizabeth J. Rideout, Lynne Marshall, Savraj S. Grewal. Clark H. Smith Brain Tumour Centre, Southern Alberta Cancer Research Institute, and Department of Biochemistry and Molecular Biology, University of Calgary, HRIC, 3330 Hospital Drive, Calgary, Alberta, T2N 4N1, Canada.

The conserved insulin/target-of-rapamycin (TOR) pathway couples nutrient availability with tissue and organismal growth in metazoans. We show that in Drosophila these effects are mediated in part by stimulation of tRNA synthesis. We find that nutrient availability controls tRNA synthesis via inhibition of the conserved RNA polymerase III repressor Maf1. Genetic inhibition of Maf1 accelerates larval growth rate and increases final body size. These phenotypes are largely due to regulation of dMaf1 function in the larval fat body, a critical nutrient-sensing tissue equivalent to vertebrate liver or adipose tissue. We show that loss of dMaf1 in the fat body stimulates an increase in peripheral insulin levels and promotes systemic insulin signaling and growth. Significantly, these effects were reproduced in transgenic flies carrying only an extra copy of a single tRNA(Met). We propose that the regulation of tRNA synthesis represents a mechanism by which nutrient availability controls tissue and organismal growth.
New Challenges, Directions and Approaches in Cell-Based RNAi Screening: Application of High-Content Imaging to Genome-Scale Interrogations of the Nucleus. Stephanie E. Mohr1, Julio Mateos-Langerak2, Joseph Dopie3, Ralph Neumuller4, Tiao Xie5, Anastasia Samsonova6, Michelle Ocana7,8, Quentin Gilly1, Benjamin MacElvany1, Ian Fleckhart1, Matthew Booker19, Claire Hu1, Giacomo Cavalli10, Maria Vartiainen11, Norbert Perrimon12,13,14. 1) Drosophila RNAi Screening Center, Harvard Medical School, Boston, MA; 2) Institute of Human Genetics, Montpellier, France; 3) Institute of Biotechnology, University of Helsinki, Finland; 4) Department of Genetics, Harvard Medical School, Boston, MA; 5) ICCB-Longwood Screening Facility, Harvard Medical School, Boston, MA; 6) Division of Biology and Medicine, Brown University, Providence, RI; 7) Howard Hughes Medical Institutes, Harvard Med Sch, Boston, MA.

The Drosophila RNAi Screening Center (www.flyrnai.org) hosts full-genome and smaller-scale RNAi screens in cultured and primary cells. Virtually any cell biological topic can be interrogated using our platform. Recently, we facilitated three full-genome RNAi screens in Drosophila cultured cells investigating distinct aspects of the nucleus using high-throughput, high-content imaging. Specifically, these studies used automated fluorescence confocal imaging to investigate chromatin structure, nuclear actin, and the nucleolus. The screens generated a large amount of data and present unique challenges for analysis and follow-up. We used multi-parametric image analysis to identify multiple phenotypic sub-classes within each image data set, defining sets of primary screen hits. Primary results have been preliminarily confirmed using dsRNAs targeting different regions of the gene as compared with the regions targeted in the screening library. Together with other improvements (e.g. better reagents, new validation methods, and integration with orthogonal datasets), the application of high-content image-based approaches to cell-based RNAi screening is making it possible to identify high-confidence associations between complex sub-cellular structures and the specific gene products or pathways that comprise and/or control these structures.

134

Univ Texas Southwestern Medical Ctr, Dallas, TX. Chih-Chiang Chan1,4, Shane Scoogg1,4, Dong Wang3, Smita Cherry1, Michael Buszczak1,4, P. Robin Hiesinger12,15. 1) Department of Physiology; 2) Green Center for Systems Biology; 3) Department of Molecular Biology, Univ Texas Southwestern Medical Center, Dallas, TX; 4) Co-first authors; 5) Co-corresponding authors.

Rab GTPases are master regulators of intracellular membrane trafficking in all cells. Neurons have high and specialized demands on membrane trafficking both during development (wiring-specific extensive arborizations) and function (neurotransmitter release). Here we test the hypothesis that neuronal Rab GTPases are key regulators of neuron-specific membrane trafficking. In order to systematically profile the entire gene family, we have developed a streamlined vector platform for ends-out homologous recombination based on Pacman, BAC recombineering and PhiC31 transgenesis. Using this technology we have generated a comprehensive set of 38 genomic rab-Gal4 transgenic flies for 25 rab genes. Surprisingly, our expression profiling shows that half of all rab GTPases in Drosophila are either neuron-specific or strongly enriched in neurons with highly variable expression patterns in the brain. Subcellular localization profiling of all neuronal Rab proteins revealed that all neuron-specific ras encode synaptic proteins. The rab-Gal4 lines presented here are designed to easily generate molecularly defined null alleles using an optimized approach to ends-out homologous recombination. We provide the protocols and three knock-ins to quantitatively evaluate the efficiency of our technique. Our findings and tools provide a powerful approach to systematically study neuron-specific membrane trafficking in a brain-in-vivo. Furthermore, the streamlined Gal4 knock-in method presented here is applicable to all genes.

135

Virtual Fly Brain. David J. Osumi-Sutherland1, Simon R. Reeve1, Nestor Milyaev2, J. Douglas Armstrong2, Michael Ashburner1. 1) Dept Genetics, Cambridge University, Department of Genetics, Cambridge CB2 3EH, UK; 2) Edinburgh University School of Informatics, 10 Crichton Street Edinburgh EH8 9AB, UK.

Navigating the Drosophila neurobiology literature and related databases is a major challenge. Finding, for example, data about the connectivity between two brain regions, the properties of the neurons involved and the genes, markers and GAL4 drivers they express can be a long, arduous and difficult task. This problem is rapidly becoming worse as yet larger datasets are produced.

One obvious way to tie neuro-anatomical data together is in an atlas. Google Earth, with its ability to zoom to different scales, overlay data and link any feature to additional information, is an obvious template. Inspired by this approach, Virtual Fly Brain (VFB) is a web-based tool that allows users to browse a 3D confocal stack of a Drosophila brain at any angle. A multicolored overlay divides the brain into regions with names and boundaries agreed by the BrainName project [Bo et al., in preparation].

An atlas alone is not sufficient. Much of our knowledge of neuro-anatomy consists of assertions about neuronal classes: what neurotransmitter they release; where they receive synaptic input; where they make synaptic connections. Our ontology of Drosophila neuro-anatomy records such assertions along with their provenance and uses them to classify neurons via an automated reasoner. This ontology drives point-and-click queries for neurons innervating regions highlighted in the atlas. It also underlies a neuron finder tool that allows simple, template-based searches for neuron classes. The ontology is used by FlyBase to annotate genes, alleles and transgenes, based on expression and phenotypes. As a result, VFB can also be used to search for genes, transgenes or alleles, expressed or causing phenotypes in any specified brain structure of neuron class.
Defining the *Drosophila melanogaster* protein complex map. Spyros Artavanis-Tsakonas1, Guruharsha Kuthethur1, Jean-François Rual1, Bo Jhai1, Julian Mintseris1, Mark Stapleton2, Rogerio Candias3,4, David Rhee1, Kadalmani Krishnan1, Christina Wong1, Chapman Beckman1, Kenneth Wan2, Charles Yu3, Xiao Chen2, Manolis Kellis3,4, K Vijay-Raghavan1, Steven Gygi1, Susan Celniker1, Robert Obar1. 1) Department of Cell Biology, Harvard Medical School, Boston, MA; 2) BDGP, Lawrence Berkeley National Laboratory, Berkeley, CA; 3) Computer Science and Artificial Intelligence Laboratory, MIT, Cambridge, MA; 4) Broad Institute of MIT and Harvard, Cambridge, MA; 5) National Centre for Biological Sciences, TIFR, Bangalore, India.

The genome of *Drosophila melanogaster* is predicted to contain ~14,000 protein-coding genes whose products are engaged in potentially hundreds of thousands of protein interactions to form functional protein complexes. Determining the composition of protein complexes is an essential step toward understanding the cell as an integrated system. Toward this end, we have generated the *Drosophila* Protein Interaction Map (DPiM) using a high-throughput co-affinity purification platform coupled to mass spectrometry analysis. We have analyzed over 5,000 high-quality data sets and developed an algorithm that incorporates peptide spectral counts to rank protein-protein interactions. DPiM contains several thousand co-complex interactions, organized into more than 500 putative protein complexes. Analysis of groups of co-purifying proteins reveals shared functional attributes such as Gene Ontology terms and expression profiles. Comparison with other protein interaction databases also indicates that DPiM is of high quality. The validity as well as the usefulness of DPiM is illustrated by our ability to purify several well-known complexes as well as the identification of potential novel members for important and evolutionarily conserved protein complexes. We have also assigned functional relationships to hundreds of protein-encoding genes that currently lack any functional annotation. Our analysis of nearly the complete predicted *Drosophila* S2R+ cell proteome makes DPiM the most extensive protein complex map for a metazoan.

Flipping In or Out: A Collection of Enhancer-trap Flippase and the FINGR Method for Mosaic Analysis. Talor Fore1, Xinyun Peng1, Audrey Ojwang’, Margaret Warner’, Chelsea Springer’, Rudolf Bohm1,2, William Welch1, Lindsey Goodnight1, Hong Bao1, Bing Zhang1. 1) Department of Zoology, University of Oklahoma, Norman, OK; 2) Department of Biology, Brandeis University, Waltham, MA.

Gal4/UAS is powerful for gene and tissue manipulation in *Drosophila*. Gal4 expression, however, is rarely tissue-specific enough for most cellular analyses. To overcome this hurdle, we recently developed the FINGR (enhancer-trap flippase-induced intersectional Gal80/Gal4 repression) method to restrict Gal4 expression (Bohm et al., PNAS 107:16378, 2010). The restriction of Gal4 lines with broad expression can be achieved by using two complementary Gal80-converting tools: tubP>stop>Gal80 (‘flip in’) and tubP>Gal80> (‘flip out’), mediated by tissue-specific ET-FLP. In the flip-in mode, Gal80 will repress Gal4 expression wherever Gal4 and ET-FLP intersect. In the flip-out mode, Gal80 will relieve Gal4 repression in Gal4/FLP overlapping cells. Coupled with UAS-GFP, the FINGR method also allows one to map the morphology of neural circuits or any other tissues. The power of the FINGR method lies in the availability of tissue-specific ET-FLP. To this end, we have generated a collection of ~1000 ET-FLPx2 lines (with two copies of FLP). To realize the full potential of these lines, we are characterizing the FLP expression pattern in each line and developing an online database available to the fly community. The ~500 lines examined to date reveal a multitude of expression patterns ranging from broad to unique, featuring adult sexual dimorphism, structure- and tissue-specific expression. Advantageous over heatshock-FLP, ET-FLPx2 lines can be used with MARCM and other FRT-based methods to generate reproducible clones. Reproducibility of mosaics is critical for both behavioral and morphological studies. Hence, we anticipate that this database along with the further expansion and characterization of the ET-FLPx2 lines will be valuable to all fly researchers interested in mosaic analysis. Supported by NSF grant IOS1025556 and internal funds from OU.


There are three essential properties of transcription regulation: the rate at which regulatory sequences interact with the target promoter, the number of transcripts produced per interaction (i.e. burst size), and the total number of mRNAs produced during a particular developmental process. We have developed methods using high-resolution light microscopy to measure each of these parameters in vivo in Drosophila blastoderm embryos. Precise timing of these events is obtained using a variety of calibration methods, such as chromosome condensation/decondensation switching during mitotic waves of syncytial nuclear divisions, and precise quantification of levels is determined by individual molecule counting.

We apply these methods to study the effects of enhancer number and promoter choice on the rate of enhancer-promoter interactions, burst size, and total amount of mRNA synthesis, with particular focus on the regulation of the genes of hunchback, short gastrulation, and snail. Our methods measure both the average behavior and the variation among cells. Evidence will be presented that promoters flicker between on and off states that either allow or disallow any polymerase. We identify the frequency of enhancer-promoter looping as a critical point of control of expression in the early embryo. We also show trade-offs between variation in transcript levels, promoter strength, and burst size.
139

Using Drosophila to find transcriptional enhancers in highly diverged arthropod genomes. Marc S. Halfon1, Jia-Yu Chen2, Majid Kazemian2, Saurabh Sinha2. 1) Dept. of Biochemistry, SUNY at Buffalo, Buffalo, NY; 2) Dept. of Computer Science, University of Illinois at Urbana-Champaign, Urbana, IL.

Computational approaches are a necessary complement to empirical methods of identifying transcriptional cis-regulatory modules (CRMs, “enhancers”). When such approaches have been applied to Drosophila, they have typically exploited detailed knowledge about specific transcriptional networks—e.g., the transcription factors (TFs) and TF binding sites (TFBS) involved in anterior-posterior or dorso-ventral patterning—and cannot readily be applied to organisms which lack Drosophila’s rich molecular genetic data, such as the arthropod emerging model organisms Nasonia, Tribolium, Apis, and Anopheles. The absence of discernable non-coding sequence alignment between Drosophila and any of these species makes comparative genomics approaches futile, and only a handful of CRMs have been characterized in these genomes so far. We previously developed methods for “motif-blind” CRM discovery that do not depend on knowledge or accurate prediction of TFBSs and that use knowledge of existing CRMs to “supervise” the search. We have also presented evidence suggesting that Drosophila TF binding specificities are largely conserved in Nasonia, Tribolium, Apis and Anopheles. We therefore reasoned that it should be possible to begin with a set of related Drosophila CRMs and locate the orthologous CRMs in other, highly diverged arthropod genomes. To test this hypothesis, we performed “supervised” CRM prediction in the four above-mentioned species for each of over 30 different regulatory networks from Drosophila. By examining if the predicted CRMs fall in the proximity of the expected genes, we found 16 networks where our approach shows statistical evidence of recovering CRMs in at least two non-Drosophila species. We will describe the statistical and visualization tools we have developed for cross-genus CRM discovery and the results of both our in silico and in vivo validation of predicted CRMs, and discuss how TFBS counts and arrangements have diverged in the course of evolution.

140

MiMIC (Minos Mediated Integration Cassette): a versatile transposable element for tagging genes in vivo. Koen Venken1, Karen Schulze1, Nele Haelterman1, Hongling Pan1, Yuchun He2, Martha Evans-Holm1, Joseph Carlson1, Bob Levis3, Roger Hoskins1, Hugo Bellen1,2,5. 1) Department of Molecular and Human Genetics, BCM, Houston, TX; 2) HHMI, BCM, Houston, TX; 3) Life Sciences Division, LBNL, Berkeley, CA; 4) Department of Embryology, HHMI, CI, Baltimore, MD; 5) Program in Developmental Biology, BCM, Houston, TX.

We developed a new versatile transposon based on Minos. Minos integrates randomly into the genome. Hence, about 1/3 of insertions are in introns and therefore ideal for protein trapping. Between the 255 nt Minos inverted repeats, we integrated a pair of attP cassettes for Recombination Mediated Cassette Exchange (RMCE) using \( \Phi C31 \). This allows the integration of any DNA fragment in vivo. Moreover, we incorporated a gene trap cassette to increase mutagenicity and a visible dominant marker to facilitate screening. We created a few thousand MiMIC insertions so far and determined their insertion sites. We selected several intronic insertions for subsequent testing. For insertions in coding introns, we integrated several constructs containing artificial exons encoding different protein tags to allow protein visualization and manipulation. For 5'UTR intronic insertions, we integrated gene trap cassettes encoding recombinases for subsequent fate mapping or components of binary systems, such as GAL4 or QF, to control expression of other genes. Interestingly, almost all insertions in introns of essential genes that are predicted to truncate the protein cause lethality and fail to complement characterized lethal mutations in the gene. This lethality can be reverted by RMCE using a reversion cassette or a protein trap cassette, demonstrating that the original insertion is responsible for the lethality. Second, when an insertion is in the opposite non-mutagenic orientation, lethality can be induced by RMCE using a gene trap cassette. Third, the integration of tags by RMCE can be used for protein detection and visualization. In summary, the MiMIC technology is extremely versatile.

Stem cells generate progenitor cells with restricted potential to amplify their output in generating post-mitotic progeny and to safeguard their genomic integrity by minimizing proliferation. Expanded progenitor cell potential leads to aberrant stem-like cells contributing to developmental defects and tumor initiation, but regulation of the progenitor cell potential is not understood. Type II neuroblasts in Drosophila larval brains repetitively generate immature intermediate neural progenitors (INPs) that assume the INP fate during the maturation process. Immature INPs in brain tumor (brat) mutant brains fail to differentiate into INPs and rapidly de-differentiate back into type II neuroblasts. Expression of a brat transgenic protein containing only the B-boxes and the coiled-coil domain rescues the brat mutant brain phenotype, indicating that Brat establishes the INP identity via a novel mechanism. We identified multiple genes including pointed P1 (pntP1) and adenomatous polyposis coli 2 (apc2) that are required for Brat to establish the INP potential. Heterozygosity of pntP1 or apc2 further enhances the ectopic neuroblast phenotype in brat mutant brains whereas ectopic expression of pntP1 or apc2 suppresses the brat mutant phenotype. PntP1, a transcription factor, is specifically detected in type II neuroblasts and immature INPs in wild type brains, but fails to localize in immature INP nuclei in brat mutant brains. PntP1 functions to promote timely differentiation of immature INPs via a RTK-independent mechanism. Apc2, a negative regulator of β-Catenin, is virtually undetectable in brat mutant type II neuroblasts. Importantly, ectopic expression of constitutively active β-Catenin enhances the brat mutant brain phenotype while ectopic expression of dominant negative Tcf suppresses the brat mutant phenotype. Thus, we conclude that Brat establishes the INP potential by promoting differentiation via PntP1 and suppressing de-differentiation through Apc2.

142 **Cyst stem cell development and regulation of germ line stem cell maintenance during Drosophila testes morphogenesis.** Matthew Wawersik, Matthew Badgett, Jake Fry, Erika Matunis, Rebecca Obniski, Xuteng Sheng, Amanda Simmons, Daniel Sinden. 1) Biology Dept, College of William & Mary, Williamsburg, VA; 2) Dept of Cell Biology, Johns Hopkins School of Medicine, Baltimore, MD.

Establishment and maintenance of functional stem cells is critical for organ development and tissue homeostasis. Drosophila testes are among the best characterized systems for studying stem cell behavior, with GSCs and a second population of somatic stem cells, termed cyst stem cells (CySCs), localized to a discrete stem cell niche at the testes apex. Communication between the niche, GSCs, and CySCs regulates the balance between stem cell maintenance and differentiation. But how does such a complex system with multiple stem cell types form? Recent data show that functional, asymmetrically dividing GSCs are first established at ~24 hrs AEL during Drosophila testes morphogenesis (Sheng et al, 2009). This process correlates with coalescence of the testes stem cell niche, or hub, but timing and development of CySCs was not examined. Here we show that functional CySCs are present at the time of GSC establishment, and examine the process of CySC development with respect to hub formation. We also examine the role of stat gene function in regulation of both GSC and CySC maintenance and differentiation. Together, our observations indicate that a fully function GSC niche, with all the cell types present in adult Drosophila testes, has formed by the end embryogenesis.

143 **Mitochondrial dynamics in larval neuroblasts.** Rachel T. Cox, Aditya Sen, Vanessa T. Damm. 1) Department of Biochemistry and Molecular Biology; 2) Center for Neuroscience and Regenerative Medicine, Uniformed Services University, Bethesda MD.

Functional mitochondria are important for all cells. While mitochondria supply cells with energy and other metabolites, they are particularly important in neurons. For normal function, neurons require high levels of ATP as well as mitochondrial movement to concentrate them at the synapse. Underscoring their importance in this cell type, mitochondrial dysfunction occurs in many different neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease. A promising avenue of treatment for neurodegenerative disease is the application of stem cell therapy. This includes addition of exogenous stem cells, as well as stimulating endogenous stem cells. To better understand how mitochondrial dynamics contributes to neural stem cell maintenance and health, we are studying mitochondrial dynamics in larval neuroblasts (NB). Using confocal microscopy and live imaging to label and view mitochondria, we have characterized mitochondrial dynamics during the NB cell cycle in third instar larval brains. Mitochondria are highly abundant in NB cytoplasm, and unlike other cell types in the larval brain they are present exclusively in discreet small spheres. In addition, mitochondria show stereotypical changes in localization during the cell cycle and at cell division. Since NBs divide asymmetrically, only a small proportion of mitochondria are inherited by the daughter cell due to the relatively low volume of cytoplasm it receives during mitosis. Our studies are the first looking at mitochondria in larval NBs. We are currently using a candidate gene approach to identify and characterize genes that perturb the normal mitochondria segregation pattern during NB cell divisions and that are also important for maintaining NBs. These studies would increase the knowledge of how mitochondria contribute to NB health and function in the brain.
The Drosophila Pez phosphatase restricts intestinal stem cell proliferation via Hippo signaling. Ingrid Poernbacher, Roland Baumgartner, Ernst Hafen, Hugo Stocker. Institute of Molecular Systems Biology, ETH Zürich, Wolfgang-Pauli-Strasse 16, 8093 Zürich, Switzerland.

The adult Drosophila midgut epithelium contains a distinctive population of intestinal stem cells (ISCs) that preserve the normal homeostasis of the gut and enable rapid tissue turnover in response to intestinal stress. ISC divisions give rise to an ISC and an enteroblast (EB), which differentiates into an enterocyte (EC) or an enteroendocrine (EE) cell. The increase in ISC proliferation that occurs in the case of tissue damage is mediated by the Hippo (Hpo) signaling pathway, a conserved regulator of organ size and tumor suppression. Hpo signaling generally restricts activity of the pro-proliferative and anti-apoptotic transcriptional co-activator Yorkie (Yki). Upon intestinal stress, Yki is activated in ECs, promotes expression of unpaired genes and thereby triggers a non-autonomous upregulation of ISC proliferation. Here, we identify the tyrosine phosphatase Pez as a novel component of Hpo signaling that functions in the Drosophila midgut to restrict ISC proliferation rates. Complete loss of pez is not lethal but induces a midgut phenotype similar to that observed under stress situations or in situations where Yki is specifically activated in ECs, including increased ISC proliferation that can be reversed by reducing Yki function. In addition, pez mutant midguts upregulate the expression of a minimal Hpo responsive element, and Pez physically interacts with the Hpo signaling upstream component Kibra. Our findings therefore suggest that Pez functions in Hpo signaling and is essential for the regulation of ISC proliferation and gut regeneration.

Follicle stem cells have a unique polarity that regulates niche competition. Todd G. Nystul1, Maria R. Kronen1, Kevin E. Schoenfelder1, Allan C. Spradling2. 1) Anatomy Dept., UC San Francisco, San Francisco, CA; 2) HHMI/Carnegie Institution, Dept. of Embryology, Baltimore, MD.

Follicle stem cells (FSCs) produce a polarized epithelium in the Drosophila ovary. This system provides the opportunity to study an epithelial stem cell and its associated niche in vivo and at single cell resolution. Previously, we found that exactly two FSCs per ovariole reside in separate niches and divide to self-renew and produce prefollicle cell daughters. We also observed that FSCs are replaced during adulthood through interniche migrations and apparent competition for niche occupancy. However it is not clear what causes FSC and prefollicle cell fates to diverge. In a screen for niche competition mutants, we found that loss of lethal giant larvae (Lgl) causes “hyper-competition,” in which mutant follicle cells replace wildtype stem cells at abnormally high rates. In mature follicle cells, loss of Lgl causes both neoplasia and a disruption of polarity, but Lgl⁻/⁻ prefollicle cells do not overproliferate, indicating the hyper-competition phenotype is more likely due a defect in cell polarity. Surprisingly, we found that FSCs have a unique polarization: they have basal and lateral domains but lack an apical domain. FSCs do not express apical markers such as bazooka, and the two lateral domains meet at a point. In addition, the adherens junctions, which are localized to the apical/lateral junction in mature follicle cells, are distributed broadly along the lateral domains in FSCs. In contrast, prefollicle cells have a rudimentary bazooka⁺ apical domain and more restricted adherens junction localization. Lgl is required to maintain the apical domain in mature follicle cells and is likely to play a similar role in prefollicle cells. Therefore, our data support a model in which an Lgl-mediated acquisition of an apical domain establishes the prefollicle cell fate. We are currently investigating whether loss of Lgl causes hyper-competition by interfering with this early step in the specification of prefollicle cells.

Steroid hormone ecdysone signaling is required for germline differentiation. Annekatrin Klepzig, Andriy Yatsenko, Miriam Weiss, Halyna Shcherbata. Gene expression and Signaling group, Max-Planck Institute for Biophysical Chemistry, Göttingen, Germany.

Previously it has been shown that in Drosophila steroid hormones are required for progression of oogenesis during later stages of egg maturation; however, roles of ecdysone signaling in the germlarium have not been reported. Here we show that ecdysteroids regulate the niche formation and the early differentiation of a germline stem cell daughter. This effect is heterochronic, in the absence of the hormone cystoblasts are blocked in a single cell state which can be overcome by ecdysone supply. Ecdysone signaling has a cell-autonomous function in germline to modulate the strength of TGF-β signaling and cell non-autonomous in soma to coordinate a crosstalk between germline and escort stem cell progeny via cell adhesion. The specificity to this systemic signaling is achieved as a result of spatial expression of EcR/USP co-activators and its interaction with repressors.
Chromosome Strand Segregation During Drosophila Male Germline Stem Cell Division. Swathi Yadlapalli, Yukiko Yamashita. Dept of Cell and Developmental Biology, Life Sciences Institute, University of Michigan, Ann Arbor, MI.

Throughout the life of an organism, stem cells are required to proliferate and supply differentiated cells while avoiding the potentially deleterious effects of DNA mutations resulting from repeated cell cycles. It has been hypothesized that stem cells might be accomplishing this remarkable feat by retaining older ("immortal") DNA strands during asymmetric cell divisions, thereby excluding all replication-induced mutations into the differentiating daughters (Immortal Strand Hypothesis - ISH). In addition, other models have also been proposed in which stem cells asymmetrically segregate only a subset of chromosomes for different reasons such as retention of epigenetic memories. Recently, the idea has emerged, provoked by a finding from our laboratory that Drosophila male germline stem cells consistently inherit the mother centrosome, that the mother centrosome might be utilized as a means of asymmetrically segregating DNA strands. In order to test if germline stem cells (GSCs) follow the immortal strand model, we scored the outcome of DNA strand segregation by using 5-bromo-2-deoxyuridine labeling combined with direct visualization of GSC-gonialblast (differentiating daughter) pairs. Our data unambiguously demonstrate that Drosophila male GSCs do not follow the immortal strand model. We are currently probing the possibility whether GSCs might be segregating only a subset of chromosomes asymmetrically. To this end, we recently adapted the CO-FISH (chromosome orientation fluorescence in situ hybridization) technique to follow the segregation of template DNA strands in vivo. Briefly, the CO-FISH method involves 1) removal of newly formed strand by creating nicks at the sites of BrdU incorporation and treating with exonuclease, 2) hybridization of single stranded template DNA to chromosome-specific satellite repetitive probes. Strikingly, our preliminary study indicates that GSCs preferentially inherit a certain chromosomal strand of Y chromosome. We are currently probing potential asymmetries in the segregation of other chromosomes.

Imaging BMP receptor activation at an adherens junction associated stem cell niche synapse. Christian Boekel, Marcus Michel, Isabel Raabe, Raquel Perez Palencia, Adam Kupinski. CRTD, TU Dresden, Dresden, Germany.

According to the stem cell niche synapse hypothesis, spatial specificity of stem cell niche signals is maximised by subcellularly restricted, directional, and contact dependent signal transduction at cadherin based adherens junctions. Niche signalling between individual stem and stromal cells should thus resemble intercellular communication at neuronal or immunological synapses. However, signalling at such a synapse has never been observed directly, in part because tools to detect active growth factor receptors with subcellular resolution are as yet missing. We have developed a novel fluorescence based reporter that is able to directly visualize activation of type I TGF-B receptors such as Drosophila Tkv. We show here that in the fly testis a niche signal by the bone morphogenetic protein (BMP) growth factors Dpp and Gbb is transmitted at subcellular foci associated with adherens junctions between individual niche and stem cells. Local generation of the signal is achieved by exocyst mediated co-targetting of junctional proteins and BMP growth factors, and RNAi mediated knockdown of the exocyst core components Sec6 or Sec8 abolishes generation of the niche signal. Surprisingly, we find that exocyst function is also essential for the generation of the Dpp signal in the wing disc, where Dpp forms a long range morphogen gradient, and show that BMP signalling coincides with adherens junctions in the zebrafish embryo. Subcellularly restricted signal generation may therefore be a more general property of BMP signal transduction.

Autophagy, the process of cytoplasmic remodeling by lysosomal degradation, is integral to cellular homeostasis in response to stress and developmental cues. While the core molecules and interactions required for the formation of autophagosomes have been identified, less is known about the identity and integration of autophagy regulatory pathways. In the Drosophila fat body, systemic insulin signaling promotes the TOR-dependent inhibition of autophagy under fed conditions, offering an excellent system to study the coordination of pathways leading to autophagy regulation. Previous work established that membrane-associated lipids regulated by PI3-kinases control multiple steps in autophagy. Class I PI3-kinase produces PI(3,4,5)P₃ at the plasma membrane within the insulin signaling pathway to inhibit autophagy, while the Class III PI3-kinase, Vps34, synthesizes PI(3)P with a conserved role in autophagosome formation. We identified the Class II PI3-kinase, Pi3K68D, as a central regulator required for TOR-dependent, starvation induced autophagy. In addition, Pi3K68D expression in the fat body is sufficient to induce autophagy under fed conditions, and this role is dependent on Vps34. We show that a kinase-dependent Pi3K68D function is responsible for autophagy induction, as well as the redistribution of Pi3K68D from the cell periphery to intracellular Atg8-positive autophagosomes. We discovered a second surprising and kinase-independent Pi3K68D function that promotes persistent insulin receptor activity and subsequent PI(3,4,5)₃ synthesis, uncoupling the inhibitory insulin/Class I PI3-kinase signaling from autophagy progression. Taken together, these data point to distinct and interrelated roles for all three classes of PI3Ks via regulation of specific phosphoinositide lipid pools, likely to recruit specific effectors that lead to differential regulation of autophagy. We propose that Class II PI3K acts in both the regulation and formation-maturation of autophagosomes membranes, providing important insights into the integration of systemic and cell autonomous regulation of autophagy.

Eye transformer is a negative regulator of Drosophila JAK/STAT signaling. Henna Myllymäki¹, Jenni Kallio¹, Juha Grönholm², Morag Armstrong¹, Leena-Maija Vanha-aho¹, Leena Mäkinen², Olli Silvennoinen², Mika Rämet¹. 1) Laboratory of Experimental Immunology, Institute of Medical Technology, University of Tampere, Tampere, Finland; 2) Laboratory of Molecular Immunology and Cytokine Receptor Signaling, Institute of Medical Technology, University of Tampere, Tampere, Finland; 3) Department of Pediatrics, Tampere University Hospital, Tampere, Finland.

The Janus kinase / signal transducer and activator of transcription (JAK/STAT) signaling pathway regulates multiple cellular processes involved in development and cell proliferation as well as immune and inflammatory responses to hematopoietic cytokines in mammals and stress response in the fruit fly Drosophila melanogaster. The core signaling pathway is highly conserved in evolution. In flies it consists of one receptor, Domeless; one Janus kinase, hopscotch and a single transcription factor, Stat92E. In order to identify genes that regulate Drosophila JAK/STAT pathway we carried out a luciferase reporter-based genome-wide RNAi in vitro screen, and found five novel regulators. Of these, CG14225 is a negative regulator structurally related to Domeless as well as mammalian IL-6 receptor and the signal transducer gp130, which are required for activation of JAK/STAT signaling. CG14225 co-immunoprecipitates with Domeless and hopscotch in S2 cells. Moreover, CG14225 RNAi caused hyperphosphorylation of Stat92E upon stimulation with the pathway ligand unpaired, indicating hyperactivation of signaling. Accordingly, CG14225 RNAi in vivo hyperactivated JAK/STAT target genes upon septic injury. CG14225 in vivo RNAi also enhanced overgrowth caused by ectopic expression of unpaired in the developing eye, and was thus named eye transformer (ET). In gastrointestinal infection model with S. marcescens, ET RNAi was protective, likely because increased JAK/STAT signaling activity is advantageous in infected flies for renewal of the injured gut wall. The exact mechanism of ET’s function remains to be studied, but one hypothesis is that ET blocks JAK/STAT signaling by interfering with the Domeless-hopscotch-signalsosome.


Wnt signaling is an evolutionarily conserved pathway that directs cell-fate determination and morphogenesis during development of metazoans. Wnt ligands are secreted glycoproteins that act at a distance. Many of the components of this pathway have been identified including the receptors Frizzled and Arrow/LRP, but several aspect of signaling specificity remain unclear. Here we describe a novel function for Off-Track/PTK7 as a co-receptor for Wnt4 in inhibitory Wnt signaling. We show that Wnt4/Otk oppose canonical Wnt signaling in embryonic patterning, and propose a model where Otk functions by localizing Dsh away from the β-catenin degradation complex keeping β-catenin dependent signaling off while activating a new non-canonical pathway.

The evolutionarily conserved Wnt/Wingless (Wg) signalling pathway is used reiteratively, both spatially and temporally, to control the development of metazoans. In the absence of the Wnt/Wg ligand, cytosolic β-catenin/Armadillo (Arm), the transcriptional effector of the pathway, is constitutively degraded by a protein destruction complex. The sequential phosphorylation of β-catenin/Arm in the destruction complex triggers its ubiquitination and subsequent degradation by the ubiquitin/proteasome machinery. Binding of the Wnt/Wg ligand to its Frizzled receptor and LRP/Arrow co-receptor activates the pathway through the inhibition of the destruction complex, thereby allowing stabilized β-catenin/Arm to translocate into the nucleus and direct the expression of Wnt/Wg target genes. Our study characterizes the role of Drosophila Hipk as only the third kinase to directly regulate the stability of Arm in vivo. Genetic interaction studies and phenotypic analyses demonstrate that hipk functions as a positive regulator of Wg signalling. Loss-of-function clones for hipk result in loss of stabilized Arm while over-expression of hipk elevates Arm levels and leads to an increase in Wg-responsive target gene expression. We show that Hipk uniquely acts to stabilize Arm by inhibiting its ubiquitination and degradation, without any effect on its phosphorylation. Hipk does so by impeding the function of SCF\(^{\text{Slimb}}\), the E3 ubiquitin ligase responsible for Arm ubiquitination. Vertebrate Hipk2 displays a similar ability to prevent SCF\(^{\text{Slimb}}\)-mediated ubiquitination of β-catenin in a functionally conserved mechanism. Unexpectedly, we find that this ability of Hipk to inhibit SCF\(^{\text{Slimb}}\)-mediated ubiquitination is not restricted to Arm and extends to other substrates, including the Hedgehog signalling effector Ci. Thus, similar to CK1 and GSK3, Hipk dually regulates both Wg and Hedgehog signalling, but it is the first kinase identified that promotes the stability of both Arm and Ci.

The deubiquitinase enzyme USP36/SCNY regulates selective p62-dependent autophagy in Drosophila and in human cells. Emmanuel Taillebourg1, Dominique Thevenon1, Isabel Gregoire1, Mathias Faure2, Marie-Odile Fauvarque1. 1) IRTSV, CEA, Grenoble, France; 2) INSERM U851, Université Lyon 1, Lyon, France.

Autophagy is the major lysosomal degradation pathway in cells. Whereas long described as a non-specific degradation process limited to bulk cytosol in response to starvation, several recent works contributed to identify selective autophagy pathways for the targeting of specific substrates to autophagy, including senescent organelles, bacteria, viruses and aggregated proteins. Ubiquitination was then identified as a key signal targeting protein aggregates, bacteria and mitochondria to autophagic degradation. Although the mechanisms that convey ubiquitinated cargos to autophagosomes are being elucidated, those responsible for their ubiquitination remain largely unknown except for mitochondria, which have been shown to be polyubiquitinated on VDAC1 by the PARKIN E3 ligase. We hypothesised that by removing ubiquitin residues from ubiquitinated substrates, some deubiquitinases might interfere with selective ubiquitin-mediated autophagy. We report here that the USP36 deubiquitinase controls selective autophagy activation in Drosophila as well as in human cells. We show that dUSP36 loss of function inhibits cell growth and activates autophagy. Interestingly, despite the phenotypic similarity, DUSP36 acts independently of the TOR signalling pathway. We also show that autophagy induced by dUSP36 loss of function depends on p62, an adapter for delivering cargo marked by polyubiquitin to autophagosomes. dUSP36 mutant cells display nuclear aggregates of ubiquitinated proteins, including Histone H2B, and cytoplasmic ubiquitinated aggregates which are eliminated by autophagy. Importantly, USP36 function in selective autophagy is conserved in human cells as we show that reduced expression of hUSP36 triggers autophagy via a p62-dependent pathway while it does not modulate starvation-induced autophagy. Our work identifies for the first time a crucial role for a deubiquitinase in selective autophagy regulation.

The complex nature of Notch pathway regulation by O-glucose residues on Notch. Jessica Leonard1, Rodrigo Fernandez-Valdivia1, Nadia A. Rana2, Yi-Dong Li1, Amanda Simcox1, Robert S. Haltiwanger2, Hamed Jafar-Nejad3, 1) Program in Developmental Biology, Baylor College of Medicine, Houston, TX; 2) Stony Brook University, Stony Brook, NY; 3) Ohio State University, Columbus, OH; 4) IMM, UT Health Science Center, Houston, TX.

Mutations in Drosophila rumi, which encodes a protein O-glucosyltransferase, result in a temperature-sensitive loss of Notch signaling. The Notch extracellular domain contains 18 EGF repeats that can be O-glucosylated. To study the contribution of these O-glucose residues to the regulation of the Notch pathway and to avoid the potential artifacts of overexpression, we performed in vivo structure-function studies using a series of Notch genomic transgenes with mutations that abolish O-glucosylation sites. To ensure comparable expression, we inserted all transgenes in the same docking site in the fly genome. In gain-of-function experiments, the presence of 2 additional copies of mutant transgenes in wild-type background show that the more O-glucose sites are mutated, the less active Notch becomes at high temperature, indicating that the temperature-dependent effects of rumi mutations on Notch signaling can be explained by loss of O-glucose from multiple EGF repeats in the Notch protein. A transgene with mutations in all 18 O-glucosylation sites rescues the lethality of a Notch null allele at 18°C, but the rescued flies show a mild loss of Notch signaling, similar to rumi animals raised at 18°C. Rescue experiments with Notch transgenes mutated in various subsets of Rumi targets indicate that while no single O-glucose mutation causes a significant decrease in Notch activity, O-glucose on EGF 10-15 has a more important role in Notch signaling compared to other regions. In addition to a redundant, positive role for O-glucose on EGF 16-20 in Notch signaling, rescue and genetic interaction experiments reveal an unexpected negative regulatory role for Rumi and O-glucose on these EGF repeats in a specific context. Together, our results highlight the complex nature of the Notch pathway regulation by O-glucose residues on the Notch protein.
Visualizing physically interacting Wg pathway components in vivo. Marcel Wehrli1, Amber Jones-Hackathorne1, David Roberts2, Mark Peifer2. 1) Cell & Dev Bio, OHSU, Portland, OR; 2) Biology, UNC, Chapel Hill, NC; 3) Franklin & Marshall Coll., Lancaster, PA.

For many biological processes we lack a refined understanding of dynamic interactions during signaling events in vivo, as current knowledge is largely derived from overexpression experiments and biochemistry. This problem is exacerbated in the Wg pathway, where most components have additional functions in other pathways, for which these components are abundant. In order to circumvent these problems and visualize only a specific pair of interacting proteins, we use a protein complementation assay (PCA), which provides molecular resolution in vivo. PCA can then be used in combination with conventional co-localization within the limit of confocal microscopy. The key regulator in the Wg pathway is a multi-protein complex, assembled around the scaffold protein Axin, which contains APC and Shaggy; this complex functions to phosphorylate and target Armadillo (Arm) for degradation. When Wg binding activates the intracellular signaling cascade, the pathway appears to branch downstream of the receptor and re-converge on the Axin complex, which it then inhibits. This inhibition is key to the accumulation of Arm and results in signaling. Many questions remain, thus it is unclear where the active Axin complex resides and whether its disassembly and degradation represents part of its inactivation. Therefore, our initial experiments focused on visualizing the assembled Axin-APC2 complex in the wing imaginal disc through PCA. The complex is readily detectable using rescue constructs for Axin and APC2, respectively. The Axin-APC2 complex co-localizes with Cadherin, α-catenin and Arm at adherens junctions, but is also present in discrete puncta in the cytoplasm. Although a Wg signaling gradient is present in this epithelium, we did not detect a change in subcellular distribution or amount of the assembled Axin-APC2 complex, suggesting the complex is inhibited by mechanisms other than degradation or trafficking. We are extending our PCA analysis to other modes of regulation.

Role of Monosaccharide O-fucose modification of Notch for the folding of the Notch receptor in Drosophila. Akira Ishio1, Tomonori Ayukawa1, Naoki Aoyama1, Tetsuya Okajima1, Kenji Matsuno2. 1) Department of Biological Science and Technology, Tokyo University of Science, Chiba, Japan; 2) Nagoya University Graduate School of Bioagricultural Sciences, Department of Applied Molecular Biosciences.

Notch (N) is a transmembrane receptor with homology to epidermal growth factor (EGF)-like repeats and mediates cell-cell interactions necessary for many cell-fate decisions. Some of these EGF-like repeats are O-fucosylated by the protein O-fucosyltransferase 1 (O-fut1), which is essential for N signaling. However, roles of monosaccharide O-fucose modification in N signaling became elusive, because it was proposed that N-specific chaperon function of O-fut1, which is independent of its O-fucosyltransferase activity, per se is essential for N signaling in Drosophila. In this study, we generated a knock in mutant of O-fut1 (O-fut1 knock in) in which single amino acid substitution is introduced into a catalytic domain of O-fut1, although N-specific chaperon activity is probably maintained. We found that monomeric O-fucose modification of N was essential for Delta-N but not Serrate-N signaling activity in imaginal organs in Drosophila. This novel function of monomeric O-fucose modification was irrelevant to the further addition of GlcNAc to the O-fucose by Fringe, which is know to regulate N signaling in a subset of organs. In agreement with this finding, we found that lack of monomeric O-fucose modification of N caused a temperature-sensitive neurogenic phenotype in embryos. In addition, disruption of N signaling associated with the lack of O-fucose modification was partly rescued by upregulation of the unfolded protein response. These results suggest that monomeric O-fucose modification of N has a novel role for the ligand-dependent activity of N, which collaborates with the proper folding of N.
Generating a suite of endogenously-expressed, EGFP-tagged core proteins to study the dynamics of planar polarity establishment in vivo. Jessica Allen, Samantha Warrington, David Strutt. Department of Biomedical Science, University of Sheffield, Sheffield, South Yorkshire, United Kingdom.

Planar polarity is the process by which cells orient themselves in the plane of an epithelium. The components of the planar polarity pathway can be grouped into three functional modules: the 'upstream' proteins, the 'core' proteins and the 'effectors'. Exactly how these modules mediate planar polarity, and how they relate to each other, remains unclear.

The core polarity proteins adopt characteristic asymmetric localisations at cell boundaries. In addition to their asymmetric localisation, the core proteins on one side of a cell colocalise with the core proteins at the neighbouring cell edge in a punctate manner. It has been hypothesised that these puncta are clusters of complexes of the core proteins.

We are investigating the in vivo behaviour of the core proteins, to understand how they interact to establish their polarised distributions. As essential tools, we are using both recombineering and in vivo homologous recombination methods to generate a suite of EGFP-tagged core proteins which are expressed at endogenous levels under their own promoters. The resulting fly strains allow us to follow core protein distribution and dynamics in real time. We use these to compare the relative levels of different core proteins in different membrane subdomains, and to measure the relative stable and unstable fractions of the core proteins using FRAP. These experiments provide important information regarding the composition of core protein complexes, and how such complexes form. Preliminary data shows that the behaviour of the core proteins in membrane complexes is highly sensitive to protein expression levels. Furthermore, we have found that EGFP fluorescence levels are a suitable proxy for amount of protein present.

Role of the Drosophila Phosphatidylinositol-4-kinase β Four Wheel Drive in Planar Cell Polarity, Sophie M Balmer, Ursula Weber, Marek Mlodzik. Dept of Developmental and Regenerative Biology, Mount Sinai School of Medicine, New York, NY.

In Drosophila melanogaster, all adult cuticular structures display planar cell polarity (PCP) features. PCP studies have identified several genes that regulate this process, notably the Frizzled (Fz) receptor and its downstream signaling cascade, the Fz/PCP pathway and associated regulatory proteins. Fz/PCP signaling and the core regulatory protein cassette are conserved in vertebrates, and also regulate cellular polarization and migration during vertebrate gastrulation (convergence and extension). Many other potential PCP genes have been identified, but their molecular and mechanistic interactions remain largely obscure. Importantly, the activating (or inhibiting) ligands and the mechanisms of extracellular regulation of the Fz/PCP signaling cascade are unknown. To identify missing components of core PCP regulation and downstream effector pathways, an in vivo genetic screen has been performed and identified several new genes implicated in the Fz/PCP pathway. One of them, the Drosophila phosphatidylinositol-4-kinase β or Four Wheel Drive (Fwd), showed a strong PCP phenotype and could be a good candidate as modulator of PCP signaling. Fwd has been involved in several biological processes in Drosophila, such as meiosis or mitotic cytokinesis and spindle stabilization. RNAi depletion of Fwd showed PCP defects in the eye, e.g. microtubule and microtubule diameter defect, and in the wing, e.g. hair misorientation, suggesting that Fwd might be implicated in the PCP establishment process. Accordingly, we also demonstrated that Fwd mutant alleles display PCP phenotypes in eyes and wings. Our data are consistent with a role of Fwd in the Fz/PCP signaling and further experiments are now being conducted to determine the exact function/position of Fwd in the PCP cascade and its genetic interaction with other PCP components.


Two isoforms of the Prickle protein, Pk and Sple, are active in Frizzled Planar Cell Polarity (PCP) signaling during adult fly development. Both isoforms contain a PET domain and three LIM domains, but differ in their N-termini. Specifically, the 13 N-terminal amino acids in the Pk isoform are replaced by 349 amino acids in the Sple isoform. Viewed at a gross level, loss of individual Prickle isoforms affects PCP in mutually exclusive regions of the adult fly. Loss of Pk affects PCP in the wings and thorax; loss of Sple affects PCP in the legs, abdomen and eye. However, in each of these tissues, loss of both isoforms does not give an identical phenotype to the individual loss of either isoform. This suggests that both Pk and Sple are active in all these tissues, but are playing distinct roles. Significantly, the over-expression of individual isoforms in the wing, and other tissues, gives strikingly different phenotypes suggesting that Pk and Sple also have distinct molecular activities. It seems likely, therefore, that the fly achieves normal PCP by employing the specific molecular activities of Pk and Sple in diverse ways in different tissues. We refer to this differential use of Pk and Sple during adult PCP development as the Prickle Isoform Code. To attempt to crack the code, we are employing spatially and/or temporally restricted expression of Pk and Sple in various adult tissues in both wild-type and prickle mutant flies. Our findings on the activities of Pk and Sple with respect to the specific signaling landscape of various adult tissues will be presented.

Lethal giant larva (Lgl) promotes neural stem cell differentiation by antagonizing Notch. Jill Haenfler1,2, Cheng-Yu Lee1,4, 1) Graduate Program in Cellular and Molecular Biology; 2) Center for Stem Cell Biology, Life Sciences Institute; 3) Cell and Developmental Biology; 4) Division of Molecular Medicine & Genetics, Dept of Internal Medicine, Univ of Michigan, Ann Arbor, MI.

Precise regulation of the stem/progenitor cell population ensures normal development and homeostasis, but how stem cells and progenitor cells are functionally distinguished remains unknown. We investigate the mechanisms that promote differentiation of neural stem cells (neuroblasts) into progenitor cells in Drosophila larval brains. While type I neuroblasts provide neural progenitors called GMCs that divide once to generate two neurons, type II neuroblasts generate immature intermediate neural progenitors (INPs) that acquire the INP fate and undergo limited rounds of asymmetric divisions to generate GMCs. Here, we show that Lgl promotes asymmetric segregation of Numb into GMCs and INPs and specifies the progenitor cell fate in both type I and II neuroblast lineages by antagonizing the polarity regulator atypical Protein Kinase C (aPKC). While larval brains lacking the lgl function or expressing constitutively active aPKC show symmetric partition of Numb and ectopic type I and II neuroblasts, heterozygosity of aPKC restores asymmetric segregation of Numb and suppresses ectopic neuroblasts in lgl mutant brains. Over-expression of Numb restores the progenitor cell fate in lgl mutant larval brains dependent of the amino terminal acyl-Coenzyme A binding domain containing 3 (ACBD3) motif, which specifically mediates Numb suppression of Notch signaling in the central nervous system. Consistently, reduced function of Notch efficiently suppresses ectopic neuroblasts in lgl mutant brains. Thus, we conclude that Lgl functionally distinguishes neuroblasts from progenitors by suppressing Notch signaling in part by a novel mechanism mediated by the ACBD3 motif of Numb.

Macroglulin complement related (Mcr) is required for tracheal morphogenesis and septate junction function during embryogenesis and imaginal disc morphogenesis during metamorphosis. Sonia Hall, Bone Courtney, Robert Ward. Molecular Biosciences, University of Kansas, Lawrence, KS.

We isolated an allele of Macroglulin complement related (Mcr) as a dominant modifier of brh for leg morphogenesis during metamorphosis (prior to verifying the identity of the mutation we referred to it as sidewinder). Mcr encodes a protein with an alpha-2-macroglulin domain and an LDL receptor A motif, and has been shown to specifically bind Candida albicans, and thus may play a role in innate immunity. Lethal phase and terminal phenotypic analyses of Mcr loss of function alleles, however, revealed that Mcr has additional essential functions during development. Mcr mutant animals are embryonic lethal with defects in cuticle deposition, tracheal tube length and diameter control, and to a lesser extent dorsal closure. This suite of phenotypes is frequently seen in mutations that affect the septate junction, an invertebrate specific junction analogous to the vertebrate tight junction. Septate junctions are indeed defective in Mcr mutant embryos as determined by the localization of the septate junction proteins Coracle, Dlg and FasIII, and by functional assays using 10 kD rhodamine dextran. RNA interference (RNAi)-based knockdown of Mcr in embryos recapitulated the loss of function phenotypes, indicating that these phenotypes are due to the specific loss of Mcr. In addition, Mcr mutations dominantly enhance the malformed leg phenotypes associated with hemizygous brh mutation indicating a role for Mcr in imaginal disc morphogenesis. Consistent with this finding, RNAi of Mcr in leg and wing imaginal discs results in malformed legs and wings often accompanied by reduced size of the adult structures. We are currently testing whether cell growth or proliferation is also affected by loss of Mcr in imaginal discs.
POSTER: Cell Biology & Signal Transduction
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

162C
Examining the role of Rap1 in regulating the actin cytoskeleton and apical polarity. Nathan J. Harris1, Mark Peifer1,2. 1) Biology, UNC-Chapel Hill, Chapel Hill, NC; 2) Lineberger Comprehensive Cancer Center, UNC-Chapel Hill, Chapel Hill, NC.

163A
Novel Kinases regulating Wnt/β-Catenin and Fz/planar cell polarity signaling. Andreas Jenny1, Ekatherina Serysheva2, Heibist Berhane1, Kubilay Demir3, Michael Boutros3, Marek Mlodzik2. 1) Department of Molecular & Developmental Biology, Albert Einstein College of Medicine, New York, NY; 2) Department of Developmental and Regenerative Biology, Mount Sinai School of Medicine, New York, NY; 3) Signaling and Functional Genomics, German Cancer Research Center, Heidelberg, Germany.

164B
The function of the Bazooka-aPKC interaction for the establishment of cell polarity in Drosophila. Michael P Krahm, Andreas Wodarz. Stem Cell Biology, University Goettingen, Goettingen, Germany.

165C
Role of DaPKC in the organization of the Follicular Epithelium of the Drosophila egg chamber. Anabel R Learte1, Sol Sotillos2, Rosario Hernández3, Sonsoles Campuzano3. 1) Centro de Biología Molecular Severo Ochoa, CSIC-UAM , Madrid, Spain; 2) Centro Andaluz de Biologia del Desarrollo, CSIC-UPO, Sevilla, Spain.

166A
βⅠS-spectrin is part of a feedback loop in the regulation of the apical domain by Rac1. Seung-kyu Lee, Graham Thomas. Dept Biochem & Molec Bio, Pennsylvania State Univ, University Park, PA.

Here we report a strong negative relationship between βⅠS-spectrin and Rac1 small GTPase signaling, which appears to represent a feedback loop wherein βⅠS-spectrin also downregulates Rac1 activity. Four lines of evidence lead to this conclusion: (i) Activation of Rac1 signaling by expression of the exchange factor Trio, is strongly enhanced by reducing βⅠS levels, and heterozygosity for βⅠS alleles results in a detectable increase in the levels of active Rac1. (ii) Sustained expression of a C-terminal fragment of βⅠS (βⅠS33) in the eye induces a dominant phenotype that is similar to expression of dominant negative Rac1. Co-expression of βⅠS33 and Trio suppresses this phenotype, as does knockdown of RacGAP50C. (iii) Loss-of-function alleles in pak, a Rac1 effector and negative regulator of βⅠS, dominantly suppresses larval lethality arising loss-of-function karst (βⅠS) alleles. (iv) Expression of constitutively active Pak1+1 in the larval salivary gland induces expansion of the apical membrane and destabilization of the apical polarity determinant Crumbs. These effects resemble Rac1 activation and are suppressed by βⅠS33.

Our data suggest that βⅠS is negatively regulated by Rac1 activation, but that Rac1 signaling is suppressed in the apical domain by βⅠS, probably via its C-terminal domain. Such a system would be bistable with either Rac1 or βⅠS predominant and may provide a mechanism for apical domain maintenance.
The Drosophila Planar Polarity Gene Multiple Wing Hairs interacts with diaphanous to Locally Inhibit the Actin Cytoskeleton. Qiuhen Lu, Paul Adler. Biology Department, Department of Cell Biology, Morphogenesis and Regenerative Medicine Institute and Cancer Center, University of Virginia, Charlottesville, Virginia.

The evolutionarily conserved frizzled signaling pathway has been extensively studied in wing planar cell polarity (PCP); however, it remains unclear how the PCP signal is read out as single distally pointing actin-rich hair. We have found that the downstream PCP gene Multiple Wing Hairs (mwh) interacts with diaphanous (dia), which encodes a formin that promotes actin polymerization. Our working model is that Mwh interacts with Dia to locally inhibit the actin cytoskeleton and hence to insure a single distally pointing hair is produced. Multiple lines of evidence argue that Mwh functions as an inhibitor of the actin cytoskeleton. For example, loss of function mutations result in ectopic hairs, Mwh localizes to proximal side of the cell prior to hair initiation (which is the opposite side from where the hair forms) and during hair elongation Mwh localizes to the base and proximal part of the hair (the region where ectopic hairs form in mwh mutants). The amino half of the Mwh protein shows similarity to Diaphanous family forms. This is thought to be the regulatory part of Dia and it is also thought to mediate Dia dimerization. This suggested the hypothesis that Mwh might form heterodimers with Dia that could not promote actin polymerization. In this model Mwh would be acting like a dominant negative formin to inhibit actin polymerization. Consistent with this model we found that expression of a constitutively active Dia leads to multiple hair cells. We have also found genetic interactions between mwh and dia that are consistent with the two proteins acting antagonistically. Further we established that the two proteins could be co-immunoprecipitated from wing discs. Additional tests of our model are in progress.

168C

Planar cell chirality contributes to left-right asymmetric epithelial morphogenesis in Drosophila. Reo Maeda1,2, Kiichiro Taniguchi3,4, Tadashi Ando5, Naotaka Nakazawa1, Ryo Hatari1, Mitsutoshi Nakamura1, Takashi Okumura1, Kenji Matsumo1,2. 1) Dept. Biol. Sci./Tech., Tokyo Univ. of Sci; 2) Res. Ins. Sci./Tech., Tokyo Univ. of Sci; 3) Equal contribution.

The left-right (LR) asymmetric morphology of organs are formed according to LR polarity information. However, the cellular mechanism how LR asymmetric morphology is generated is still elusive. To elucidate this mechanism, we have been studying the LR asymmetric development in Drosophila. Here, we analyzed the LR polarity in the epithelial cells of the hindgut tube during LR asymmetric morphogenesis of this tissue, which makes a 90 degrees left-handed rotation. We found that the shape of cells at the apical plane of hindgut epithelium showed LR bias before the left-handed rotation. Consistent with this observation, we also revealed that the position of centrosomes in these epithelial cells showed LR bias. These results showed that hindgut epithelial cells have LR polarity in their plane before the left-handed rotation of the hindgut epithelial tube. We refer to this LR biased cell shape "planar cell-shape chirality (PCC)". Previously, we reported that Myo31DF (Myo31D), encoding MyoID, was required for the LR asymmetric development of various organs. In embryos homozygous for Myo31D, the PCC of hindgut epithelial cells were reversed. We also found that the PCC of epithelial cells were randomized in Drosophila E-cadherin (DE-cad) mutant embryos in which the laterality of the hindgut is randomized. The distribution of DE-cad in the apical cell boundaries showed LR bias in Myo31D-dependent manner, which may account for the formation of t he PCC. In addition, mosaic analysis in hindgut epithelium showed that LR biased distribution of DE-cad is attributed to the cell-autonomous function of MyoID. Using in silico simulation, we demonstrated that PCC was sufficient to induce the left-handed rotation of the hindgut epithelium tube. Taken these results together, we propose a novel mode for developing LR asymmetric organs.

169A

Study of a new epithelial polarity pathway. Vincent Mirouze1, Dan Bergstrahl2, Caroline Vachias1, Daniel St johnson1. 1) GReD, CNRS, INSERM, Clermont University, Clermont-ferrand, France; 2) The Wellcome Trust Gurdon institute, Cambridge University, UK.

Acquisition and maintenance of cell polarity are key steps of the morphogenesis of many cell types. We have identified a new pathway of epithelial polarity that is specifically required under low energy conditions, linking for the first time metabolism and cell polarity (Mirouze et al, J Cell Biol, 2007). At the molecular level, we identified several members of this pathway such as the kinases AMPK and LKB1 or the transmembrane complex Dystroglycan and its ligand Perlecan (Mirouze et al, Dev Cell, 2009). Moreover, it has been shown that this pathway is conserved in human epithelial cells and requires the Myosin II. Our current work indicates that, contrary to what had been proposed, AMPK regulates the Myosin II activity not directly but rather via a mechanism involving the Rho kinase both in fly and in human cells. Moreover, we have shown that cell growth regulator Tor is also involved in this energetic stress dependent polarity pathway. Beside its basic interest, study of this pathway might be relevant for cancer biology. More than 80% of cancers arise from epithelial tissues and both the loss of cell polarity and energetic stress conditions are common features of these cancers, at least at some point of their development. In agreement with an important function of this pathway in tumor biology, many of its components are tumor suppressors or oncogenes in human.

170B

Asymmetric cell signaling: new lessons learned from symmetry breaking of EGFR signaling during bract cell fate determination in Drosophila. Ying Peng, Jeff Axelrod. Dept. of Pathology, Stanford University School of medicine, Stanford, CA.

While cells communicate with each other in a highly coordinated spatial manner during development, how a signaling cell can distinguish among different neighbors and trigger selective signaling outputs via is a poorly understood process. We studied the mechanisms of cell fate determination of bract cells on Drosophila legs as a model to address this question. Specifically one bract cell arises on the proximal side of each mechanosensory bristle, and the bract cell fate is dependent on the signals from the sensory organ precursor lineage. We found while Notch and EGFR signaling both contribute to the fate determination of bract cells, their specific roles are distinct: Notch signaling only controls the threshold of bract fate determination, while EGFR signaling, serving as the inducing signal, is responsible for the spatial bias in which a bract cell arises only from the proximal side of the signaling cell. Through genetic mosaic studies, we have identified the socket cell from sensory organ precursor lineage, as the signaling cell responsible for sending EGFR ligand, Spitz. During the process of bract cell fate determination, socket cells send planar polarized protrusions specifically to their proximal neighbors. The polarity of the cellular protrusions is under the control of PCP genes: in mutants of core PCP genes, the directions of socket cell protrusions fail to maintain strict proximal directionality, and those failures correlate to the mistakes in which bract cells arise at incorrect positions. The significance of the planar polarized protrusions in biasing EGFR signaling is further confirmed by the fact that bract cells fail to be induced when the protrusions are suppressed in the signal sending cells. Overall, we have unveiled a novel mechanism that EGFR signaling can be potentiaded through dynamic cellular protrusions emanating from the signaling cells. When those protrusions are polarized on a planar axis, the spatial symmetry of EGFR signaling is broken to favor neighbors on a particular side.

171C

Analysis of the Crumbs-Yurt interaction in the organization of Drosophila photoreceptor cells. David ter Stal1, Juan Huang2, Ying Hong2, Ulrich Tepass1. 1) Cell and Systems Biology, University of Toronto, Toronto, Ontario, Canada; 2) Department of Cell Biology and Physiology, University of Pittsburgh, Pittsburgh, Pennsylvania.

The Crumbs (Crb) protein complex is a key regulator of epithelial polarity of photoreceptor cells (PRCs) in the Drosophila eye. Loss of Crb function results in a shortening of apical stalk membranes in PRCs and the converse for Crb overexpression. Previous work showed that the ability of Crb to regulate polarity is in large part due to its cytoplasmic tail, which contains a C-terminal PDZ binding site linking it to other core components of the Crb complex, and a FERM domain binding site (FDB), which mediates interactions with other FERM domain containing proteins including Yurt. Crb recruits Yurt from the basolateral to the apical domain of PRCs late during PRC development, where it regulates Crb activity in apical membrane growth. However, neither the mechanisms by which Yurt is recruited by Crb, nor how Yurt is able to regulate Crb activity are known. Previous work has suggested that the FDB of Crb is phosphorylated at four Serine/Threonine residues by aPKC, which is important for Crb function in polarizing simple epithelial cells. However, recent gene replacement of crb with a Mutant isoform in which these S and T residues were replaced by Alanine do not show developmental defects in simple epithelial. The effect of the non-phosphorylatable isoform of Crb in PRC morphogenesis is unknown. We hypothesize that phosphorylation of the Crb FDB by aPKC serves as a mechanism to regulate Yurt recruitment to the stalk membrane in PRCs. To test this we have made a series of Crb constructs expressing either a phosphomimetic or non-phosphorylatable
form of Crb, which will be used to analyze the effects of Crb phosphorylation on Yurt distribution in PRCs. Additionally, using transmission electron microscopy we have examined the length of the apical stalk membrane as a measure of Crb activity. Preliminary findings show that flies which express mutant forms of Crb lacking either two or all four phosphorylation sites cause a significant increase in stalk membrane size.

172A


We probed interaction between the relay loop and converter domain of Drosophila muscle myosin in vitro, within muscle fibers and in intact organisms. We generated a transgenic line expressing myosin with a mutation in the converter domain (R759E) at the relay loop interaction site. The mutation depresses calcium, basal or actin-activated MgATPase values by 60% and actin-sliding velocity by 35%. Ultrastructure of 2-day-old adult indirect flight muscle (IFM) fibers shows minor disruption of the myofilament lattice, which becomes more severe in 1-week-old adults. Flight ability is reduced in 2-day-old flies compared to controls and is absent in 1-week-old adults. Thus appropriate relay/converter interaction is essential for formation of filaments, myofilament stability and locomotion. We examined interaction specificity by making putative compensatory mutations designed to restore function of the R759E mutant. Our molecular modeling indicates that relay residues I508, S509 and D511 interact with converter residue R759. To test this, we generated three transgenic lines that express R759E and either the I508K, S509R or D511K mutations. Interestingly, N509K/R759E calcium, basal, and actin stimulated ATPase values are restored to 70% of wild-type levels and actin-sliding velocity is nearly normal. IFM fibers from 2-day- or 1-week-old N509K/R759E appear morphologically normal and flight ability is the same as the wild-type control line. For D511K/R759E myosin, ATPase and motility defects are generally worse than for R759E. Further, myofilament structure is not improved compared to R759E and flight ability is reduced. I508K/R759E myosin shows no enzymatic activity and does not stimulate actin filament sliding. S509K/R759E myosin assembles abnormally and no flight is observed. Overall, our results reveal that residues at the relay-converter interface are important for myosin and muscle function and that interaction of residue 509 in the relay loop with residue 759 in the converter is essential for normal locomotion.

173B

Actin dynamics in larval epidermal wound closure. Amanda R. Brock, Yanyang Liu, Susanne Berger, Yujane Wu, Renate Renkawitz-Pohl, Michael J. Galke. 1) Biochem & Mol Bio, UT MD Anderson Cancer Center, Houston, TX; 2) Philippus-Universität Marburg, Marburg, Germany.

Drosophila is ideal for dissecting the cellular and molecular mechanisms of wound closure. We developed an assay to create reproducible, sterile wounds by pinching third instar larvae. This produces a gap in the epidermal sheet without damaging the cuticle. In a pilot RNAi screen, we found that chickadee (Profilin) is required for wound closure. We have ruled out nonspecific effects of the RNAi, confirmed epidermal knockdown of the protein, and confirmed the phenotype using an RNAi-independent loss-of-function allele.

In vitro studies show that Profilin, a conserved protein, binds to actin monomers and facilitates the binding of these monomers to the barbed end of actin filaments, though less work has been done in vivo. Following a wound, we have shown that Profilin is upregulated and relocated from the perinuclear region to the cytoplasm of epidermal cells proximal to the wound. Some of this upregulation is transcriptional, as shown by chic-lacZ activation around wounds.

We are also using LifactGFP to examine Actin phenotypes. Lifact-GFP is a 17 amino acid peptide fused to GFP that binds F-actin without interfering with actin polymerization dynamics. This tool allows us to visualize the actin-based protrusions that form at the wound edge and enable cells to move into the wound gap. In control larvae, F-actin is concentrated at the leading edge in cells adjacent to the wound. In cha124k-expressing larvae, actin does not concentrate at the wound edge nor do the epidermal cells extend filopodial and lamellipodial processes. We are using this assay to examine the actin phenotype of other genes identified in our screen and will present this analysis.

174C

AIP1-mediated Actin Dynamics is Essential for Adherens Junction Rearrangement during Epithelial Morphogenesis in the Drosophila Eyes. Dandan Chu, Ping Wan, Jing Chen. Model Animal Research Center, Nanjing University, 12 Xue Fu Road Nanjing, China 210061.

Dynamic assembly and disassembly of the actin cytoskeleton are required for such essential processes as cell division, cell motility, endocytosis and morphogenesis. Actin interacting protein 1 (AIP1) was first identified in yeast and was later shown to collaborate with cofilin to disassemble F-actin in vitro. It is a WD-repeat protein (~67-kD), and like cofilin it is highly conserved across species. Drosophila AIP1 is encoded by the gene flare (flr). Recently, mutations of flr have been reported to affect planar polarity and morphogenesis of wing hairs. We show here that epithelial morphogenesis is severely affected in the larval eye discs of flr mutants. Specifically, adherens junctions (AJ, E-cadherin/?-catenin/?-catenin complex) within ommatidial pre-clusters and clusters are severely altered in flr mutant tissues, while other epithelial polarity markers, including the apical Par3/Par6/aPKC complex and the basolateral Dlg complex (septate junction) do not show gross defects. Immuno-staining study shows that AIP1 is preferentially expressed behind the MF, and it specifically colocalizes with the AJ within ommatidial clusters. Furthermore, F-actin level was dramatically increased in the mutant flr clones behind the MF but not anterior to it, indicating AIP1 is required for F-actin assembly and its actin depolymerizing activity is mostly required in regions of epithelia that undergo active morphogenesis (eg. dynamic rearrangement of AJ). These F-actin and AJ defects could also be detected in ts (encoding cofilin) temperature-sensitive alleles. Overexpressing cofilin in flr/RNAi flip-out clones rescued AJs structure, suggesting that AIP1 enhance the efficiency of cofilin-induced actin depolymerization specifically around AJs. Taken together, our results suggest that AIP1 and cofilin-mediated actin dynamics is required in AJ formation and rearrangement during epithelial morphogenesis in the eye.

175A

Integrin function is developmentally regulated through distinct interactions with talin. Stephanie J. Ellis, Mary Pines, Michael J. Fairchild, Guy Tanentzapf. Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, Canada.

Cell adhesion molecules perform diverse, essential functions during morphogenesis. Integrins are the main adhesion receptors in metazoan cells that mediate binding to the ECM. In addition to binding extracellular ligands, integrins assemble a large intracellular adhesion complex (IAC) with important roles in cytoskeletal organization and signalling. The adapter molecule talin is known to be essential for linking integrins to the IAC. Biochemical studies have revealed that talin contains two different integrin binding sites (IBSs): IBS1 and IBS2. However, the respective role of each IBS has not been systematically examined in vivo. In our present study, we analyzed the role of these IBSs in the context of fly development. Our results show that the interaction between talin IBS2 and integrin helps to maintain the link between integrins and the IAC. This function is particularly important for mediating dynamic adhesive processes during large-scale morphogenetic tissue movements. In comparison, the interaction between IBS1 and integrin helps maintain the link between integrins and their ECM ligands which was of particular importance in maintenance of long-lasting adhesions at myotendinous junctions. Loss of the interaction between integrins and both IBS1 and IBS2 leads to complete loss of integrin-mediated adhesion in the fly. Altogether, these results indicate that while the interactions of integrin with the IBS domains of talin are partially redundant, each IBS mediates a distinct set of integrin functions in the context of development. Our work provides insight into the role of talin in regulating integrin and IAC function and gives developmental context for the relative importance of different talin interactions with the integrin cytoplasmic tails. Moreover, we illustrate how specific interactions between an adhesion receptor and different domains of a single cytoplasmic adapter can comprise a mechanism to regulate cell adhesion during embryogenesis.

176B

Overlapping regulation of Myosin Regulatory Light Chain phosphorylation and tissue morphogenesis by Drosophila Drak and Rok kinases. David R. Hipfner1,2, Dagmar D.A. Neubueser1. 1) Institut de recherches cliniques de Montréal (IRCM), Montréal, QC, Canada; 2) Département de Médecine, Université de Montréal, Montréal, QC, Canada; 3) Department of Anatomy & Cell Biology, McGill University, Montreal, QC, Canada.

Dynamic regulation of cytoskeletal contractility through phosphorylation of the nonmuscle Myosin-II regulatory light chain (MRLC) provides an essential source of tension for
shape epithelial tissues. Rho GTPase and its effector kinase ROCK have been clearly implicated in regulating MRLC phosphorylation in vivo, but evidence suggests that other mechanisms must exist. We have identified a previously uncharacterized Drosophila kinase belonging to the Death-associated protein kinase (DAPK) family, which we called Drak, as a regulator of MRLC phosphorylation. Based on analysis of genetic null mutants, we find that Drak broadly promotes proper morphogenesis of epithelial tissues during development. Drak activity is largely redundant with that of Drosophila ROCK (Rok), such that it is essential only when Rok levels are reduced. We demonstrate that these two kinases synergistically promote phosphorylation of Spaghetti squash (Sqh), the Drosophila MRLC orthologue, in vivo. The lethality of drak/rok mutants can be rescued by restoring Sqh activity, indicating that Sqh is the critical common effector of these two kinases. These results provide the first evidence that DAPK family kinases regulate actin dynamics in vivo and identify Drak as a novel component of the signaling networks that shape epithelial tissues.

177C
The LIM protein PINCH suppresses defects associated with mutations in the myosin phosphatase flapwing. Julie L. Kudrmas1,2, Stephen M. Pronovost1, Mary C. Beckerle1,2. 1) Department of Oncological Sciences; 2) Huntsman Cancer Institute; 3) Department of Biology, University of Utah, Salt Lake City, UT.

Non-muscle myosin II is a major cytoskeletal motor that drives changes in both cell shape and position in a wide variety of cell types and biological processes. Actomyosin contraction is regulated by phosphorylation of the Myosin regulatory light chain, encoded by spaghetti squash (sqh), via a variety of protein serine/threonine kinases. Actomyosin relaxation requires dephosphorylation of Sqh. flapwing (flw) encodes Protein phosphatase 1f, with the single essential function of Sqh dephosphorylation. Strong loss-of-function mutants in flw exhibit hyper-phosphorylated Sqh and are semi-lethal. The vast majority of flw mutants die due to larval muscle detachment. Rare adult escapers are sterile, with poor ambulatory ability and grossly malformed wings. Genetic screens have identified many suppressors of flw lethality. One class of flw suppressors affords viability by decreasing signaling through the Jun kinase signaling cascade. The molecular pathways connecting flw dependent myosin relaxation to Jun kinase signaling are not well understood. PINCH, a LIM domain containing scaffolding protein encoded by steamer duck (stick), has well-established roles in stabilizing actin-integrin linkages in muscle, in maintaining adhesion between the epithelial sheets of the wing, and in regulating Jun kinase signaling. The phenotypes of stick and flw mutants show considerable overlap. As such, PINCH is an attractive candidate to serve as a molecular link between Jun kinase signaling and the activity of flw on myosin. We tested for a genetic interaction between stick and ics, and demonstrate that ectopic expression of PINCH indeed potently suppresses the lethality and sterility of flw mutants. Ongoing studies to determine the precise mechanism of this suppression will provide a broader understanding of the integration of signaling pathways key to actomyosin function.

178A
β-spectrin and Annexin B9 have roles in protein recycling and multivesicular body function in Drosophila. Mansi R. Khamma1, Monika Tjotsa2, Seung-Kyu Lee2, Juan Wu1, Janice A. Williams1, Graham H. Thomas1,2. 1) Dept Biol, Penn State Univ, State College, PA; 2) BMB, Penn State Univ, State College, PA; 3) Vanderbilt Univ Med Center, Nashville, TN.

Spectrin is a peripheral membrane protein that has roles as a molecular scaffold in endocytosis and in apical-basal polarity. Annexins are a family of proteins that bind phosphatidyls in a calcium-dependent manner and have widespread roles in the endosomal system, cell proliferation and migration. Previous data from our lab has suggested a role for β-spectrin (βH) in the modulation of endocytosis and recycling; Loss-of-function karst (βH) alleles perturb the early endosome and overexpression of the 33rd segment of βH results in plasma membrane expansion. These membrane protrusions sequester dyamin and Annexin B9, suggesting direct or indirect association of this spectrin fragment with components of the endocytic pathway. Here we report and map a direct interaction between Annexin B9 and the C-terminus of a subset of βH isoforms. We also show that knockdown in the levels of Annexin B9 results in an increase in the number of vesicles positive for the multivesicular body (MVB) markers Hrs, Vps16 and Endo15, as well as an accumulation of ubiquitinated proteins and of DE-Cadherin in the cytoplasm. These results are consistent with a role for Annexin B9 in MVB formation and function. Annexin B9 knockdown also results in perturbation of the polarity of βH at the plasma membrane and its appearance on internal vesicular structures where it closely colocalizes with MVB markers. Knockdown of βH also results in the accumulation of ubiquitin, but in a distinct pattern to Annexin B9, and epistasis places βH upstream of Anx B9. We suggest model in which Annex B9 suppresses the efficient transport of cargo through the MVB, whereas βH has a role in cargo trafficking from the plasma membrane to the MVB.

179B
Dynamic expression and the asymmetric distribution of the homophilic adhesion molecule Echinoid controls the polarisation of the actin cytoskeleton. Arsida Noçka1, Caroline Laplante1,2, Laura Nilsson1. 1) Department of Biology, McGill University, Montreal, Canada; 2) Molecular, Cell and Developmental Biology, Yale University, New Haven, CT.

Epithelial morphogenesis is driven by cell shape changes and movements. We study the role of the homophilic cell adhesion molecule Echinoid (Ed) in the morphogenesis of the follicular epithelium and during dorsal closure, where Ed disappears from defined populations of cells, thus creating interfaces where cells expressing Ed abut cells not expressing Ed. These interfaces are smooth, develop a contractile actomyosin cable, and are essential for proper morphogenesis. In embryogenesis, Ed disappearance coincides with the disappearance of its mRNA, suggesting a negative regulation at the transcriptional level. Due to its homophilic binding property, Ed disappears from the interfaces, generating a planar polarized Ed distribution in the Ed expressing cells. We show that this asymmetric distribution is essential for actin cable formation at Ed interfaces, and that this function requires the Ed intracellular domain but not its C-terminal PDZ-binding motif. Thus, the homophilic binding property of Ed provides cells with a spatial cue to recognize their neighbors, and the resulting planar polarized distribution of Ed instructs cells to target actin cable formation to the appropriate face.

180C
Roles of Ena/VASP and Capping Protein in Drosophila development. Stephanie Nowotarski1, Julie Gates2, Mark Peifer1. 1) Biology, Univ North Carolina, Chapel Hill, NC; 2) Biology Dept, Bucknell University, Lewisburg, PA.

Proper development requires cells to build a range of different actin-based structures. Each actin structure presumably arises from differing underlying actin geometries dictated by the effect of different suites and levels of actin regulators. We use Drosophila morphogenesis and oogenesis as models for understanding requirement of proper actin regulation in vivo, especially the actin regulator Enabled VASP (Ena) and Capping protein (CP). In oogenesis both Ena and CP play roles in the formation of bundled actin filaments in nurse cells during the dumping process. Ena localizes to the ends of these actin structures and appears to be negatively regulated by Ableson kinase in this context. Analysis of ena mutants affecting different domains suggests the EVH1 domain is not as critical as the oligomerization and EVH2 domains in oogenesis. We found CP is important for nurse cell dumping, and also plays a surprisingly critical role in oocyte specification, perhaps as part of the dynactin complex. Together these data support an antagonistic relationship between Ena and CP in oogenesis. I am currently exploring the contribution and importance of individual domains of Ena during morphogenesis by examining site directed mutants in both Drosophila cell culture and in embryos. We are also continuing to explore the relationship between Ena and CP in morphogenesis, looking at the dynamic actin-dependent process of dorsal closure and at axon outgrowth in the CNS.

181A
Screening chromosone 2R for regions that genetically interact with Ab1 kinase during cell migration. Kristina Reiss, Christopher Moline, Caron Leonard, Traci L. Stevens. Dept. of Biology, Randolph-Macon College, Ashland, VA.

Cell migration is vital during development, wound healing, and immune responses, and errors in migration can contribute to disease states such as cancer. Ab1, a tyrosine kinase, directs cell migration through control of actin polymerization. Ab1, an actin regulator, is one protein in Ab1 signaling pathways, but other proteins that mediate Ab1’s effects on actin and molecules that link Ab1 to the cell surface remain largely unknown. To dissect Ab1 pathways, we use an activated version of Ab1, Ber-Ab1. The Ber-Ab1 fusion protein results from a translocation that has been linked to leukemia in humans. In the epithelium of Drosophila, expression of Ber-Ab1 disrupts developmental processes that require cell
migration, such as dorsal closure and head involution. To identify other components of Abelson pathways, we are screening the genome for genes that, when one copy is removed, dominantly modify Bcr-Abelson phenotypes in the epithelium. We have found several candidate genes that interact with Abelson during cell migration. Mutations in shg, which encodes DE-cadherin, strongly enhanced phenotypes associated with Bcr-Abelson expression. It has previously been shown that shg mutations enhanced loss-of-function abl mutations and that Abelson acts at adherens junctions. Thus, this genetic interaction validated the idea that expression of Bcr-Abelson in the Drosophila epithelium provides a sensitized background in which to identify components of normal Abelson signaling pathways. We also found that mutations in Egfr and dNotc, which encode epidermal growth factor receptor and kinesin heavy chain respectively, modified Bcr-Abelson-dependent phenotypes, suggesting that these proteins may play roles in Abelson signaling. The rest of chromosome 2R was screened using heterozygous deficiencies, and using this strategy, several interacting regions were identified. We are currently using smaller deficiencies and candidate genes in these interacting regions to identify the gene(s) responsible. By identifying genes that interact with Abelson, we can develop a better understanding of the molecular pathways that direct cell migration.

182B
Somatic myoblast fusion in Drosophila is independent of β-Tubulin isotypes, while two non-muscle myosins are required in redundancy as actin motor proteins. Anja Rudolf1,2, Carolina Boms1, Detlev Buttgereri1, Renate Renkawitz-Pohl1. 1) Developmental Biology, University of Marburg, Germany; 2) Pediatric Hematology and Oncology, University hospital of Gießen, Germany.

The multi-nucleated somatic musculature of Drosophila is formed by fusion of two different cell types, the founder cells and the fusion-competent myoblasts (FCMs). After establishment of the cell-cell contact between FCM and growing myotube in the somatic mesoderm, electron-dense vesicles accumulate on opposing membranes. It has been shown previously that regulated F-actin assembly is not only required in this vesicle transport, but also in fusion pore formation and integration of the FCM into the growing myotube (reviewed in Onel and Renkawitz-Pohl, 2009). Here we investigate whether solely the F-actin cytoskeleton or in addition newly synthesized microtubules are essential for myogenesis. β3-Tubulin is the only β-Tubulin isotype expressed in the somatic mesoderm during myoblast fusion (Leiss et al., 1988;Kimble et al., 1989; Buttgereri et al., 1996). We are able show that in embryos deficient for β3-Tubulin, the body wall musculature develops correctly and attachment to the epidermis occurs. This is surprising as we further demonstrate that β3-Tubulin is not replaced by β1- or β2-Tubulin. Formation of the gut muscles functions correctly as well. So we propose that the transport of electron-dense vesicles to the fusion site does not require an active microtubule network but depends solely on the actin cytoskeleton. We identified two myosin-heavy-chain-encoding genes which are expressed in the mesoderm and act in functional redundancy. Double mutants show myoblast fusion as well as strong outgrowth and guidance defects in the somatic musculature. Using Eve as a marker to visualize the nuclei of DA1 muscles, it became obvious that double mutants stop at the level of precursor cells, which implements an involvement of the myosins in the second fusion phase. We propose that both myosin isoforms act in redundancy during migration of FCMs towards the growing myotubes or that they are involved in vesicle transport or widening of the fusion pore.

182C

Zasp (Z-band alternatively spliced PDZ-domain protein) is an Alp/Enigma PDZ-LIM domain protein in Drosophila melanogaster which co-localizes with integrin and α-actinin in myotendinous junctions (MTJs) and muscle Z-lines, respectively. We have previously shown that Zasp is crucial for alpha-actinin recruitment to the Z-line and muscle attachment at MTJs (Jani & Schlick, 2007). The Drosophila Zasp protein has various isoforms produced by alternative splicing. In this study, different exons and mRNA transcripts were identified using RT-PCR and EST sequencing. The developmental stage- and tissue-specific expression of Zasp variants was determined by Western blot and antibody stainings. Furthermore, the alternative splicing of Zasp isoforms was investigated in a new Zasp EMS allele, which lacks indirect flight muscle-specific isoforms.

184A
Role of Moesin in maintaining Ecadherin in the late-stage follicular epithelium. Kristin Sherrard, Richard Feigon. Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL.

Cadherin-based cell-cell adhesion is a fundamental property of epithelial tissues, but we do not understand how epithelial integrity and cadherin levels are dynamically maintained while adherens junctions are remodeled during morphogenesis. Moesin, the sole ERM protein in Drosophila, has been implicated in a wide variety of processes involving organization of the actin cytoskeleton and cell membrane domains, including maintenance of epithelial integrity. However, the precise role of Moesin in maintaining epithelial integrity has been elusive to ascertain: cells lacking Moesin function do not develop normally and show uncontrolled cell proliferation. The follicular epithelium is a promising candidate for investigating Moesin function because we find that mutant cells are stably maintained in this epithelium. Interestingly, as we showed previously for the Moesin regulator Sip1, null Moesin clones in the late-stage follicular epithelium lose all Ecadherin staining, though Moesin do not develop. The follicular epithelium is a promising candidate for investigating Moesin function because we find that mutant cells are stably maintained in this epithelium. Interestingly, as we showed previously for the Moesin regulator Sip1, null Moesin clones in the late-stage follicular epithelium lose all Ecadherin staining, though sepalte junction markers are unaffected. We have therefore been investigating the mechanism by which Moesin maintains Ecadherin and adherens junctions in stage 11-14 follicle cells. We are using mitotic clones UAS-RNAi screening in combination with live imaging and FRAP analysis to distinguish among the (non-exclusive) possibilities that 1) Moesin septate junction markers are unaffected. We have therefore been investigating the mechanism by which Moesin maintains Ecadherin and adherens junctions in stage 11-14 follicle cells. We are using mitotic clones UAS-RNAi screening in combination with live imaging and FRAP analysis to distinguish among the (non-exclusive) possibilities that 1) Moesin regulates clearance of Ecadherin from the cell surface by endocytic trafficking. In addition, we are asking if these effects are mediated through Moesin’s role in regulating Rho1 activity. We expect that these studies will provide a better understanding of Moesin’s role in regulating the actin cytoskeleton and epithelial integrity.

185B
A Drosophila high-content genome-wide RNAi screen identifies novel conserved regulators of the actin cytoskeleton. David Sim1, Jennifer Rehn2, Tao Lin3, Marina Fedorov2, Frieder Schoeck1, Maria Vartiainen1, Amy Kiger1, Norbert Perrimon2, Buzy Baum2. 1) The Institute of Cancer Research, London, UK; 2) MRC Laboratory for Molecular Cell Biology, University College London, UK; 3) Department of Biology, McGill University, Montreal, Canada; 4) Institute of Biotechnology, University of Helsinki, Finland; 5) Division of Biological Sciences, University of California, San Diego, CA; 6) Department of Genetics, Harvard Medical School, Boston, MA.

The dynamic regulation of the actin cytoskeleton of eukaryotic cells provides the mechanical force and structural integrity required for many key cellular functions including cell division, cell migration and the establishment of the specialised cell morphologies required for multi-cellular development. Although many of the core components of the actin machinery have been well characterised we are still far from understanding the pathways regulating the generation of specific sub-cellular actin structures. We have used RNAi screening in conjunction with high-content imaging to systematically interrogate the Drosophila genome for genes required for the development of lamellipodia in the adherent haemocyte cell line S2R+. This analysis revealed roles for many of the known core actin regulators (Arp2/3, cofilin etc), and phenotypic clustering identified several new members of the previously characterised Rac pathway. Furthermore, several clusters of novel actin phenotypes were identified suggesting contributions from a large number of genes and pathways to the morphology of S2R+ cells. For example, we have identified a novel role for a previously uncharacterised member of the formin family of actin regulators, CG32138, in cell spreading. As important genes are likely to have conserved functions across species, we validated the phenotypes of a subset of hits using siRNAs in mammalian cell culture. Using this approach we were able to establish conserved functions of both known and novel regulators and to explore the effects of functional redundancy in large-scale mammalian siRNA screens.

186C
A role for Slik in regulating apical membrane integrity and growth of terminal branches in the tracheal system. Fiona P. Ukken1,2, Maria Leptin1,2, Nair Jayanandanar1. 1) University of Cologne, Cologne, Germany; 2) EMBL, Heidelberg, Germany.

The Drosophila Sterile-20-like kinase Slik is involved in maintaining epithelial integrity and promotes tissue growth during development. It regulates activity of members of the band 4.1/Ezrin/Radixin/Moesin (ERM) superfamily proteins through phosphorylation. Apart from its kinase activity, Slik also interacts with Raf to promote cell survival and...
growth. Raf is an important downstream effector of the bnl/btl RTK pathway crucial for tracheal development. An immediate target of the RTK-MAPK signalling is src (serum response factor), a transcription factor known to be indispensable for terminal cell development. Here, we show that Slik contributes to terminal cell development through both its kinase dependent and independent functions. Both Slik and activated Moesin (p-Moesin) are enriched at the apical membrane in terminal cells. slik mutant or knockdown terminal cells show branching defects and destabilised tubes similar to the phenotype of moesin mutants, suggesting that slik is an essential factor in terminal cell growth and development. In addition, slik depletion results in the loss of p-Moesin at the apical terminals indicating that Slik through its kinase dependent function toward Moesin regulates terminal cell development. This is further supported by the effect of expressing a kinase-dead form of Slik, which causes a multilumen phenotype similar as the one seen in slik mutant cells. In addition to the luminal defects, slik depletion also resulted in reduced branching of terminal cells. The same phenotype is observed upon knockdown of Raf. As Raf is thought not to be a kinase substrate of Slik but a binding partner, the results suggest an additional kinase independent function of Slik in tracheal development. Therefore, the disruption of src or the downstream target of bnl/btl signalling pathway src, exhibits a similar branching defect. We propose that slik acts in the development of terminal cells through activation of Moesin at the apical membrane and a possible regulation of the bnl/btl RTK pathway through its interaction with Raf.

187A Membrane and Cytoskeleton Responses during Single Cell and Multicellular Wound Repair in Drosophila. Jeffrey M Verboom, Maria Teresa Abreu-Blanco, Raymond Liu, Susan Parkhurst, Fred Hutchinson Cancer Research Center, Seattle, WA.

Despite differences in magnitude and scope, injuries to single cells or to tissues require rapid and robust wound repair responses to prevent cell death, loss of tissue integrity, and invasion by microorganisms. We have established the early syncytial embryo as a model for single cell wound repair. This single cell repair model, along with the previously developed model using dorsal closure stage fly embryos for multicellular wound repair, provide systems in which to investigate the molecular and cellular mechanisms of the two repair processes. Using 4D, high-resolution microscopy to monitor the expression of and effects of perturbations to components of the cytoskeleton and plasma membrane, we have defined a specific series of phases for each type of wound repair. Both types of repair undergo Expansion, which occurs immediately post wounding, Contraction during which the wound decreases in area, and Closure, in which wound edges come together. Multicellular wounds have an additional step between Expansion and Contraction, Coalescence, during which an acanthocytosis cable is assembled. We find that single cell repair requires plasma membrane mobilization, assembly of a contractile actomyosin ring, and surprisingly, an important role for E-Cadherin. In contrast, epithelial wound repair uses a combination of actin-rich protrusions and an actomyosin purse-string anchored intercellularly by cadherin-based adherens junctions. We are currently identifying additional components of and pathways required for these two repair processes.

188B Egfr interacts with Dpp signaling during dorsal closure. Xi Chen1, Weiping Shen2, Nicholas Harker1. 1) Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, B.C., Canada; 2) Laboratory of Genetics, National Institute on Aging, NIH Biomedical Research Center, Baltimore, MD.

Following retraction of the germband in the Drosophila embryo, a hole is left in the dorsal epidermis occupied by the amnioserosa. In dorsal closure (DC), this hole is sealed by migration of the epidermal flanks up over the amnioserosa until they meet at the dorsal midline. DC is driven in part by myosin-dependent cell shape change in the epidermis and the amnioserosa. Myosin transcription in these two tissues is dependent on Dpp signaling. We have found that mutants in Egfr have DC defects and that Egfr genetically interacts with Dpp signaling during DC. We have identified two routes by which Egfr negatively impacts Dpp function in DC-inhibition of Dpp expression in the epidermis and inhibition of myosin transcription. Preliminary results indicate that Egfr function in the amnioserosa negatively regulates a signal controlling myosin transcription in conjunction with Dpp. We have previously demonstrated that ACK, a non-receptor tyrosine kinase known to negatively regulate Egfr in other species, functions in the amnioserosa to positively control myosin expression and we are currently addressing interactions between ACK and Egfr during DC. Our results indicate the existence of a complex signaling network dedicated to coordinating tissue morphogenesis during DC.

189C Mummy Mediates Embryonic Dpp Signal Restriction. Gregory B. Humphreys, Kate Monroe, Anthea Letsou. Human Gen, Univ Utah, Salt Lake City, UT.

mummy (mmy), a member of the raw group of signaling antagonists, encodes the single Drosophila UDP-N-acetylglucosamine pyrophosphorylase. Mmy’s effects on signal antagonism are most evident in the context of embryonic dorsal closure. In this developmental context the JNK/AP-1 signaling cascade transcriptionally activates Dpp signaling in leading edge (LE) epidermal cells. Whereas dpp is confined to LE cells in wild-type embryos, it expands ectopically into the dorsolateral epidermis in mmy mutant embryos, establishing Mmy as a dpp antagonist. Western studies indicated that Jun and P-Jun are maintained at wild-type levels in mmy mutants. In addition, in immunohistochemical stains of embryos in situ we observed restricted accumulation of Jun to LE cells in wild-type and mmy mutants, implying that Mmy-mediated effects on dpp are downstream of AP-1. Considering that Jun is required for LE dpp transcription, and as Jun activity and localization are unaffected in mmy mutants, we have utilized a mmy Jra double mutant to specifically test if Jun-initiated LE dpp expression is required to ectopically express Dpp in a mmy mutant background. mmy Jra mutant embryos lack both LE and ectopic dpp expression, indicating a requirement for an initiating round of LE dpp to enact ectopic expression. Taken together, these data indicate that Mmy antagonizes paracrine Dpp in the embryonic epidermis, with earlier evidence identifying Dpp-dependent dpp transcription in the LE epidermis. To test this model, we assayed embryonic Dpp signaling activity by probing Mad phosphorylation. P-Mad is found broadly in the epidermis in early embryos, but undergoes a Mmy-dependent restriction to LE cells during germband extension and dorsal closure. P-Mad activity remains broad in the dorsolateral epidermis in mmy mutant embryos, suggesting that Dpp undergoes a Mmy-dependent transition from paracrine to autocrine signaling in the embryonic epidermis. A Mmy requirement for limiting epidermal Dpp signaling during dorsal closure highlights a role for glycosylation in defining a highly restricted Dpp activity fields.

190A The role of the JNK signaling antagonist, Raw, during Drosophila dorsal closure. Molly C. Jud, Melissa Ratcliffe, Gregory B. Humphreys, Anthea Letsou. Department of Human Genetics, University of Utah, Salt Lake City, UT.

Signaling pathways are important to several life processes including development, differentiation, growth, homeostasis, and apoptosis. One major family of signaling pathways is the Mitogen-activated protein kinase (MAPK) cascade. The MAPK family includes ERKs, JNKs/SAPKs, and p38/HOG; these kinases activate transcription factors in response to cell growth and/or stress signals. The positive regulation of the MAPK pathways is well characterized; however, less is known about their negative regulation. Our lab studies the Raw antagonist of JNK signaling using dorsal closure as a model. The novel gene, raw, is a member of the Raw group antagonists including ribbon, puckered, and mummy. The Raw group members share similar loss of function phenotypes such as dorsal closure defects, hypotrophy of ventral denticle belts, and ectopic expression of the JNK target gene, dpp, in cells beyond the leading edge (LE). Using genetics, we have previously shown that Raw is widely expressed during embryogenesis and is required to suppress Basket (JNK)-independent AP-1 activity in the lateral epidermis of embryos undergoing dorsal closure. Therefore, Raw functions to silence basal levels of the epidermal AP-1 transcription factor. Furthermore, we show biochemical data that activated phospho-Jun accumulates to high levels in raw and raw basket mutant embryos, about 2.5 fold higher than wild type. Since Jun is active in raw mutants even in the absence of basket, this indicates that another kinase is responsible for activating the AP-1 transcription factor. As Raw does not act through the JNK signaling negative feedback loop involving the MAPK, Puckered, our findings indicate Raw functions in a previously unrecognized JNK/AP-1 regulatory system. To better understand this regulatory system, here we test: (1) whether the ectopic dpp expression in raw mutants is due to Jun mislocalization, and (2) that a MAPK other than the basket-encoded JNK activates Jun in the epidermis of raw mutants.
POSTER: Cell Biology & Signal Transduction
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

Integrative Biology, Rutgers, The State University of New Jersey, Camden, NJ 08102.

Tissue patterning and cell fate determination are mediated by a handful of signaling pathways. The Bone Morphogenetic Protein (BMP) is one such pathway, which is an essential regulator of tissue development throughout the animal kingdom. In D. melanogaster, early BMP signaling is found exclusively along the anterior-posterior boundary of the two-dimensional monolayer of follicle cells (FCs), which surround the developing oocyte. The type I BMP receptor, thickveins (tkv), regulates this pattern; however, Tkv is not sufficient to transduce signaling, so we focused on the type II receptor, wishful thinking (wit). We found that WIT is expressed in an evolutionarily conserved pattern in the FCs of numerous Drosophila species, which overlaps the BMP signaling domain. Of importance, null clones of WIT coincide with a cell autonomous loss of BMP signaling and, consequently, modification in downstream gene patterning. Furthermore, we found WIT to be self-regulated by a positive feedback loop, which we demonstrate works to sharpen the BMP signaling gradient. Previous studies have shown WIT to regulate BMP signaling only during neurogenesis. However, for the first time, our results establish a role for WIT in non-neuronal tissue and demonstrate WIT as an active type II receptor during FC patterning.

192C

Cell fate determination and cellular homeostasis are finely regulated through a balance of many different signaling cascades that ultimately results in either cell division, cell growth or cell differentiation. Misregulation of cell signaling at various levels is often at the origin of uncontrolled malignant cell growth. Furthermore one of the hallmarks of cancer is self-sufficiency in the production of mitogenic signal, often coupled with cell inability to arrest the cell cycle and prevent mitosis. Recent studies carried out using an unbiased forward genetic approach in the fruitfly Drosophila melanogaster defined a new class of Tumor Suppressor Genes (TSGs) that encode for components of the Endosomal Sorting Required for Transport (ESCRT) endocytic sorting pathway. After endocytic internalization vesicles carrying cargo proteins, including signaling receptors fuse to early endosomes. There cargoes to be sorted by ESCRT proteins toward lysosomal degradation are internalized in the forming the Multi Vesicular Bodies (MVBs). MVBs eventually fuse with lysosomes, whereas cargoes are degraded. Consistent with the ability of ESCRT to degrade signaling receptors, ESCRT mutant cells fail to differentiate and to polarize, and grow uncontrollably forming tumors. To ward the identification of the missorted receptors responsible for the tumor phenotypes of ESCRT mutant cells, we set out to determine the proteome of cells deficient for MVB sorting. To this end we combined a set of the art proteomics approach (Stable Isotope Labeling Amino acid in Cell culture (SILAC)) with Drosophila genetics. SILAC is a metabolic protein labeling technique that allow both quantitative and qualitative mass spectrometry analysis. We are applying this technique both in fly cell culture and in vivo in fully labeled animals and at the meeting we will discuss our progress.

193A
The role of V-ATPase in Notch trafficking and signaling activation. Serena Duchi, Thomas Vaccari. IFOM - FIRC Institute of Molecular Oncology.

Evidence indicates that endosomal entry promotes signaling by the Notch receptor, but the mechanisms involved are not clear. In a search for factors that regulate Notch activation in endosomes, we isolated mutants in Drosophila genes that encode subunits of the vacuolar ATPase (V-ATPase) proton pump. V-ATPase mutant cells internalize Notch and accumulate it in lysosomes, and show a strong loss of Notch activation in endosomes. V-ATPase activity is required in signal-receiving cells for Notch signaling downstream of ligand activation but upstream of /g534-secretase-dependent S3 cleavage. These data indicate that V-ATPase, promotes not only the degradation of Notch in lysosomes but also the activation of Notch signaling in endosomes. Interestingly, lack of V-ATPase activity prevents the ectopic Notch signaling activity observed in mutants that fail to degrade the Notch receptor. By virtue of their ability to sustain pathologic Notch signaling in Drosophila, V-ATPase could be at least in principle therapeutic target for human cancers that display ectopic Notch signaling, such as breast and lung cancer and leukemias. To ascertain the consequence of the V-ATPase mutation of Notch signaling in in mammals, we assayed Notch signaling activation in human normal anf cancer breast cells in culture. We found that, similar to inhibitors of Notch cleavage, drugs that impair V-ATPase function (such as Bafilomycin and Concanamycin) reduce strongly Notch signaling activation. Overall, our results underline the existence of a conserved tightly controlled balance between endosomal trafficking, sorting and degradation of the Notch receptor that is critical to maintain physiologic levels of signaling and prevent tumorigenic Notch signaling. We hope in the future to develop strategies to pharmacologically alter the endocytic control of Notch signaling to counteract the ectopic activation frequently associated to Notch tumors.

194B
Identification of GAP proteins regulating RAB11 during development. Carl Lalamme, Jonas Dorn, Paul Maddox, Gregory Emery. Research Institute for Immunology and Cancer (IRIC), Montreal, Quebec, Canada.

Endocytosis is a key mechanism to control the plasma membrane expression of receptors and molecules involved in cell signalling. Internalized proteins are either degraded or redirected to the plasma membrane by trafficking through the so-called recycling endosome. Consequently, the small GTPase Rab11 that regulates this later trafficking pathway is an important regulator of cell signalling. In particular, we have recently involved Rab11 in the regulation of Notch signalling during asymmetric cell division and in the spatial regulation of Receptor Tyrosine Kinase during border cell migration. As other small GTPases, Rab11 cycles between an active GTP-bound state and an inactive GDP-bound state. GTPase activating proteins (GAPs) are important regulators of Rab proteins: they promote the hydrolysis of GTP into GDP and thus inactivate the Rab protein. We hypothesize that GAPs proteins acting on Rab11 are potentially important regulators of signaling events requiring trafficking through the recycling endosome. By using a combination of colocalization and functional experiments in S2 cells, we identified 3 candidate GAP proteins for Rab11. Interestingly, we found in preliminary experiments that one of those GAP colocalizes with Rab11 during asymmetric cell division. Furthermore, we found that the downregulation of another GAP phenocopies rab11 loss of function during border cell migration. Those preliminary results suggest that different GAP proteins could specifically regulate Rab11 in vivo during diverse developmental signalling events.

195C
A gradient of apical endocytosis shapes the apical surface of the early embryo. Aleksandar S. Necakov, Piotr Fabrowski, Stefano De Renzis. Developmental Biology Unit, The European Molecular Biology Laboratory, Heidelberg, Germany.

Apical endocytosis is a post-temporal modulation of membrane trafficking plays an important role in controlling cell morphology and behavior. We have developed a modified form of Total Internal Reflection Fluorescence microscopy (TIRFM) that has allowed us to visualize for the first time both endocytic and exocytic events at the apical plasma membrane during embryonic development. Our data reveal the presence of a temporal gradient of apical endocytosis that underlies the remodeling of the apical plasma membrane during cellularization. Using a combination of genetic and pharmacological approaches we demonstrate that endocytosis is essential for the clearance of filopodial-like membrane protrusions from the apical surface. Conversely, interfering with exocytosis inhibits protrusion formation and results in widespread membrane blebbing. We propose that membrane dynamics underlie the biogenesis of membrane protrusions and that the rate of both endocytosis and exocytosis is a critical factor in determining the length of cellular protrusions during cell communication. We are currently addressing the implications of our findings with respect to the signaling systems and membrane remodeling events that operate during gastrulation.

196A
Phosphoinositide roles in muscle membrane compartmentalization and remodeling. Ines Ribeiro, Amy Kiger. Division of Biological Sciences, University of California, San Diego, La Jolla, CA.

Muscle cells, called myofibers, must maintain a highly compartmentalized plasma membrane, or sarcolemma, with cell remodeling during development and with use. The sarcolemma contains spatially restricted integrin adhesions that maintain myofiber attachments and sites of union with the transverse tubule (T-tubule) membranes, an internal membrane network continuous with the sarcolemma critical for excitation-contraction coupling. Although membrane trafficking is an important mechanism for the maintenance
and remodeling of plasma membrane organization in many cell types, little is known about the mechanisms that regulate sarcolemmal compartmentalization in muscle. Phosphoinositide lipids, under the control of dedicated phosphatases and kinases, convey transient identity to trafficking membrane compartments. We found that both integrin adhesions and t-tubules are remodeled upon myofibril turnover in abdominal persistent larval muscles during metamorphosis and that this remodeling requires myostatin (mtn) phosphoinositide phosphatase. We determined that Class II PI3-kinase, PI3K68D, and Mtn co-regulate PI(3,5)P2 involved in integrin relocalization from endosomal-related compartments to the sarcolemma necessary for myofiber attachments, but are independently required for T-tubule remodeling. Interestingly, PI3K68D from pupal thorax lysates co-immunoprecipitated discs large (Dlg), a membrane associated guanylate kinase scaffold protein localized at T-tubules and neuromuscular junctions, suggesting involvement in a common pathway. Moreover, the localization and function of Rab6 GTase, implicated in unconventional secretion in yeast, reveals an intrinsic trafficking pathway underlying sarcolemma membrane organization. Our results indicate that regulation of distinct phosphoinositide pools plays a central role in maintaining cell compartmentalization during muscle remodeling, with likely roles for integrin, Dlg and Class II PI3-kinase in MTM cellular pathways.

197B
Chmp1 protein controls wing vein development. Meagan Valentine1, Maiyun Park2, Simon Collier1. 1) Marshall University, Huntington, WV; 2) Marshall University School of Medicine, Huntington, WV.

Chmp1A is a conserved protein and a component of ESCRT-III (Endosomal Sorting Complex Required for Transport), a complex required for the recycling and degradation of activated receptor proteins. Chmp1A has been linked to pancreatic cancer in humans, as pancreatic tumors have reduced Chmp1A expression, and work in zebrafish and mammalian cell culture has shown that Chmp1A knockdown results in tumor formation and accelerated cell growth, respectively. In light of these studies, Chmp1A has been classified as a tumor suppressor. In Drosophila, there is a single Chmp1 protein [encoded by the Chmp1 gene (CG4108)] that is homologous to vertebrate Chmp1A. To date, no studies have been published on Chmp1 in Drosophila. We have previously shown, through knockdown and over-expression studies in the wing, that Chmp1 may regulate the EGFr pathway, Notch signaling, and the Frazzled (Fz) Planar Cell Polarity Pathway (PCP). We have shown that Chmp1 interacts with PCP protein Strabismus (Stbm), suggesting that Chmp1 regulates PCP through an interaction with Stbm. Additionally, Chmp1 knockdown and over-expression result in over-sized wing veins and widening of the distal tip of wing veins, respectively. Both phenotypes could be attributed to either Notch or EGFr signaling, as they are interactive in regulating the size of wing veins. It is likely that Chmp1 regulates both pathways via its involvement in ESCRT, since both Notch and EGFR require ESCRT for proper signaling. To ascertain if Chmp1 regulates EGFr or Notch signaling, we have begun wing disc staining to observe the effects of Chmp1 knockdown and over-expression on components downstream of EGFr (e.g. Blistered and dpt) and Notch [e.g. E(spl)]. Using a tagged Chmp1 protein, we are also looking at Chmp1 localization in Drosophila. Assuming Chmp1 function is conserved, we expect it to localize to both the endosome and condensed chromatin, as it does in vertebrates. We will drive tagged Chmp1 expression in the embryo to look for endosomal localization, and in the salivary gland to look for localization to condensed chromatin.

198C
Non-autonomous and context-dependent control of apoptosis by deregulated Hedgehog signaling. Andreas Bergmann, Yun Fan, Tian Ding, Audrey Christiansen. Dept Biochem & Molec Biol, MD Anderson Cancer Ctr, Houston, TX.

Hedgehog (Hh) signaling is an important signaling pathway for development and homeostasis. Deregulated, i.e. increased Hh signaling can give rise to disease including cancer. In Drosophila and mammals, deregulated Hh signaling causes excessive cell proliferation leading to overgrowth and tumor phenotypes. However, here we show in eye imaginal discs that deregulated Hh signaling caused by loss of the negative regulators coxl, ptc and pka also promotes cell survival by increasing apoptosis resistance. Surprisingly, it is not the cells with deregulated Hh activity that have increased apoptosis resistance, instead, these mutant cells promote apoptosis resistance of neighboring wild-type cells. This non-autonomous effect is mediated through Hh-induced accumulation of the protein levels of Drosophila Inhibitor of Apoptosis-1 (DIAP-1) in neighboring wild-type cells, conferring apoptosis resistance. This activity is context-dependent and occurs only in and anterior to the morphogenetic furrow in the eye disc. The posterior part of the eye disc is inert to deregulated Hh activity with respect to DIAP-1. We also observe non-autonomous and context-dependent up-regulation of DIAP-1 by deregulated Hh activity in wing imaginal discs. In summary, we demonstrate that deregulated Hh signaling not only promotes proliferation, but also cell survival of adjacent tissue. Potentially, in humans a similar non-autonomous effect on apoptosis by deregulated Hh signaling may be needed to generate a supportive micro-environment for tumor growth.

199A
Ihog and Boi are essential for Hedgehog signaling in Drosophila. Darius Camp1,2, Ko Currie1, Alain Alain Labbé1, Donald van Meyel3, Frédéric Charmon1,2. 1) Experimental Medicine, McGill University, Montreal, QC, Canada; 2) Molecular Biology of Neural Development, IRCM, Montreal, QC, Canada; 3) Centre for Research in Neuroscience, MUHC, Montreal, QC, Canada.

Background: The Hedgehog (Hh) signaling pathway is important for the development of a variety of tissues in both vertebrates and invertebrates. The molecular signaling mechanism underlying the function of Hh is not fully understood. In Drosophila, Ihog (Interference hedgehog) and Boi (Brother of Ihog) are related transmembrane proteins of the immunoglobulin superfamly (IgSF) with orthologs in vertebrates. Both members of this IgSF subfamily have been shown to bind Hh and promote pathway activation but their exact role in the Hh signaling pathway has remained elusive. To better understand this role in vivo, we generated loss-of-function mutations of the ihog and boi genes, and investigated their effects in developing eye and wing imaginal discs. Results: While mutation of either ihog or boi alone had no discernible effect on imaginal tissues, cells in the developing eye disc that were mutant for both ihog and boi failed to activate the Hh pathway, causing severe disruption of photoreceptor differentiation in the retina. In the anterior compartment of the developing wing disc, where different concentrations of the Hh morphogen elicit distinct cellular responses, cells mutant for both ihog and boi failed to respond at activation thresholds of Hh signaling. They also lost their affinity for neighboring cells and aberrantly sorted out from the anterior compartment of the wing disc into posterior territory. We found that ihog and boi are required for the accumulation of Hh signaling mediator Smoothened (Smoo) in Hh-responsive cells, providing evidence that Ihog and Boi act upstream of Smoo in the Hh signaling pathway. Conclusions: The consequences of boi;ihog mutations for eye development, neural differentiation and wing patterning phenocopy those of smoo mutations and uncover an essential role for Ihog and Boi in the Hh signaling pathway.

200B
G protein-coupled receptor kinase 2 promotes high-level Hedgehog signaling by regulating the active state of Smoo through kinase-dependent and kinase-independent mechanisms in Drosophila. Yongbin Chen1, Shuang Li1, Chao Tong1, Yun Zhao1, Bing Wang1, Yajuan Liu2, Jin Jiang1. 1) Department of Developmental Biology, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75390, USA; 2) Markey Cancer Center, University of Kentucky, Lexington, Kentucky 40536, USA.

G protein-coupled receptor kinase 2 (Gprk2/GRK2) plays a conserved role in modulating Hedgehog (Hh) pathway activity, but its mechanism of action remains unknown. Here we provide evidence that Gprk2 promotes high-level Hh signaling by regulating Smoothened (Smoo) conformation through both kinase-dependent and kinase-independent mechanisms. Gprk2 promotes Smoo activation by phosphorylating Smoo C-terminal tail (C-tail) at Ser741/Thr742, which is facilitated by PKA and CK1 phosphorylation at adjacent Ser residues. In addition, Gprk2 forms a dimer/oligomer and binds Smoo C-tail in a kinase activity-independent manner to stabilize the active Smoo conformation, and promotes dimerization/oligomerization of Smoo C-tail. Gprk2 expression is induced by Hh signaling, and Gprk2-Smoo interaction is facilitated by PKA/CK1-mediated phosphorylation of Smoo C-tail. Thus, Gprk2 forms a positive feedback loop and acts downstream from PKA and CK1 to facilitate high-level Hh signaling by promoting the active state of Smoo through direct phosphorylation and molecular scaffolding.

201C
Temporal regulation of lipid-droplet transport via Helio. Gurpreet K Arora, Susan L Tran, Nicholas P Rizzo, Michael A Welte. Department of Biology, University of
Bidirectional transport along microtubules is essential for proper spatial and temporal distribution of cellular components. Regulated back-and-forth motion of lipid droplets, powered by kinesin-1 and cytoplasmic dynein, in *Drosophila* embryos provides an excellent *in vivo* system to understand how motors are controlled to achieve net transport. Net transport is temporally regulated in three distinct phases (I, II, and III), and the key factor controlling this timing is the novel protein Halo. Halo is transiently expressed in Phase II where biophysical measurements show that Halo upregulates kinesin and downregulates dynein activity on lipid droplets. Other cargos are unaffected by Halo. This cargo specificity is likely due to Halo’s intracellular distribution: we find that the protein is enriched on lipid droplets, both by immunolocalization in centrifuged embryos and by Western analysis of purified droplets. Halo physically interacts with both kinesin-1 and dynein, providing a rationale how one regulator controls motion in both directions. Halo likely acts on motors and droplets via other proteins; however, the known transport regulators Klar, LSD-2 and Sfo are dispensable for Halo’s droplet localization or physical interactions with motors. Previous physical interaction screens have suggested a number of Halo partners. For one candidate, Protein Phosphatase 2A (PP2A), we show that it indeed co-immunoprecipitates with Halo from embryo lysates. In addition, reduced expression of either Halo or PP2A results in delayed droplet transport in Phase II. We are now investigating whether Halo recruits PP2A to droplets or regulates PP2A activity. Halo protein levels drop precipitously in Phase III. We find that this rapid downregulation is disrupted in embryos impaired for either Drop-out or Dappled/Wech function. In such embryos, net droplet transport is disrupted in Phase III, suggesting that regulated Halo turnover is crucial for proper control of transport.

**202A Schizo/Loner functions as an unconventional GEF for the Arf1-GTPase.** Verena Groth, Christine Dottermusch, Renate Renkawitz-Pohl, Susanne-Filiz Önel. Philipps-Universität Marburg, Marburg, Hessen, Germany.

The *Drosophila* body wall muscles are multicellular syncytia formed by successive fusions between so-called founder cells (FCs) and fusion competent myoblasts (FCMs). The fusion process itself requires basic events of developmental biology such as cell-cell recognition, adhesion and remodeling of the plasma membrane. Hereby, the recognition and adhesion of FCs and FCMs is mediated by transmembrane molecules of the immunoglobulin superfamily (IgSF). In *Drosophila* S2-cells the localization of the guanine nucleotide exchange factor (GEF) Schizo/Loner seems to depend on the IgSF members Dumbounded (Dsu/Krire) and its parologue Roughset (Rst/Inre). The GEF Schizo mediates the GDP-to-GTP exchange on Arf1-GTPases. We aimed to rescue schizo mutants by expressing the activated form of each of the three *Drosophila* Arf1-GTPases daArf, daArf1 or daArf2. Only daArf1 was able to partially restore the muscle pattern in schizo mutant embryos, indicating that daArf1 acts downstream of Schizo during myoblast fusion. Aiming to identify potential Schizo interaction partners we have performed a yeast-2-hybrid screen. We will present first interaction partners and discuss their role for myoblast fusion.

**203B Kosh accumulates at the prefusion complex stage during *Drosophila* myoblast fusion.** Christina Hornbruch1, Barbara Griemert1,2, Detlev Buttgerisi, Renate Renkawitz-Pohl1.

1) Developmental Biology, Philipps-Universität Marburg, 35043 Marburg, Germany; 2) Institute for Biochemistry, Justus-Liebig-Universität, 35392 Giessen, Germany.

Somatic myoblast fusion in *Drosophila* consists of two phases: the first one leads to trinucleated precursor cells while the second phase proceeds to the syncytial myoblast. To build such a myotube two cell types are needed: founder cells (FCs) and fusion competent myoblasts (FCMs), which come in close proximity, adhere and fuse. This process depends on the formation of a protein complex named Fusion-Restricted Myogenic-Adhesive Structure (FuRMAS), which contains a local F-actin accumulation at the sites of cell-cell contact. Within these FuRMAS, Kosh, a putative Calcium-binding protein, is expressed solely on the site of FC and accumulates in foci. These foci colocalize with F-actin, but they appear more transient. Based on electron microscopy studies it is known that after adherence between precursor cell and FCM so-called electron-dense vesicles appear at the side of fusion that are part of the prefusion complex, which leads to the formation of electron-dense plaques. Afterwards, membrane vesiculation starts and a fusion pore is built to integrate the FCM into the growing myoblast. To address the question, during which step of myogenesis Kosh is involved, Kosh distribution in a number of fusion mutants was analysed: sns mutants reveal that the enrichment of Kosh requires successful cell adhesion between FCMs and FC/growing myotubes and signaling by the cytoplasmic domain of Sns. Instead of that Kosh accumulates in sing mutants, which arrest fusion establishment after completion of prefusion complexes. blow mutants, which stop myogenesis beyond the prefusion complex stage, show reduced Kosh foci. In loss-of-function mutants for Kette, Wip or Ap3, which are known as actin regulators, Kosh foci are transient like in wild-type embryos. Therefore we hypothesize, that Kosh might act as a regulator leading to eocytosis of electron-dense vesicles to resolve fusion complexes during myoblast fusion.

**204C Sbf pseudophosphatase coregulates PI(3)P homeostasis and Rab21 activity in endocytic control of dynamic hemocyte shape.** Steve Jean, Sarah Cox, Amy Kiger. Division of Biological Sciences, University of California, San Diego, California, San Diego, CA.

The specific combination of different lipid phosphoinositides and Rab GTPases define and control membrane compartment identities and trafficking. It is therefore critical that localized regulation is coordinated between the proper phosphoinositide kinases and phosphatases, and the appropriate Rab stimulatory and inhibitory factors. Myotubulins (MTMs) encode for phosphoinositide phosphatases, with some members curiously encoding catalytically inactive or ‘pseudo’-phosphatases. In humans, mutations in either the MTM2 catalytic phosphatase or MTMR13 pseudophosphatase are associated with similar diseases, indicating that pseudophosphatases are not simply pseudogenes and may functionally collaborate with other MTM members in shared pathways. We found that *Drosophila* MTM pseudophosphatase, Sbf, plays a central role in policing membrane traffic control by co-regulating Class II PI3-kine (PI3Kδ/δ), Mtm phosphatase and Rab21 functions in endocytosis. We show that Sbf is necessary and sufficient to recruit Mtm protein to promote turnover of a PI(3)P pool and endocytic recycling important for hemocyte cell protrusions, antagonistic to PI3Kδ/δ. Surprisingly, we found that Sbf has a second independent role through physical interactions with PI3Kδ/δ to promote lyssosomal influx, here acting antagonistic to Mtm. The shared phenotypes and physical interactions suggest that Sbf scaffolds both Class II PI3-kine and Mtm phosphatase to balance PI(3)P-mediated functions. Furthermore, we found that Sbf directly interacts with Rab21 GTPase, previously implicated in endocytic recycling. Sbf showed preferential binding with GDP-bound Rab21, indicating potential Sbf stimulatory Rab21 GEF activity. Moreover, Rab21 and Sbf genetically interact to promote hemocyte cell protrusions. We showed that Sbf is a critical central regulator of membrane compartment identities through the coordination of PI(3)P cycles and Rab21 activity, controlling distinct endocytic trafficking functions in hemocytes and with implications in human disease.

**205A Identification of nuclear localization mechanisms of the cell polarity regulator Bazooka.** Michael P Krahm, Andreas Wodarz. Department of Stem Cell Biology, University of Goettingen, Germany.

The PAR (partitioning-defective) / aPKC (atypical protein kinase C)-complex is one of the key regulators of cell polarity which functions at the top of a hierarchy determining the apical plasma membrane domain in epithelial cells and the apical cortical domain in neural stem cells. Bazooka (Baz), one of the core components of the complex, functions as a scaffold to recruit the serine/threonine aPKC and its regulator PAR-6 to the cortex. In addition to their cortical localization, aPKC and PAR-6 have been detected in the nucleus of mammalian cells. Nuclear phosphorylation targets of aPKC have been identified, but a nuclear function of PAR-6 remains elusive. PAR-3, the mammalian homologue of Baz, was also found in the nucleus and has been assigned a function in DNA double strand break repair. However, no further investigation of potential nuclear functions of Baz/PAR-3 has been performed so far. Here we describe the mechanisms, which are implicated in the nuclear import and export of Baz and present an experimental set-up by which the nuclear function of Baz can be tested independently of its cortical localization. We identified three functional nuclear import signals in Baz, which are also conserved in human PAR-3. Vice versa, we detected two nuclear export signals. Deletion of both motifs leads to accumulation of Baz in the nucleus. Interestingly, only one of these motifs is sensitive to Leptomycin B, a drug, which inhibits Exportin-1-mediated nuclear export, indicating that Baz can also be shuttled out of the nucleus by a different mechanism. Nuclear accumulated Baz is capable to associate with the chromosomal DNA and we are currently testing to which regions of the genome it can bind by Chip-Seq and whether this binding is direct or indirect via interaction partners. Finally, we inactivated all nuclear import signals in a GFP-tagged Baz construct and established transgenic flies, which are now
suggest that the Dlg5 functions in intracellular trafficking to regulate cell migration and cell survival. Overexpressed GFP-Dlg5 fusion protein partially colocalizes with several Rab markers. These results and kidneys, and also required for polarization of citron kinase in mitotic neural precursors. Here we reported the genetic analysis using dlg5 mutants and in vivo RNAi. Our cell junction assembly, apical-basal polarity, signaling and cell proliferation. It has been reported that mouse Dlg5 is required for epithelial tube maintenance in mammalian brain and kidneys, and also required for polarization of citrin kinase in mitotic neural precursors. Here we reported the genetic analysis using dlg5 mutants and in vivo RNAi. Our results show that dlg5 is required for border cell migration and follicle cell survival. Dlg5 antibody staining and genomic RFP tagging transgenic flies indicate that Dlg5 is expressed in Drosophila ovaries and localized in vesicle-like structures. Overexpressed GFP-Dlg5 fusion protein partially colocalizes with several Rab markers. These results suggest that the Dlg5 functions in intracellular trafficking to regulate cell migration and cell survival.

Border cell migration during Drosophila oogenesis is an excellent model system for investigating the genetic requirements for cell migration in vivo. In a P-element based loss-of-function screen to identify new genes required in border cells migration, we found Dlg5, also named as CG6509, as an essential gene for migration. Dlg5 is conserved between Drosophila and vertebrate but is distinct from the Dlg subfamily members. It contains an N-terminal coiled-coil domain, four PDZ domains, a Src homology (SH) 3 domain, and a guanylate kinase domain. It is a member of the MAGUK (membrane-associated guanylate kinase) super family proteins that mainly function as scaffolding proteins involved in cell junction assembly, apical-basal polarity, signaling and cell proliferation. It has been reported that mouse Dlg5 is required for epithelial tube maintenance in mammalian brain and kidneys, and also required for polarization of citrin kinase in mitotic neural precursors. Here we reported the genetic analysis using dlg5 mutants and in vivo RNAi. Our results show that dlg5 is required for border cell migration and follicle cell survival. Dlg5 antibody staining and genomic RFP tagging transgenic flies indicate that Dlg5 is expressed in Drosophila ovaries and localized in vesicle-like structures. Overexpressed GFP-Dlg5 fusion protein partially colocalizes with several Rab markers. These results suggest that the Dlg5 functions in intracellular trafficking to regulate cell migration and cell survival.

Klar interacts physically with Kinesin-1 and Dynemin and is involved in pole plasm assembly during oogenesis. Michael A. Welte1, Yanxun V. Yu1, Sean L. Cotton2. 1) Dep Biol, Univ Rochester, Rochester, NY; 2) Dep Biomed Eng, Boston Univ, Boston, MA. Microtubule motors play critical roles in transporting and positioning many intracellular cargoes, from protein complexes to entire nuclei, but how they are regulated remains largely unknown. The Klar protein is widely expressed throughout Drosophila development and controls a number of intracellular transport processes. In the two best-studied instances (motion of embryonic lipid droplets and of photoreceptor nuclei), Klar is present on the cargo whose transport it controls. It has been proposed that Klar either anchors motors to the cargo or increases transport efficiency by coordinating opposing motors. However, no physical or functional connections between Klar and motors have been demonstrated. We find that in oocytes Klar is present in distinct puncta that accumulate in a posterior crescent in stages 9 and 10. Posterior Klar accumulation does not depend on formation of pole plasm since Klar localization is normal in oocytes lacking either Oskar or Staufen protein. Klar accumulation, however, requires a correctly polarized microtubule network and motor activity: In the absence of Klar, Staufen and Vasa particles can be detected away from the oocyte surface, consistent with a delay in transport. When Kinesin-1 function is compromised, the ectopic localization is enhanced in both penetrance and severity. These defects do not simply reflect altered microtubule organization since posterior accumulation of Klar-1 is minimally affected. We propose that Klar allows Klar-1 to efficiently transport or anchor pole plasm components.

Guidance Receptor Degradation Is Required for Neuronal Connectivity in the Drosophila Nervous System. W. Ryan Williamson1, Taehong Yang2, Jonathan R. Terman2, P. Robin Hiesinger2, Juicy Chen. Model Animal Research Center, Nanjing University, Nanjing, China. Axon pathfinding and synapse formation rely on precise spatiotemporal localization of guidance receptors. However, little is known about the neuron-specific intracellular trafficking mechanisms that underlie the sorting and activity of these receptors. Here we show that loss of the neuron-specific v-ATPase subunit a1 leads to progressive endosomal guidance receptor accumulations after neuronal differentiation. In the embryo and in adult photoreceptors, these accumulations occur after axon pathfinding and synapse formation is complete. In contrast, receptor misorting occurs sufficiently early in neurons of the adult central nervous system to cause connectivity defects. An increase of guidance receptors, but not of membrane proteins without signaling function, causes specific gain-of-function phenotypes. A point mutant that promotes sorting but prevents degradation reveals spatiotemporal specific guidance receptor turnover and accelerates developmental defects in photoreceptors and embryonic motor neurons. Our findings indicate that a neuron-specific endosomally degradation mechanism is part of the cell biological machinery that regulates guidance receptor turnover and signaling. (References: Williamson WR, Wang D, Herberman AS, Hiesinger PR (2010) A dual function of V0-ATPase a1 provides an endosomal degradation mechanism in Drosophila photoreceptors. J Cell Biol. 189: 885-899. Williamson WR, Yang T, Terman JR, Hiesinger PR (in press) Guidance receptor degradation is required for neuronal connectivity in the Drosophila nervous system. PLoS Biology.)


**POSTER: Cell Biology & Signal Transduction**

See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

JAK signaling is involved in many processes during development, including embryogenesis, immune system development, and aging. As the first step of triggering the signaling, three ligands have been identified in the pathway: Upd, Upd2 and Upd3. The sequence similarity among the three proteins is very low, and only 6 conserved blocks with unknown function are shared, therefore the relationship of the ligands to each other is not known. The necessity of Upd to stimulate JAK signaling has been verified in many developmental processes, whereas evidence also suggests Upd is not sufficient to fully regulate the signaling. Both Upd2 and Upd3 are involved in the signaling regulation, but how they cooperatively function with Upd is not clear yet. upd2 mutants are viable with no visible defect. However, only limited work has been carried out to investigate the function of this protein and the factors required for its dimerization. Here we describe a structure/function analysis of Dome. We have generated a series of mutations and deletions which define the domains within the Dome intracellular region required for binding of Hopscotch and STAT92E and demonstrate the effects of these on pathway activation and receptor post-translational modification in response to ligand. Interactions of the SOCS3/E negative regulator will also be presented. In addition, previous reports have demonstrated that Dome forms homodimers in vivo to transduce canonical pathway signalling; this dimerization only occurs in a subset of tissues, but takes place independently of pathway ligand. We have carried out a genome-wide RNA interference (RNAi) screen at the Sheffield RNAi Screening Facility (SRSF) to identify genes required for Dome dimerisation. This approach, using a bi-molecular split β-galactosidase complementation assay, has identified a number of gene ontologies and candidate genes responsible for both the dimerisation and stability of Dome. Characterisation of these loci and their requirement for JAK/STAT pathway activity and Domeless trafficking will be presented.

**212B A molecular characterisation of the JAK/STAT pathway receptor Domeless.** Katherine H Fisher1, Wojciech Stec1, Amy Taylor1,2, Stephen Brown1,2, Martin P Zeidler1. 1) The MRC Centre for Developmental & Biomedical Genetics, Dept of Biomedical Science, University of Sheffield, UK; 2) Sheffield RNAi Screening Facility, Dept of Biomedical Science, University of Sheffield, UK.

The JAK/STAT signalling pathway is a mediator of tumourigenesis in various human cancers playing a key role in the development of multiple solid tumours, as well as a wide range of haematopoietic neoplasia. The Drosophila JAK/STAT pathway represents a lower complexity pathway that retains functional homology to vertebrate systems in its requirement for immunity, haematopoiesis and stem cell maintenance. By contrast to vertebrate systems, comprised of multiple cytokine receptors, all three Drosophila JAK-like molecules signal through a single receptor termed Domeless (Dome). However, only limited work has been carried out to investigate the function of this receptor and the factors required for its dimerisation. Here we describe a structure/function analysis of Dome. We have generated a series of mutations and deletions which define the domains within the Dome intracellular region required for binding of Hopscotch and STAT92E and demonstrate the effects of these on pathway activation and receptor post-translational modification in response to ligand. Interactions of the SOCS3/E negative regulator will also be presented. In addition, previous reports have demonstrated that Dome forms homodimers in vivo to transduce canonical pathway signalling; this dimerization only occurs in a subset of tissues, but takes place independently of pathway ligand. We have carried out a genome-wide RNA interference (RNAi) screen at the Sheffield RNAi Screening Facility (SRSF) to identify genes required for Dome dimerisation. This approach, using a bi-molecular split β-galactosidase complementation assay, has identified a number of gene ontologies and candidate genes responsible for both the dimerisation and stability of Dome. Characterisation of these loci and their requirement for JAK/STAT pathway activity and Domeless trafficking will be presented.

**213C Regulation of a STAT signaling circuit that mediates border cell determination and migration.** Amanda J Monahan, Michelle Starz-Gaiano. University of Maryland, Baltimore County, Baltimore, MD.

In many developmental processes, gene expression is controlled by gradient-dependent activation of positive and negative regulators. During Drosophila oogenesis, such a gradient-contingent regulatory circuit is necessary for the induction and migration of specialized follicle cells called the border cells. About mid-oogenesis, a group of anterior somatic follicle cells are fated to be the migratory portion of the border cell cluster. This cluster, including a pair of non-migratory polar cells, migrates through the egg chamber’s germ line to the oocyte border. The polar cells secrete Unpaired (UPD), which specify and maintain the motile cell population. UPD is a ligand for the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway. Two crucial genes downstream of STAT signaling are slow border cells (slbo) and apoptic (apt), both also transcriptional regulators. Previously, STAT was shown to induce both slbo and apt, which mutually inhibit each other, with SLBO promoting border cell formation and migration, and APT inhibiting migration through its repression on both slbo and STAT. APT’s mechanism of action to inhibit STAT signaling is not well characterized and not believed to be direct. We are focused on determining if APT regulates a direct inhibitor of STAT activity. We chose suppressor of cytokine signaling36e (socs36e) as a candidate gene to be a component of the STAT/SLBO/APT regulatory circuit. The SOCS protein family has been shown to work in a negative feedback loop on STAT signaling. We determined that socs36e expression in the egg chamber corresponds to STAT activation. We also confirmed that over-expression of socs36e, using a border cell-specific GAL4 driver, induces a border cell migration defect. Through genetic techniques, immunohistological analysis, and biochemical assays, we are working on characterizing socs36e’s role in border cell fate and APT’s potential role in socs36e regulation. Our preliminary analysis supports socs36e as a potential addition to the JAK/STAT genetic circuit involved in determining border cell fate and motility.

**214A The Distribution of the JAK/STAT Ligand Unpaired (Upd) During Oogenesis.** Dustin W. Perry, Travis R. Sexton, Douglas A. Harrison. Dept Biology, University of Kentucky, Lexington, KY.

Morphogens are molecules that can directly specify different cell fates in a concentration-dependent fashion. During Drosophila development, much focus has been given to the Wnt(Wg), Hedgehog(Hh) and TGF-β(Tdp) ligands and their abilities to behave as morphogens. Using a novel system in which to study morphogens, the Drosophila ovary, we report on another morphogen, Unpaired (Upd). Upd is part of the Unpaired ligand family that activates Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) signaling in Drosophila. Previous work with Upd showed that it is glycosylated, secreted, and bound to the extracellular matrix. Upd can be released from the ECM with heparin, suggesting that Upd may bind to Heparan Sulfate Proteoglycans (HSPGs). In the ovary, the JAK/STAT pathway is activated in a graded fashion and the amount of (JAK/STAT) signaling in Drosophila. Previous work with Upd showed that it is glycosylated, secreted, and bound to the extracellular matrix. Upd can be released from the ECM with heparin, suggesting that Upd may bind to Heparan Sulfate Proteoglycans (HSPGs). In the ovary, the JAK/STAT pathway is activated in a graded fashion and the amount of JAK signaling is sufficient to determine anterior follicular cell fates. Due to the juxtaposition of the germ line cells with the apical surface of the follicular epithelium the stability, retention and distribution of Upd are easy to follow. Antibody staining against the Upd protein shows that once released from its source in the ovary, there is a clear graded distribution of Upd along the apical surface of the epithelium. Most recently the functional analysis has revealed that loss of one of the four known HSPGs, Dally, causes a reduction in JAK signaling and extracellular Upd accumulation. Our data support a role for Dally in stabilization of the extracellular Upd, by retaining it to the ECM, preventing its degradation. The Drosophila ovum presents a novel model for morphogen signaling with distinct advantages for ligand tracking and manipulation of extracellular environment.

**215B The effect of Upd3 on stem cells in Drosophila testes.** Lingfeng Tang, Douglas Harrison. Department of Biology, University of Kentucky, Lexington, KY.

The Drosophila testes serves as a perfect model for investigating stem cells. Besides the sheath, the niche is only composed of 3 kinds of cells: hub cells, germline cells and somatic cells. At the tip of testes is the hub, which is composed of 9-12 cells. The germline stem cells (GSCs) and somatic stem cells (SSCs) directly attach to the hub. JAK/STAT activity which is activated by Upd, the ligand of the pathway, serves as the molecular niche of both GSCs and SSCs in the testes. It has been reported that loss of function of Stat92e leads to the loss of both GSCs and SSCs in testes, and ectopic activation of Stat92e leads to ectopic stem cell like germine cells. Upd activates SSCs directly, and then SSCs affect GSCs through TGF-beta signaling. Mutants for Upd3, another JAK pathway ligand, have recently been found to exhibit premature male reproductive senescence. The Upd3 mutant has testes with a larger diameter compared to wildtype. Because of the previously identified roles of JAK signaling in stem cell maintenance, we hypothesized that the upd3 mutation may have an effect on stem cells in the testes. Using molecular markers for testis cell types, our preliminary results show that the number of GSCs decreases and the number of SSCs increases as the wildtype fly ages. Compared to wildtype, the upd3 mutant has more GSCs but less SSCs, and the ratio of SSCs/GSCs is much lower in the mutant. Our results show that Upd3 is required for the maintenance of normal numbers of SSCs, suggesting that it contributes to the activation of JAK signaling in the niche.
Sequoia affects leading cell migratory behaviour in Drosophila trachea by regulating FGF levels. Sofia J. Araujo, Jordi Casanova. IBMB-CSIC, IRB Barcelona, C/ Baldiri Reixac, Barcelona, Spain.

Coordination and integration of cell changes during development enables organs to adapt their final function, shape and size to the proper performance of the full organism. Cells can respond to different signals by adopting different fates and/or changes in their properties and developmental programmes. The migratory ability and behaviour of each cell depends on extracellular signals and the sensing of its surroundings. The Drosophila tracheal system is a model to address this as its many features, in particular the migration of the tracheal cells, rely on a set of positional cues provided by their neighbours. At the ventral side of the embryo, a single terminal cell forms at the tip of each ganglionic branch (GB) which migrates towards the embryonic central nervous system (CNS). Here we report that the Sequoia (Seq) transcription factor, which is expressed in the nervous system, is responsible to restrict terminal fate to a single cell per GB. We show that in the GB of seq mutants some otherwise trailing cells do not follow the leading cell and also act as leading cells. Seq acts as a repressor of bnl and that ectopic expression of bnl in the ventral midline of the CNS mimics the seq phenotype. In agreement, we detect genetic interactions between seq and bnl and pointed (pnt) mutants. These data indicate that the extent of Bnl signal determines how many cells in the GB will adopt a terminal fate and controls how many cells adopt a leading migratory behaviour instead of acting as trailing cells.

Cytoskeletal polarization during collective cell migration in the Drosophila egg chamber. Maureen P. Cetera, Sally Horne-Badovinac. DRSB, University of Chicago, Chicago, IL.

Collective cell migration is critical for multiple developmental processes. The Drosophila egg chamber provides a novel system in which to study collective cell migration of an entire epithelial cell layer. During oogenesis, the egg chamber elongates from a spherical precursor to form a mature elliptical egg. At this time, the follicle epithelium migrates circumferentially around the egg chamber’s anterior-posterior (AP) axis (Hagio and Bilder). Previous work has demonstrated planar organization of actin filaments at the basal surface of the follicle cells, perpendicular to the AP axis. Our lab has found that, in addition to these actin filaments, actin-based protrusions extending from just one side of each cell are also uniformly polarized with sinistral or dextral chirality during the stages of rotation. Through live and fixed cell imaging, we have shown that these protrusions form the leading edge of the migrating cells, and that the VASP protein Enabled localizes to protrusion tips where it may function to regulate actin dynamics. Live imaging of GFP fusion proteins has also revealed Myosin II enrichment along filamentous actin at the trailing edge of the cells which may be relieving focal adhesions to allow forward migration. Additionally, we have uncovered an uncharacterized population of planar polarized microtubules at the basal follicle surface and are working to elucidate their role during the migratory process. These studies will provide insight into the cytoskeletal dynamics underlying a novel mode of collective cell migration.

Par-1 regulates the spatial activity of myosin in migrating border cells. Pralay Majumder, George Aranjuez, Jocelyn McDonald. Department of Molecular Genetics, Lerner Research Institute, Cleveland Clinic, Cleveland, OH.

Cell migration is vital for a variety of tissue remodeling processes, especially during embryonic development, wound healing and tumor metastasis. While the mechanisms regulating single cell migration are fairly well studied, less is known about collective (group) cell migration. To understand the underlying mechanism of collective cell migration, we are studying the genetically tractable migration of Drosophila border cells during ovarian development. Border cells are a group of 6-10 epithelial-derived cells that form a cluster at the anterior tip of the egg chamber (subunit of ovary) during late oogenesis and migrate collectively away from the follicular epithelium to the border of the oocyte. We have shown previously that Par1, a serine-threonine kinase implicated mostly in cell polarity, regulates border cell detachment and protrusions. Protrusions are essential for sensing guidance cues as well as providing the anchorage to pull the cluster forward. Non-muscle myosin II (myosin) has been previously shown to regulate border cell migration and protrusion morphology. Phosphorylation of the myosin regulatory light chain, called Spaghetti Squash (Squ), activates myosin. However, the regulation of Squ in border cells has yet to be examined in-depth. Live imaging of border cells mutant for squ or one of the known activating kinases, Rho-kinase (rok), show remarkably similar migration defects to those observed when border cells are mutant for par-1. Furthermore, we identify a robust genetic interaction between squ and par-1 and find that activated Squ strongly suppresses the phenotypes caused by loss of par-1. Activation of Squ by expressing different known regulators also rescues the par-1 mutant phenotype. We find that Par-1 regulates both phosphorylation of Squ and its spatial localization in migrating border cells. Moreover, functional GFP-tagged Squ exhibits a dynamic subcellular localization in live migrating border cells that is lost in par-1 mutants. Together, our data indicate that Par-1 regulates myosin dynamics in migrating border cells by activating and localizing Squ.

Molecular Changes that Enable Cell Rearrangement and Detachment from the Ovarian Epithelium. Lathiena Manning, Alvin Kennedy, Michelle Starz-Gaiano. Dept. of Biological Sciences, UMBC, Baltimore MD.

Cells that are mandated to travel a necessary distance for proper developmental function often start as part of a tightly connected epithelial layer. These cells must disconnect from their neighboring cells in order to fulfill a defined task at the new location. Prior to migration, cells in an epithelium exhibit a simple morphology with a distinct apical and basal polarity. Once designated as migratory, a cell begins to detach from an epithelium and migrates along a specific path. To do so, cell adhesions are weakened and cells are able to move from one location to another. Cells that detach as groups must remove their attachments from the epithelial layer while maintaining contact with the other migrating cells within the cluster, and may maintain apical-basal polarity. This study proposes to understand the molecular mechanisms that allow the reorganization of adhesions and the ability for cells to gain motility while in a cohesive group. During Drosophila oogenesis, a small population of migratory cells, referred to as border cells, detach from the anterior epidermis and migrate posteriorly toward the oocyte while remaining in a cluster. Border cells display the characteristics of collective cell migration as they move. We have found that border cell detachment and migration can be genetically separated and that detachment depends on proper control of STAT signaling. We are using a combination of genetic, immunohistological, and live-imaging approaches to define the changes in adhesive properties as cells disconnect from their neighbors. One adhesion molecule known to play a role in border cell migration is E-Cadherin, which mediates homophilic cellular adhesions. Our preliminary data implicates additional adhesion molecules such as E-Cadherin in border cell movement. In addition, we demonstrate a functional link between Rab11 and the exocyst complex. Finally, our data clearly show that Rab11 genetically interacts with the small Rho GTPase rac1. This suggests that Rab11 may directly regulate Rac1. We hypothesize that the recycling endosome is a central player of border cell migration by allowing correct regulation in time and space of the machinery controlling guidance, including RTKs and Rho GTPases.
Role of Drosophila Wash in cell migration.

Wiskott-Aldrich Syndrome (WAS) family proteins participate in cytoskeleton reorganization and signal transduction by acting as effectors of Rho family GTPases and polyunsaturated fatty acids (PUFAs) in the cortical membrane. WAS proteins are known to interact with the cortical cytoskeleton and the membrane, important for a number of processes, such as cell migration, endocytosis and phagocytosis. In collaboration with Trask Lab, we identified a new member of the WAS family called Wash. We are using Drosophila hemocytes as an in vivo model to study the role of Was in migration and associated cell shape changes. In Wash knockdown hemocytes the characteristic developmental dispersal is affected. The speed of their migration is not affected, however the direction of their migration is, suggesting that those hemocytes are failing to receive the cues needed to migrate along the ventral midline. Interestingly, we also find that hemocytes fail to migrate from the head to the tail, leading to a reduced number of hemocytes in the posterior end of the embryo. In hemocytes lacking Arg2/3 we find that the invasion-migration of hemocytes to the tail is also impaired, suggesting that Wash requires its own activation to drive the proper migration of hemocytes. In addition to undergoing developmental migrations, embryonic hemocytes are attracted to epithelial wounds. When called to a wound. Wash mutant hemocytes fail to polarize their lamellipodia, however they migrate at faster speed than WT hemocytes. It has been shown previously that Rho, Rac and Cdc42 small GTPases play important roles in the recruitment of hemocytes to wounds. Rho mutant hemocytes show similar phenotypes to those mutant for Was, supporting our evidence that Wash is acting downstream of Rho. We are currently dissecting the interaction between Was and Rho. These experiments will allow us to understand how cells remodel their cytoskeleton in signaling to enable movement and shape change.

A putative neurotransmitter transporter bloated tubules (blot) is required for Drosophila Border cell migration. Ping Wan, Jiong Chen. Model Animal Research Center, Nanjing University, Nanjing, China.

Border cells of the Drosophila ovary have emerged as a useful model system for studying the mechanism of collective cell migration. In a screen to uncover novel genes and mechanisms essential for border cell migration, we have identified a previously reported P-element mutation in the blot gene locus, which encodes a protein with significant sequence similarity to a subpopulation of vertebrate neurotransmitter transporters and was previously reported to be required for malpighian tubule morphogenesis. blot mutant border cells display strong migration defects, and clonal analysis shows that cells in mutant clone within a border cell cluster tend to lag behind in a greater frequency than the wild type border cells. Interestingly, there are strong pAKC, E-cadherin and β-catenin accumulation around the cell membrane in blot mutant border cells. Since border cells retain some epidermal characteristics during their collective migration, the epidermal polarity markers pAKC (apical) and E-cadherin and β-catenin (sub-apical, adherens junction) are normally present in the cells. Consistently, the strong accumulation of these junctional markers are also observed beneath the apical cell membrane in the mutant clones in the follicle epithelium. In contrast, we found no defects in the localization of Disc large(Dlg), a lateral junctional marker, in mutant cells of both border cells and follicle cells. Finally, we found that the recycling endosome marker Rab11 is also strongly accumulated around the cell membrane of mutant cells, and these Rab11 stained vesicles colocalize with the accumulated cytoplasmic E-cad staining. These results suggest that the epidermal polarity molecules are regulated by recycling endosomes and Blot probably regulates the correct targeting and fusion to the apical membrane of border cells during collective migration.

Enzymatic activity-independent functions of O-fucosyltransferase 1 in the folding and trafficking of the Notch receptor in Drosophila. Naoki Anyama1, Tonomori Abe1, Akira Ishii1, Takuya Suzuki2, Kenjiro Matsusato2, Takeshi Sasamura1, Tetsuya Okajima1, Kenji Matsuno1, 2, 1) Dept. Biol. Sci. & Tech., Tokyo Univ Sciene, Noda, Chiba, Japan; 2) G & DRC., Tokyo Univ Sciene, Noda, Chiba, Japan; 2) Nagoya University Graduate School of Bioagricultural Sciences, Department of Applied Molecular Biosciences, Japan.

Notch signaling is an evolutionarily conserved mechanism that controls many cell-fate specifications through local cell-cell interactions. Glycosylations of Notch are known as important elements that control the signaling activity and Notch localization. For example, EGF-like repeats of the Notch extracellular domain are O-fucosylated. This O-fucosylation is catalyzed by O-fucosyltransferase 1 (O-fut1), which is essential for Notch signaling in Drosophila. Besides the activity of O-fucosyltransferase, recent studies suggested that O-fut1 has two enzymatic activity-independent roles: a Notch-specific chaperone activity and activity promoting Notch endocytosis, although molecular nature of these O-fut1’s activities are still elusive. In this study, we found that up-regulation of unfolded protein response (UPR) could restore the disruption of Notch signaling associated with the absence of Notch fucosylations, including O-fucosylation. However, up-regulation of UPR can not restore the Notch signaling activity in the absence of O-fut1. This result suggested that O-fut1 is a major chaperone responsible for the folding of Notch. We previously reported that O-fut1 cell-nonautonomously promotes the endocytosis of Notch in its enzymatic activity-independent manner. Our further analysis showed that a secreting form of O-fut1 restored the subcellular localization of Notch, which was suggested to depend on the endocytosis of Notch, whereas this secreting O-fut1 could not restore Notch signaling associate with the absence of fucosylations. Taking these results together, two enzymatic activity-independent functions of O-fut1 separately contribute to folding and trafficking of the Notch receptor in Drosophila.
are co-expressed on the same cell surface. In the case of cis-interactions, the effect of the ligand appears to be inhibitory for Notch signaling. During a systematic examination of EGF-like repeats in the extracellular region of the Serrate ligand, we have been able to identify specific EGF-like repeats that encode this cis-inhibition property. Removal of specific EGF-like repeats eliminates the cis-inhibitory property of Serrate without significantly affecting the trans-activation capability of this ligand. Therefore, the transactivation and cis-inhibition properties of Serrate are functionally separate. Notch ligands can also demonstrate a second form of inhibition onto the Notch receptor. In this case, mutated ligand forms that lack the intracellular (IC) domain or lack the IC and transmembrane domains fail to activate Notch and instead exhibit dominant-negative attributes that distinctly inhibit Notch activation. We will present evidence demonstrating that the dominant-negative property of secreted Serrate molecules requires the presence of these same EGF-like repeats as does cis-inhibition. These findings demonstrate that both forms of ligand-induced Notch inhibition are likely to be mediated by a single region within Serrate that we call the Notch Inhibitory Region.

226A

dEHBP1 affects Notch signaling by impairing Delta trafficking during asymmetric divisions. Nikos Giagtzoglou1, Shirya Yamamoto2, Diana Zitserman2, Hillary Groves2, Hayley Klein3, Kazuya Schulte, Hao Wang1, and Nicole Roegers1, Hugo Beachy1, 1) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX. 77030; 2) Program in Developmental Biology, Baylor College of Medicine, Houston, TX. 77030; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX. 77030; 4) Department of Neuroscience, Baylor College of Medicine, Houston, TX. 77030; 5) Fox Chase Cancer Center, Philadelphia, PA 19111.

Notch signaling governs appropriate cell fate acquisition during asymmetric divisions of the developing external sensory organ lineages in Drosophila. In a genetic screen designed to identify novel players in the Notch pathway, we isolated mutations in dEHBP1 that lead to cell fate transformations within the external sensory organ lineages, as evidenced by the formation of supernumerary neuron and sheath cells at the expense of external cells. Our data indicate that dEHBP1 is enriched at the actin rich signaling interface of pia and pib cells, a domain that has been implicated in Delta-Notch signaling. Loss of dEHBP1 leads to a defect in Delta trafficking. Furthermore, we show that Sec15, which is also involved in Delta recycling, physically interacts with dEHBP1 and controls its subcellular localization. Therefore, dEHBP1 provides a novel link in the Delta recycling pathway during Notch signaling in asymmetrically dividing cells.

227B

dEHBP1 regulates Notch mediated lateral inhibition in the Drosophila eye. Nikos Giagtzoglou1, Tongchao Li2, Hugo Bellen1,2,4, 1) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX; 2) Program in Developmental Biology, Baylor College of Medicine, Houston, TX; 4) Department of Human and Molecular Genetics, Baylor College of Medicine, Houston, TX; 4) Department of Neuroscience, Baylor College of Medicine, Houston, TX.

Notch signaling is an evolutionarily conserved pathway that plays a central role in multiple developmental processes. dEHBP1 is a novel component of Notch signaling that controls cell fate acquisition during asymmetric divisions in sensory organ precursor lineages in the peripheral nervous system of Drosophila melanogaster. In the absence of dEHBP1, the ligand Delta is mistrafficked during asymmetric divisions of the ESO lineages. Notably, although dEHBP1 does not affect all aspects of Notch signaling, we discovered that dEHBP1 regulates Notch signaling mediated lateral inhibition during R8 photoreceptor development in the eye. Thus, decapering the role of dEHBP1 in eye development may provide a molecular basis of context dependent regulation of Notch signaling in different processes, i.e. lateral inhibition versus asymmetric divisions. We investigated the requirement of dEHBP1 in Notch signaling and identified its role in trafficking of Scabrous, a positive regulator of Notch signaling, but not in the trafficking of Notch or Delta. In wild type cells, Scabrous is secreted from the prospective R8 photoreceptor cell and uptaken by the surrounding cells, where it traffics to the late endosomes to potentiate Notch signaling. In the absence of dEHBP1, Scabrous accumulates within actin rich cellular processes that normally extend from the morphogenetic furrow via filopodial structures towards the surrounding and differentiating ommatidial clusters. Hence, it appears that Scabrous fails to be secreted from these cells. We are currently investigating the potential interaction of dEHBP1 with Scabrous, as well as the cellular compartment, where Scabrous accumulates. In conclusion, dEHBP1 is a novel molecular node in intracellular trafficking that regulate distinct aspects of Notch signaling in two different developmental contexts.

228C

Intracellular Notch activation is regulated by the core component of the ESCRT-III complex Shrub and by the ubiquitin ligase Deltex. Kazuya Horii1, Anindya Sen1, Tom Kirchhausen1, Spyros Artavanis-Tsakonas1,2, 1) Dept Cell Biol, Harvard Med Sch, Boston, MA; 2) Immune Disease Institute, Harvard Med Sch, Boston, MA, 3) Collège de France, Paris, France.

Genetic interaction screens in Drosophila, identified shrub, the homologue to the yeast protein Snf7, a core component of the ESCRT-III complex as a Notch signal modulator. We demonstrate that Shrub activity is responsible for capturing internalized receptors into Multivesicular Bodies (MVBs) leading to degradation of Notch. The interplay between the ubiquitin ligase Deltex and the non-visual /g53-arrestin Kurtz with Shrub, can bypass this traffic pathway and instead lead Notch to a ligand-independent, intracellular receptor activation path, which is linked to the ubiquitylation state of Notch.

229A

An in vivo RNAi Screen for Identification of Genes Involved in Follicle-Cell Differentiation and Cell-Cycle Switches. Dongyu Jia, Yi-Chun Huang, Wu-Min Deng. Department of Biology, Florida State University, Tallahassee, FL.

The Drosophila follicle-cell epithelium is an excellent model system for study of cell-cycle regulation and cell differentiation in development. During oogenesis, mediated by multiple signaling pathways, including the Notch, Hedgehog, EGFR, Wingless, JAK/STAT, Hippo, and JNK pathways, the follicle cells sequentially undergo three distinct cell-cycle programs: the mitotic cycle, the endodye, and gene amplification. Among these pathways, Notch signaling plays a central role. Its activation and inactivation in follicle cells are essential for the mitotic cycle-endodye and the endobody-gene amplification switches, respectively. Cut, a linker between Notch signaling and cell-cycle regulators, is specifically downregulated by Notch during the endodye stage. To determine how signaling pathways coordinate during the mitotic cycle-endodye switch and identify novel genes involved in follicle-cell differentiation, we are currently performing an in vivo RNAi screen through induced knockdown of gene expression and examination of Cut expression in follicle cells. To test the efficacy of this Drosophila RNAi system, we first examined RNAi lines targeting several Notch, EGFR, and Hippo pathway components and the results demonstrated that the in vivo screen strategy could help us identify additional genes involved in follicle-cell differentiation and cell-cycle switches. So far, we have screened about 900 RNAi lines from the Drosophila RNAi Screening Center and found some lines showing Cut upregulation in midoogenesis or Cut downregulation in late oogenesis. Furthermore, knockdown of shibire caused Cut upregulation during midoogenesis and degeneration of egg chambers during late oogenesis. Later we will continue using this approach to screen lines available from the Drosophila RNAi Screening Center. Further studies of the genes identified from the screen will help us gain more insights into the coordination of signaling pathways in the regulation of follicle-cell differentiation, proliferation, and growth.

230B

Modulation of Notch Signaling by the Drosophila Xylosyltransferase Shams. Tom Lee1, Maya Sethi2, Jessica Leonardo3, Hans Bakker2, Hamed Jafar-Nejad1,3. 1) Institute of Molecular Medicine, University of Texas Health Science Center, Houston, TX; 2) Hannover Medical School, Hannover, Germany; 3) Program in Developmental Biology, Baylor College of Medicine, Houston, TX.

One of the important posttranslational modifications identified on the extracellular domain of the Notch receptors is the addition of O-glucose to Epidermal Growth Factor-like (EGF) repeats with the C1-X-X-P-C2 consensus sequence. Previous work in Drosophila has identified the protein O-glucosyltransferase Rumi, a temperature-sensitive activator of Notch signaling which likely regulates the Notch pathway by adding O-glucose to Notch EGF repeats. The O-glucose on EGF repeats can be extended by the addition of xylose residues. Accordingly, we hypothesize that the xylose residues added to O-glucose contribute to the regulation of Notch signaling, analogous to the role of GlcNAc added by the...
The RAS1/MAPK signaling pathway is a key element of normal cellular proliferation and differentiation in metazoans. Importantly, oncogene driven activation of MAPK results in perturbed expression of genes which—like mapk—bear large introns. This suggests that intron size is an important characteristic predominantly found to impact mapk expression. In particular, the EJC, a complex traditionally associated to post-splicing regulatory events, was unexpectedly found to regulate signaling is also tightly associated to the development and progression of cancer. In order to better understand the regulatory network surrounding the RAS1/MAPK signaling pathway, we performed a screen for novel regulators of the RAS1/MAPK signaling pathway.

232A

Epsin functions as an atypical clathrin adapter in Notch signaling. Xuanhua Xie, Janice Fischer. Dept MCDB, Univ Texas, Austin, Austin, TX.

Epsin is an endocytic protein that binds clathrin, the plasma membrane, ubiquitin, and also a variety of other endocytic proteins through well characterized motifs. Although epsin is also a known endocytic factor, genetic analysis revealed that epsin is required specifically for the internalization of ubiquitinated transmembrane ligands of the Notch receptor, a process required for Notch activation. How epsin promotes ligand endocytosis, and thus Notch signaling, is unclear. Understanding the role of epsin will help to elucidate the role of ligand endocytosis. Here, by generating Drosophila lines transformed with thirty different transgenes that express wild-type epsin or different epsin deletion variants, we tested each of the five protein or lipid interaction modules of epsin for a role in Notch signaling. There are two major results of this work. First, we discovered that a single ubiquitin interaction motif (UIM) is necessary for epsin function. Second, we found that the UIM is the only indispensable type of epsin module. Although epsin requires modules for direct interaction with plasma membrane lipids and also with a variety of proteins present in clathrin cage structures at the plasma membrane (clathrin, the adapter protein complex AP-2, and accessory protein complexes), our results suggest that intron size is an important characteristic determining sensitivity to regulation of splicing by the EJC.

232B

Evidence for monomeric a-catenin as a component of cadherin catenin complex. Riddhhi Desai, Milena Pelikka, Noboru Ishiyama, Ritu Sarpal, Mitsuhiro Ikura; Ulrich Tepass. 1) Cell & Systems Biol, University of Toronto, ON, Canada; 2) Division of Signaling Biology, ON, Canada.

a-catenin is a key component of the cadherin catenin complex (CCT) that is essential for maintaining the architecture of epithelial tissues. Based on the physical linkage model, a-catenin directly links the CCT to the underlying actin cytoskeleton in order to stabilize adherens junctions (AJs). Null mutations of Drosophila a-catenin reveal that a-catenin deficient epithelial cells are unable to adhere to each other and loss of a-catenin leads to embryonic lethality. To understand how a-catenin supports cadherin activity during development, we employed a genome-wide RNAi screen in Drosophila S2 cells. This experiment led to the identification of many mTRA processing factors, including splicing factors and the exon junction complex (EJC) as pathway regulators. Further analysis revealed that these components could be positioned downstream of the MAPKK, MEK, and were predominantly found to impact mapk expression. In particular, the EJC, a complex traditionally associated to post-splicing regulatory events, was unexpectedly found to regulate the splicing of mapk. Moreover, the changes in splicing produced by EJC depletion were distinct from those observed for the other splicing factors identified in our screen. Finally, disruption of the EJC was also found to alter the splicing of other genes which—like mapk—bear large introns. This suggests that intron size is an important characteristic determining sensitivity to regulation of splicing by the EJC.

233A

The EJC Act on RAS1/MAPK Signaling by Altering the Splicing of mapk. Dariel Ashton-Beaucage, Christian Udel, Hugo Lavoie, Caroline Baril, Martin Lefrançois, Anne-Sophie Guenier, Jean Duachne, Daniel Lamarre, Patrick Gendron, Sébastien Lennieux, Marc Therrien. IURC, Univ de Montreal, Montreal, PQ, Canada.

The RAS1/MAPK signaling pathway is a key element of normal cellular proliferation and differentiation in metazoans. Importantly, oncogene driven activation of MAPK results in perturbed expression of genes which—like mapk—bear large introns. This suggests that intron size is an important characteristic predominantly found to impact mapk expression. In particular, the EJC, a complex traditionally associated to post-splicing regulatory events, was unexpectedly found to regulate the splicing of mapk. Moreover, the changes in splicing produced by EJC depletion were distinct from those observed for the other splicing factors identified in our screen. Finally, disruption of the EJC was also found to alter the splicing of other genes which—like mapk—bear large introns. This suggests that intron size is an important characteristic determining sensitivity to regulation of splicing by the EJC.

233B

Evidence for monomeric a-catenin as a component of cadherin catenin complex. Riddhhi Desai, Milena Pelikka, Noboru Ishiyama, Ritu Sarpal, Mitsuhiro Ikura; Ulrich Tepass. 1) Cell & Systems Biol, University of Toronto, ON, Canada; 2) Division of Signaling Biology, ON, Canada.

a-catenin is a key component of the cadherin catenin complex (CCT) that is essential for maintaining the architecture of epithelial tissues. Based on the physical linkage model, a-catenin directly links the CCT to the underlying actin cytoskeleton in order to stabilize adherens junctions (AJs). Null mutations of Drosophila a-catenin reveal that a-catenin deficient epithelial cells are unable to adhere to each other and loss of a-catenin leads to embryonic lethality. To understand how a-catenin supports cadherin activity during development, we employed a genome-wide RNAi screen in Drosophila S2 cells. This experiment led to the identification of many mTRA processing factors, including splicing factors and the exon junction complex (EJC) as pathway regulators. Further analysis revealed that these components could be positioned downstream of the MAPKK, MEK, and were predominantly found to impact mapk expression. In particular, the EJC, a complex traditionally associated to post-splicing regulatory events, was unexpectedly found to regulate the splicing of mapk. Moreover, the changes in splicing produced by EJC depletion were distinct from those observed for the other splicing factors identified in our screen. Finally, disruption of the EJC was also found to alter the splicing of other genes which—like mapk—bear large introns. This suggests that intron size is an important characteristic determining sensitivity to regulation of splicing by the EJC.

233C

A genome-wide RNAi screen to identify novel pathways responding to Caffeine. Francesca Di Cara, Brenda Parsons, Ran Zhuo, Eden Foley, Kirst King-Jones. 1) Biological Science, University of Alberta, Edmonton, Alberta, Canada; 2) MMI Dept, University of Alberta, Edmonton, Alberta, Canada.

Caffeine is arguably the most regularly consumed xenobiotic compound in the world. Exposure to caffeine results in a strong upregulation of detoxification enzymes, and for this reason caffeine has been frequently used to study xenobiotic response pathways. Caffeine has also been shown to affect the cell cycle checkpoint, DNA repair, cell growth, cell division as well as intracellular calcium homeostasis and apoptosis. Although it is possible that the cytotoxicity of caffeine is due to its ability to trigger apoptosis, the mechanisms of caffeine action are still unknown. In order to dissect the cellular pathways affected by caffeine, and to define how the drug induces a detoxification response, we employed a high-throughput screen in Drosophila S2 cells. Firstly, we determined the feasibility of our strategy by establishing a caffeine concentration able to induce a cell death. We then found that the knock-down of the caspases Dronc and drICE reverted the caffeine-mediated cell death. Conversely, we showed that knock-down of the anti-apoptotic gene dIAP1 increased caffeine-induced cell death. To identify genes that mediate caffeine-induced cell death, we are currently performing a high-throughput screen using an established genome-wide Drosophila library that targets 15,683 genes of the Drosophila genome. Hits from this primary screen will be organized into functional sub-networks and tested in a secondary screen that uses a reporter assay as a read out. For this, we have generated a Drosophila S2 cell line that expresses Red Fluorescent Protein (RFP) under the control of the Cyphost promoter. Cyphost encodes a cytochrome P450 monooxygenase and acts as a class I detoxification enzyme. This reporter system is designed to detect changes in RFP expression levels when dsRNA is added in the presence of caffeine. This approach will permit the identification of signaling molecules that are involved in the metabolism and detoxification of drugs and other xenobiotics.

235A

The sumoylation pathway regulates JNK pathway through the action of Hipk. Renjie Jiao1, Hai Huang1,2, Hanqing Chen1,2, Xuehong Liang1, Changqing Li1, Lei Xue3, Jun Ma4. 1) Institute of Biophysics, Chinese Academy of Sciences, Bejing, Beijign, China; 2) Graduate School of the Chinese Academy of Sciences, Bejing, China; 3) Shanghai Key Laboratory for Signaling and Diseases, School of Life Science and Technology, Tongji University, Shanghai, China; 4) Divisions of Biomedical Informatics and Developmental

183
Biology, Cincinnati Children’s Research Foundation, Cincinnati, OH, USA.

Post-translational modification by the small ubiquitin-related modifier (SUMO) is important for a variety of cellular and developmental processes. With the fact that Drosophila sumo, smt3, homologous mutants are lethal at the early 2nd instar larval stage, we used RNAi to conditionally deplete smt3 to study the impact of lacking sumoylation on tissue specific development. Smt3 knockdown in Drosophila wing discs causes phenotypes resembling JNK gain-of-function, including ectopic apoptosis and apoptosis-induced compensatory growth. We observed that Smt3 depletion leads to an increased expression of JNK target genes. While knockdown of the homeodomain-interacting protein kinase (Hipk) suppresses Smt3 depletion-induced activation of JNK, Hipk overexpression synergistically enhances this type of JNK activation. We found that Hipk is sumoylated in vivo. Currently, we are investigating how the sumoylation pathway regulates the activation of Hipk during JNK activation and analyzing the corresponding factors involved in this process.

236B

Arrestins are required for activation of Drosophila Rh1 rhodopsin kinase. Alexander V. Kiselev, Joseph E. O'Tousa. Biological Sci, Notre Dame Univ, Notre Dame, IN.

Arrestins are required for activation of Drosophila Rh1 rhodopsin kinase Alexander Kiselev and Joseph E. O'Tousa. Department of Biological Sciences, University of Notre Dame, IN 46556 The Drosophila rdgC mutant lacks Rh1 rhodopsin-specific phosphatase. Consequently, Rh1 remains highly phosphorylated upon illumination in this mutant and initiates the processes leading to retinal degeneration. Arrestin-1 and arrestin-2 are known to regulate endocytosis and proper turnover of Rh1. In sum both arrestins are present at about a 1:3 ratio to Rh1. To elucidate the role of arrestins on Rh1 light-driven posttranslational modifications and endocytosis, we developed an experimental system capable of following light-driven Rh1 phosphorylation in vivo. We show that Rh1 phosphorylation requires at least one of the arrestins. During the white light illumination all photo activated Rh1 molecules in rdgC animals are in equilibrium with arrestins, while intense blue light generates a non-equilibrium system, where about 30% of total photo activated Rh1 forms complexes with arrestins, and about 70% of molecules are in arrestin-free state. Nonetheless, both white and blue light illuminations result in almost complete phosphorylation of Rh1. These observations indicate that following activation by Rh1/arrestin complexes, the Rh1 kinase phosphorylates all photo activated Rh1 molecules, both in the arrestin-bound and arrestin-free states. Arrestin binding and receptor phosphorylation are common elements of the deactivation process for G protein-coupled receptors (GPCRs) including Rh1. The prevailing view has been that GPCR's phosphorylation is necessary for arrestin recruitment. However, through use of the Drosophila rdgC arrestin mutants, we demonstrate that arrestins are required for Rh1 kinase activation. We propose that the sequence of molecular events in the GPCRs regulation is: (1) arrestin binds to stimulated receptor; (2) GPCR/arrestin complex activates GPCR kinase; and (3) the activated GPCR kinase phosphorylates all activated receptors, irrespective of whether arrestin is bound to the GPCR.

237C

Engrailed homeoprotein acts as a signaling molecule in the developing fly. Sophie Layalle1, Michel Volovitch1, Bruno Mugart1, Nathalie Bonneau1, Marie-Laure Parmentier1, Alain Prochiantz2, Alain Jolles3, Florence Maschat1. 1) Institut de Génomique Fonctionnelle, Neurobiology Department, CNRS UMR203 INSERM U661, Montpellier, France; 2) Institut de Génétique Humaine, CNRS UPR1142, Montpellier, France; 3) Collège de France, Ecole normale supérieure. CNRS UMR 7233, Paris, France.

Homeodomain transcription factors classically exert their morphogenetic activities through the cell autonomous regulation of developmental programs. In vertebrates, several homeoproteins have also been shown to have direct non-cell autonomous activities in the developing nervous system. We present here the first in vivo evidence for homeoprotein signaling in Drosophila. Focusing on wing development as a model, we first demonstrate that the homeoprotein Engrailed is secreted. We show that Engrailed is a short-range signaling molecule and using single-chain anti-Engrailed antibodies expressed under the control of a variety of promoters, we delineate wing territories in which secreted Engrailed acts. Thus we found that the Engrailed signaling participates in anterior crossove development, interacting with the Dpp signaling pathway.

238A

Forward Genetic Screening to Identify Genes in Cell Competition. Chang Hyun Lee, Gerard Rimesso, Nicholas Baker. Genetics, Albert Einstein College of Medicine, Bronx, NY.

Cell competition may model features of tissue repair and tumor development. Genetic as well as microarray screens have revealed genes in the loser cells that participate in their being eliminated by winner cells. Genes that are required in the winner cells have been identified less systematically. To fill this gap, we are performing a genetic screen for mutations that are required in wild type cells to eliminate Minute cell losers. Wild-type clones generated in the adult eye in a Minute heterozygous background are being assessed. So far, we have isolated 40 candidate mutant strains on 3R.

239B

Separating planar cell polarity and Hippo pathway activities of the protocadherin Fat. Hitoshi Matakatsu, Seth Blair. Dept Zoology, Univ Wisconsin, Madison, WI.

The Drosophila protocadherin Fat (Ft) affects the planar cell polarity (PCP) of hairs in wing and abdomen and ommatidia in eye. Ft also controls the growth of imaginal discs via a 1.6P6 zygotic phenotype, two were specifically expressed in oenocytes and in chordotonal organs. Subsequent characterization showed that Ft's ECD can act independently of its ICD in PCP, and can trigger dominant negative and boundary effects on Hippo activity, likely via binding to the protocadherin Dachsous (Ds).

240C

egghead and brainiac regulate chordotonal organ precursor recruitment. Stephanie M Pontier1, Xue Li Guan2, François Schweisguth1. 1) Development, Instituto Pasteur, Paris, France; 2) Swiss Tropical and Public Health, Institute, Basel, Switzerland.

Glycophospholipids (GSL) are structural components of cellular membranes that have been shown to regulate the function and the properties of number of essential signalling molecules in vitro. With its single class of GSLs, Drosophila constitutes a perfect model system in order to assess the actual influence of GSL on signaling networks in vivo. egh and brn genes encode for enzymes catalyzing respectively the 2nd and the 3rd steps of the sugar chain elongation of glucosyleraminides. Their maternal mutation correlates with detrimental perturbations in embryo epithelium integrity and central nervous system organization. In contrast, while their zygotic mutation is still lethal, mutant flies die around eclosion without obvious anatomical defects. These observations suggest that egh and brn zygotic mutations may only interfere with the development of specific organs or tissues. Following this rationale, we have overexpressed the brn gene under a UAS promoter in a brn106 genetic context using a panel of Gal4 drivers in order to rescue brn106 flies. Among the Gal4 drivers that proved to rescue brn106 zygotic phenotype, two were specifically expressed in oenocytes and in chordotonal organs. Subsequent characterization showed that egh and brn embryos and larvae show increase number of both oenocytes and chordotonal precursors compared to controls. Correlating with those results, maternal mutation of brn using a viable homozygous allele, brn106, is accompanied by a Lch6-7 phenotype in 80% of the abdominal segments (n=50) compared to the typical and systematic Lch5 phenotype observed in wild type embryos. Such an increase in the recruitment of chordotonal mechanical precursors has been linked to gain of function mutations in the EGFR pathway. Our results, hence, suggest that elongated GSL insure the proper downregulation of the EGFR pathway in vivo. Further studies will determine the molecular basis of this modulation.

241A

ecal is probably a non-coding RNA that negatively regulates Jun N-terminal Kinase (JNK) signaling during embryonic dorsal closure. Luis Daniel Rios-Barrera, Juan Rafael Riesgo-Escovar. Developmental Biology Dept., Neurobiology Institute, Universidad Nacional Autónoma de México, Queretaro, Juriquilla, Mexico.
Embryonic dorsal closure, a model to study JNK signaling, consists in the stretching of the lateral epidermis towards the dorsal side of the embryo over the amnioserosa. The only Drosophila JNK member, Basket (Bsk), is activated at the most dorsal row of epidermal cells, where it regulates closure. Mutants for Bsk pathway components do not completely close and die with a ‘dorsal open’ cuticular phenotype. We have isolated a novel mutant with this phenotype, and generated an allelic series bearing defective closure. This gene was named “acal”, which means “boat” in a Mexican native language. Mapping and cloning of acal revealed that despite lacking significantly long or conserved ORFs, this gene is conserved in nucleotide sequence between Drosophila and other species. Predicted nucleotide structure of its transcript is severely altered in at least one mutant allele. Of note, there are annotated Acal-associated transcripts within this locus. By in situ hybridization, acal is seen early in development. At germ band retraction it is restricted to prospective nervous and epidermal tissues. During closure, it is found in the lateral epidermis. Then, it disappears from this tissue but persists in the nervous system. Genetic interactions between acal and hsk show that both genes antagonize each other, which means that Acal negatively regulates Bsk signaling. This was confirmed by analyzing the expression of a Bsk signaling reporter gene, puc-LacZ. Ectopic expression of puc-LacZ is seen in acal mutants. To determine how Acal may counteract Bsk signaling, we studied genetic interactions with other negative regulators: Anterior open (Aop) and Raw. aop is epistatic to acal and, consistent with above results, its mutant phenotype is enhanced by mutations in acal. Mutations in acal and raw suppress each other. Altogether, these results place Acal as a novel negative Basket regulator during dorsal closure, acting in the epidermis after Aop, opposing Raw function.

242B Identification and Functional Characterization of Novel Components of the Insulin/TOR Pathway in Drosophila melanogaster, Ralf B. Schittenhelm1, Timo Glatter2, Oliver Rimmer3, Matthias Gstaiger1, Ruedi Aebersold1, Hugo Stocker2, Ernst Hafen3. 1) Institute of Molecular Systems Biology, ETH Zurich, Switzerland; 2) Proteomics Core Facility, Biocenter, University of Basel, Switzerland.

The insulin/TOR pathway is one of the major signaling cascades controlling cell and animal growth, and its deregulation has been linked to a number of severe diseases like cancer or diabetes. During the last decades, extensive genetic analyses have been used to identify and characterize essential components of the insulin/TOR pathway and their regulatory relationships. However, their organizations into distinct complexes as well as the underlying processes regulating their spatial and temporal assembly are just poorly understood. Using a systematic AP-MS/MS approach to further elucidate the insulin/TOR signal transduction network in Drosophila melanogaster, we observed 112 specific protein-protein interactions among 79 identified network components. Using label-free quantification, we could demonstrate that 20% of all identified protein-protein interactions are sensitive to changes in insulin levels providing important information about insulin-induced rearrangements of protein complexes within the insulin/Tor pathway. Moreover, we found a substantial overlap between our data and protein-protein interactions known from other organisms, highlighting the conservation of this pathway. In addition, we identified several proteins that have not been linked to the insulin/TOR pathway so far. Ongoing genetic and biochemical analyses suggest novel protein complexes and regulatory mechanisms controlling cell growth by the insulin/TOR pathway.

243C The retinoblastoma protein RBF and the PHD zinc-finger domain protein Rhinoceros coordinately regulate EGFR signaling and the induction of photoreceptor differentiation, Madina Z. Sukhanova1, Latishya J. Steele1,2, Wei Du1. 1) Ben May Dept Cancer Res, Univ Chicago, Chicago, IL; 2) Department of Cell Biology, Harvard Medical School, Boston, MA.

Inactivation of the Drosophila retinoblastoma protein (RBF) leads to deregulated cell proliferation, increased apoptosis, and a subtle differentiation defect. To identify genes that can modulate the consequence of rbf inactivation, we carried out a genetic screen and identified a mutation corresponding to the gene Rhinoceros (Rno), which encodes a PHD zinc-finger protein. Inactivation of both rbf and rno causes synergistic differentiation defect characterized by a multiple-R8 phenotype and a delay of neuronal differentiation after specification of R8 photoreceptors. We reported that RBF in cooperation with Rno regulates the recruitment of R8 photoreceptors through control of the expression of Notch ligand, Delta [Steele et al., Dev. Biol. 335, 2009]. Here, we demonstrate that RBF and Rno coordinately regulate Epidermal Growth Factor Receptor (EGFR) signaling within the morphogenetic furrow (MF) of eye imaginal disc and modulate the initiation of photoreceptor differentiation. We show that RBF plays an important role in the activation of EGFR signaling near the MF through the control of the expression of Rhomboid protein required for a successful cleavage of EGFR ligand, Spitz. Although Rno was previously reported to be a negative regulator of EGFR signaling, our results suggest that Rno is required for the nuclear function of the signaling. Here, we also present that Rno directly controls the expression of Ebi, an F-box/WD40 repeat protein involved in EGFR-dependent degradation of Tramtrack88, an antagonist of neuronal and cone cells development. Our results provide new insights into the role of RBF and Rno in photoreceptors differentiation and regulation of EGFR signaling.

244A Ligand Specificity in EGFR Signaling, Christina L. Austin, Amanda Simcox. Molecular Genetics, The Ohio State University, Columbus, OH.

Egfr is a receptor tyrosine kinase which signals through the canonical Ras/MAPK pathway, mediating diverse developmental processes by evoking both proliferative and differentiation cues. Signaling through Egfr is activated by four ligands: vein (a neuregulin-like ligand), and the TGF-alpha-like ligands spitz, gurken, and keren. The developmental consequences of removing any one of these four ligands are different, reflecting their diverse roles in development, yet all activate signaling through the Ras/MAPK pathway. gurken is only maternally required and keren null mutants are apparently normal. This suggests zygotic patterning is mediated by spitz and vein. However, vein, spitz double mutants are less severely affected than Egfr mutants. Here, we examined triple mutant embryos that are deficient for keren as well as spitz and vein. These resemble Egfr nulls, revealing a cryptic role for keren and excluding the possibility of ligand-independent signaling by the receptor. A further role for keren in wing development is demonstrated by the exacerbation of vein hypomorphic phenotypes when keren dose is reduced. Finally, I address the question of ligand specificity by determining whether a given ligand can rescue the mutant phenotype of another if expressed in the correct endogenous pattern. This last series of experiments will test whether ligand differences exist, perhaps engendered by differential affinity for the receptor, which can result in different signaling outcomes, or indeed if ligands are equivalent when appropriately expressed.

245B Characterization of novel epidermal growth factor receptor targets with apparent roles in Drosophila egg and wing development, Lisa A. Kadlec, Jacqueyn Gallo, Dawn Gregor, Hannah Laimer, David Marr. Dept of Biology, Wilkes University, Wilkes-Barre, PA.

Signaling by the Drosophila epidermal growth factor receptor (Egfr) plays an important role in many aspects of development, including oogenesis, embryogenesis and proper development of both the eye and the wing. In the ovary, the Egfr pathway plays a key role in the establishment of the body axes during oogenesis. In the wing, Egfr signaling plays an important role in vein tissue specification. Microarray screens by our lab and others have been used to identify potential downstream transcriptional targets of the Egfr receptor using the Drosophila ovary as a model system. Our initial work compared gene expression using fly ovaries in which the activity of the Egfr-pathway was reduced (grk HK36), and excluding the possibility of ligand-independent signaling by the receptor. A further role for keren in wing development is demonstrated by the exacerbation of vein hypomorphic phenotypes when keren dose is reduced. Finally, I address the question of ligand specificity by determining whether a given ligand can rescue the mutant phenotype of another if expressed in the correct endogenous pattern. This last series of experiments will test whether ligand differences exist, perhaps engendered by differential affinity for the receptor, which can result in different signaling outcomes, or indeed if ligands are equivalent when appropriately expressed.

246C β-arrestin Kurtz inhibit MAPK and Toll signaling in Drosophila development, Alexey Veraksa1, Marla Tipping1, Yoosik Kim2, Stanislav Y. Shvartsman2. 1) Department of Cell Biology & Signal Transduction, See page 16 for presentation schedule.
The *Drosophila* β-arrestin Kurtz (Krz) has been implicated in controlling signaling via Notch and G protein coupled receptors. In this study, we report a novel function of Krz in the regulation of two distinct developmental signaling modules: MAPK ERK and NF-κB, which transmit signals from the activated receptor tyrosine kinases (RTKs) and the Toll receptor, respectively. Analysis of expression of the effectors and target genes of Toll and Moesin reveals that Krz limits the activity of both pathways in the early embryo. Protein interaction studies suggest a previously uncharacterized mechanism for ERK inhibition: Krz can directly bind and sequester an inactive form of ERK, thus preventing its activation by the upstream kinase, MEK. A simultaneous dysregulation of different signaling systems in *krz* mutants results in an abnormal patterning of the embryo and severe developmental defects. Our findings uncover a new in vivo function of β-arrestins and present a novel mechanism of ERK inhibition by the *Drosophila* β-arrestin Krz.

247A

**RTK/Ras/MAPK signaling in the visceral mesoderm does not require Pnt as a transcriptional effector to specify founder cell fate.** Yiyan Zhou, 1,2 Amanda L. Neisch, Richard G. Fehon. Dept MGCB, Univ Chicago, Chicago, IL.

We have previously identified the transmembrane protein Evenness interrupted (Evi/Wls) as a core component of the Wnt signaling pathway that is strictly required for the specification of founder cell (FC) lineages in *Drosophila* wing imaginal discs. Here, we report that AP-1 and clathrin are essential for secretory granule biogenesis in the FCs of the visceral mesoderm (VM). In the absence of AP-1 activity, FCs fail to differentiate into mucin-expressing somatic FCs. This is reflected in profound defects in secretory granules. These findings establish a novel role for AP-1/clathrin-dependent trafficking in the formation of mucin-containing secretory granules.

248B

**Investigating the function of a RhoGAP, Conundrum, that interacts with Moesin.** Amanda L. Neisch, Richard G. Fehon. Dept MGCB, Univ Chicago, Chicago, IL.

Moesin, a membrane-associated protein, is involved in regulating the actin-cytoskeleton, and plays a role in epithelial integrity, cell survival, and Hedgehog signaling in *Drosophila*. Moesin interacts with a predicted GAP, CG17082 or Conundrum, in co-immunoprecipitation experiments in Drosophila S2 cells and that the localization of epitope-tagged protein at the cell cortex is dependent on Moesin. Increasing their intrinsic GTPase activity, suggesting a model where Moesin regulates Rho1 by promoting Conundrum activity. We have confirmed that Conundrum interacts with Moesin, a predicted RhoGAP. RhoGAP's are known to regulate Rho family GTPases by decreasing Rho1 activity. Currently, we are examining the in vivo target of Conundrum GAP activity in epithelia and the functional significance of Moesin-Conundrum mutants, which are viable and lack the epithelial defects found in *Moesin* mutants, suggesting that it cannot exclusively regulate Rho activity downstream of Moesin. We have also found that excessive Conundrum GAP activity at the cell cortex results in the localization of the epithope-tagged protein at the cell cortex is dependent on Moesin. Conundrum mutants, however, are viable and lack the epithelial defects found in *Moesin* mutants, suggesting that it cannot exclusively regulate Rho1 activity downstream of Moesin. We have also found that excessive Conundrum GAP activity at the cell cortex results in the localization of the epithope-tagged protein at the cell cortex is dependent on Moesin. Conundrum mutants, however, are viable and lack the epithelial defects found in *Moesin* mutants, suggesting that it cannot exclusively regulate Rho1 activity downstream of Moesin. We have identified the protein Evi/Wls as a core component of the Wnt signaling pathway that is strictly required for the Wnt/Wg signaling cascade as a key regulator of specific cell fates in the visceral mesoderm.
secretion of Wnt/Wg. Evi shuttles Wnt/Wg from the Golgi to the plasma membrane and is then recycled by the retromer complex. However it remains unclear, how and when Evi releases Wnt/Wg into the extracellular space.

We are interested in understanding how Wnt/Wg proteins are secreted and finding other factors that might be required for their secretion. We are addressing these questions using both cell culture and Drosophila in vivo RNAi screening approaches. A focused RNAi library subset was chosen, containing genes with functions in trafficking, such as Rab-protein family member, exocyst complex genes, retromer and others. Here, we present our RNAi screening data along with the candidates that are under further detailed investigation. Moreover, we also present preliminary data on novel functional roles of Evi in Wnt/Wg secretion.

252C

Cell death factors, tumor suppressors and cytoskeletal proteins control prepupal activities of Drosophila salivary glands preceding edcsyone-triggered programmed histolysis. Robert Farkas1, Lucia Mentelova1,7, Erika Halaszova1,7, Daniel Vleck1, Milan Beno1, Alena Hercegová1,7, Peter Danis1,7, Ivan Raska1, Bernard Mechler1,2, 1) Inst Experimental Endocrinology, Slovak Academy Sciences, Bratislava, Slovakia; 2) Department of Genetics, Faculty of Science, Comenius University, Bratislava, Slovakia; 3) Institute of Embryology, Biology and Pathology, Faculty of Medicine, Charles University, Prague, Czech Republic; 4) Department of Developmental Genetics, Deutsches Krebsforschungszentrum-ZMBH Allianze, Heidelberg, Germany.

At the onset of Drosophila metamorphosis the steroid edcsyone activates a cell death program that leads larval salivary glands (SGs) to rapidly disintegrate about 14-16 hr after puparium formation. During this process edcsyone acts through the edcsyone receptor (Ecr/R) and heterodimer that regulates primary response genes, including the Broad-Complex (BR-C) critical for SG death. Timing of SG histolysis and preceding cellular activities including unconventional secretion depends upon the level of p127(2)gl, a cytoskeletal tumor suppressor that interacts with nonmuscle myosin II heavy chain (nmHIC) encoded by the zip (zip) gene. Several other factors, including Ecr and directly edcsyone-regulated genes BR-C and E74 as well as some components of apotpttic machinery appears to be also involved in the control of this unconventional secretion. On the other hand, programmed cell death and the secretory activity can be dissected using ectopic expression of baculovirus anti-apoptotic protein p35. Using co-immunoprecipitation we have shown that several of these factors can form precipitable complex indicating they can physically interact. We will present evidence for the sequence of so far neglected phases of prepupal salivary gland development and clues differ them from early prepupal development and final histolysisic stage. (Supported by the EEA & NFM Norwegian Fund # SK-0086/3655/2009/ORINFM).

253A

Wg regulation by the cytoplasmic proteins, Tum and Pav/Wingless/Wnt. Elisabeth Greer, Anna Chao, Amy Bejsovec. Biology, Duke University, Durham, NC.

Wingless/Wnt (Wg/Wnt) signaling is essential for specifying body pattern in flies and other animal species. In humans, deregulated Wnt signaling is associated with cancer; this highlights the importance of understanding how this developmental pathway is regulated. We have found that the cytoplasmic kinesins proteins Tumbleweed/RacGAP50C (Tum) and Pavarotti (Pav) act as negative regulators of Wg/Wnt signaling in Drosophila embryos and in mammalian cell lines. While these proteins act in the cytoplasm to position the contractile ring during cell division, we find that they are required in the nucleus to perform their regulation of Wg/Wnt signaling. Mutating the nuclear localization sequences (NLS) in either protein abolishes their ability to modulate Wg-mediated in embryos and target gene expression in human HEK293T cells. These mutated NLS forms retain the ability to participate in cytoplasmic: David Glover’s laboratory has shown that Pav/NLS can rescue the cytoplasmic defects of pav mutant embryos (Minestrini et al. 2002 J.Cell Sci. 115:725) and we find that the same is true for the Tum/NLS protein. Our genetic epistasis analysis has placed Tum and Pav downstream of Armadillo (Arm) stabilization, but upstream of or in parallel to Arm and Tcf in modulating target gene expression. We are now studying these recently uncovered role for Tum and Pav in the nucleus and cellular activities throught the Arm/broad Complex (BC) and further explore the role of these two nuclear factors. We are using genetics and co-immunoprecipitation to determine how Tum and Pav interact with other nuclear components of the Wg/Wnt pathway, including Legless, Pygopus, Tcf and Groucho. We are testing whether Tum and Pav regulate endogenous gene expression in SG1, Kc167, and Clone-8 cells, and will use these cells in chromatin immunoprecipitation assays to determine whether Tum and Pav modulate Wnt signaling by binding to and modifying the activity of transcription factors.

254B

Examination of cell autonomous and non-autonomous effects of APC loss in the developing Drosophila wing. Kellie Kravarik, Sandra Zimmerman, Amy Fuller, Brooke McCartney. Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA.

Adenomatous Polyposis Coli (APC) is a negative regulator of the Wnt signal transduction pathway whose loss is implicated in >80% of human colon cancers. However, the early cellular effects of APC loss in polyp initiation are not well understood. Like humans, Drosophila have two APC homologues, APC1 and APC2, which function together in partially overlapping roles throughout development. To better understand the effects of both APC1 and APC2 loss in a model epithelial tissue, we are examining clones of APC2 APC1 (APC) null tissue in the developing Drosophila wing. We have previously shown that APC null clones exhibit dramatic Wnt dependent apical constriction and invagination in the wing disc that requires the activation of Rho1 and Myosin II. We are now examining how APC loss and activation of Wnt signaling affects tissue morphology, cell death and cell fate specification throughout wing development. In the pupal wing, preliminary data suggests that clones may exhibit an increase in apoptosis. Further, APC null clones exhibit both cell autonomous and non-autonomous fate changes leading to alterations of bristle and vein development. To better understand the temporal organization of these effects, we are now utilizing a novel live-imaging technique to observe changes in APC null clones during the larval and pupal stages. This characterization of the effects of APC loss in the developing wing will contribute to our understanding of how Wnt signaling can affect epithelial tissues and may help us understand the cellular changes that accompany the development of colon cancer.

255C


The colon cancer tumor suppressor A-Denomatus polyposis coli (APC) negatively regulates Wnt signal transduction through its activity in the destruction complex. APC binds directly to the main effector of the pathway, β-catenin (Armadillo in Drosophila), and targets it for proteosome-mediated degradation. Biochemical and biological studies have suggested that phosphorylation and salt bridge interactions between the 20 amino acid repeats (20Rs) of APC and β-catenin are important for their interaction and function in Wnt signaling. However, there has been no new in vivo model of this study. In this model, we investigated the functional role of these molecular interactions by making targeted mutations in Drosophila APC2 that disrupt phosphorylation and salt bridge interactions, and deletion mutants missing the 20Rs and/or 15 amino acid repeats (15Rs). Vertebrate 20R3 has the highest affinity for β-catenin, suggesting a significant role in destruction complex function. Drosophila APC2 has five 20Rs with 20R3-R5 having the highest homology to the vertebrate 20R3. Thus, we generated two versions of the phosphorylation and salt bridge mutants, one mutating relevant residues in 20R1-R5, and one mutating only 20R3-R5. We tested the ability of these mutant proteins to regulate Wnt signaling in APC2 null (APC2 15), and APC2 APC1 double null (APC2 15 APC1 15) backgrounds. While all of the mutants exhibited some rescuing ability in the APC2 15 background, the ability of 20R1-R5 and the deletion background to rescue was dependent on APC1. 20R3-R5 mutants rescued as well as the wild type APC2, suggesting that 20R1 and 20R2 are sufficient for APC2’s destruction complex function. These in vivo data support the biochemical model of a role for phosphorylation and salt bridging in APC’s destruction complex function. Furthermore, the dependence on APC1 suggests that APC2 and APC1 may act cooperatively in the destruction complex.
The recent discovery of ~22nt microRNAs (miRs) as global regulators of gene expression make them ideally suited to be involved in modulating the activity of signaling pathways that often display dose-dependent phenotypes. We aimed to uncover putative miR-controlled regulatory mechanisms of the transcriptionally poised Armadillo (Arm)/TCF complex, the key transcriptional regulators of the Wnt/Wg pathway. Thus, we have performed a screen for microRNAs (miRs) that can modulate Wg signaling activity downstream of the degradation complex, in Drosophila cells. The directed nature of the primary screen design was achieved by activating the pathway via dsRNA-mediated knockdown of Axin, an inhibitor of cytosolic Arm. In this context, we assessed the effect of exogenous overexpression of a library of primary miR constructs on an Arm responsive TOPFlash reporter. Candidate miR regulators of the pathway were first tested using epistasis analyses to investigate their relationships with known components of the pathway. We demonstrate that expression of a selected candidate miR cluster that inhibits reporter activity also reduces levels of Arm protein. Overexpression of the miR cluster in the wing and leg imaginal discs phenocopies Wg loss of function. Furthermore, expression of an Arm construct lacking the 3UTR rescues miR overexpression phenotypes. Genetic interaction studies in the fly eye suggest that the candidate cluster antagonizes phenotypes resulting from the expression of high levels of endogenous Arm. Using P-element mobilization, we have generated a genomic excision of the analyzed cluster and observed that F1 flies lacking the miR function exhibit a significant degree of male-specific sterility, suggesting a miR-regulated role for the Wg pathway in the male germline. Finally, the mammalian ortholog of the fly candidate cluster exhibits similar inhibitory properties towards the activity of a Wnt pathway in a variety of human/Wnt-relevant cancer cell lines.

257B
Testing a Paracrine Signaling Role for Wg in Drosophila Ostia Formation. Gloriana Trujillo, Candice Lovato, Jill Hendren, Lynda Helander, Richard Cripps. Department of Biology, University of New Mexico, Albuquerque, NM, USA 87131.

Drosophila melanogaster is a simple, tractable invertebrate model for heart development. The similarities of the Drosophila dorsal vessel to vertebrate cardiac formation provide opportunities to study complex processes, in a system whose embryonic development does not hinge on heart function. The dorsal vessel has an aorta and a chamber termed the heart. Crucial structures for the proper functioning of the dorsal vessel are the inflow tracts, or ostia, located in the heart chamber.

The question remains as to how formation of ostia differ from non-ostial cardiac cells. Initial heart specification requires the homeotic selector protein Abdominal A (Abd-A). Ostial cells specifically express the COUP-TFII homolog Seven-up (Svp), while neighboring non-ostial cardiac cells express Tinman (Tin)/Nkx2.5. The signaling glycoprotein Wingless (Wg)/Wnt is important for initial specification of cardiac cells and has roles beyond specification to morphogenesis. We are addressing the contribution of Wg to formation of Drosophila cardiac inflow tracts.

Our approach was to analyze the roles of three genes:svp,abd-A, and wg. Ostia do not form in svp mutants, while wg is partially required for ostia formation. Expressing ectopic Abd-A throughout the cardiac tube results in ostia formation in the aorta. By contrast, if Abd-A is expressed in Svp-expressing cells, ostia do not form. We hypothesize that ostial formation involves a combination of signals from both the Wg pathway and from cells expressing Tinman (Tin)/Nkx2.5. When Wg signaling is inhibited in the neighboring Tin cells, ostia do not form, suggesting that Wg signals in a paracrine fashion. Our findings support an additional role for Wg after initial heart cell specification and that interactions between ostial and non-ostial cells is required for inflow tract formation.
The DNA damage checkpoint is one of the first pathways activated in response to DNA damage. Checkpoint activation temporarily arrests the cell cycle to provide time for involved in the vital regulation replication and cell cycle check points.

261C Developmental analysis and tissue specificity of checkpoint genes in Drosophila melanogaster. Jeffrey P Chmiielewski, Laura Henderson, Tim W Christensen. Biology, East Carolina University, Greenville, NC.

The GINS complex is a heterotrimeric complex of the protein subunits Psf1, Psf2, Psf3, and Sld5. The GINS complex is one of several proteins recruited to DNA in transition from G1 to S phase. Recent research in our lab using the yeast two hybrid system confirms a direct interaction of Psf2 with Sld5, Cdc45, and MCM2. Our lab has also shown that a null mutation for Psf2 is characterized by M phase delays, anaphase bridges, and defects in condensation. The goal of our research is to use an in vivo and in vitro approach in D. melanogaster to establish a relationship between Psf2 and loki, encodes Chk2. We believe there is interaction between Chk2 and loki that mediates the speed at which the replication fork progresses. Recent research in mice has show Psf2 to have an indirect interaction with Chk2 proteins. Using the yeast two hybrid system we have tested the direct interaction between Psf2 and loki. Taking a genetic approach to characterize the interaction of Psf2 with loki, we have used null mutations both separately and in combination. We have analyzed brain tissue in combination with acridine orange staining to look for apoptotic events. We have also examined the histone variant H2AX, indicative of double stranded breaks, as an indication of DNA damage. DNA fiber analysis has been used to gauge the speed of the replication fork during the elongation phase.

259A In Vivo and in vitro analysis of the interaction between Psf2 and Chk2 in D. melanogaster. Jeffrey P Chmiielewski, Laura Henderson, Tim W Christensen. Biology, East Carolina University, Greenville, NC.

The DNA damage checkpoint is one of the first pathways activated in response to DNA damage. Checkpoint activation temporarily arrests the cell cycle to provide time for DNA repair. The DNA damage checkpoint is mediated by the evolutionarily conserved ATM/ATR kinase cascade. The mechanism by which DNA damage activates the ATM/ATR kinases is not fully understood. We conducted a genome-wide RNAi screen in Drosophila cells to identify novel genes required for the G2/M checkpoint, a type of checkpoint activated at the interface of G2 and M. We identified ~60 genes that could be classified into particular biological functions including DNA repair, DNA replication, cell cycle control, chromatin regulation and RNA processing. We report in vivo characterization of two genes, mus101 (TOPBP1) and mus312 (BTBD12/SLX4), which were checkpoint activated at the interface of G2 and M. We identified ~60 genes that could be classified into particular biological functions including DNA repair, DNA replication, cell cycle control, chromatin regulation and RNA processing. We report in vivo characterization of two genes, mus101 (TOPBP1) and mus312 (BTBD12/SLX4), which were originally identified as DNA damage-sensitive loci but have not been implicated in the G2/M checkpoint.


The DNA damage checkpoint is one of the first pathways activated in response to DNA damage. Checkpoint activation temporarily arrests the cell cycle to provide time for DNA repair. The DNA damage checkpoint is mediated by the evolutionarily conserved ATM/ATR kinase cascade. The mechanism by which DNA damage activates the ATM/ATR kinases is not fully understood. We conducted a genome-wide RNAi screen in Drosophila cells to identify novel genes required for the G2/M checkpoint, a type of checkpoint activated at the interface of G2 and M. We identified ~60 genes that could be classified into particular biological functions including DNA repair, DNA replication, cell cycle control, chromatin regulation and RNA processing. We report in vivo characterization of two genes, mus101 (TOPBP1) and mus312 (BTBD12/SLX4), which were originally identified as DNA damage-sensitive loci but have not been implicated in the G2/M checkpoint.

261C Developmental analysis and tissue specificity of checkpoint genes in Drosophila melanogaster. Sara Morais da Silva1, Nicolas Malmanche2, Claudio Sunkel1. 1) Molecular Genetics, IBiMC, Porto, Portugal; 2) Stowers Institute for Medical Research, Kansas City, USA.

Assessing the functions of cell-cycle regulators in developing organisms is a rigorous confirmation of the observations made in cultured cells. Conflicting data about the role of checkpoint genes in neoplasia and tumour progression require further studies in an in vivo situation such as the analysis of clones of cells mutant for a checkpoint gene in an otherwise healthy tissue. This study addresses the link between the role of mitotic checkpoint genes and tissue specificity. Data resulting from mutations in a checkpoint gene, Bub3, in a tissue context will be presented. Bub3 mutant clones, in the follicle cells of the ovary display a highly abnormal morphology, delaminate from the epithelium and die. In addition, a developmental time course revealed that these abnormal phenotypes are always derived from progenitor cells indicating that Bub3 may have a specific role in progenitor cells, in particular in the ovary follicle stem cells. Also, Bub3 knockdown by RNA interference revealed that Bub3 depletion confers phenotypes in the notum and brain indicating tissue specific roles for this mitotic checkpoint gene. This work contributes towards a better understanding of the general mechanisms that link cell division, development, maintenance of tissue integrity and cancer.

262A The oocyte-specific function of dPds5 in insulator body assembly is monitored by a new meiotic checkpoint. Raquel A.M. Santos, Patricia Silva, Vitor Barbosa. Instituto Gulbenkian de Ciência (IGC), Rua da Quinta Grande, 6, 2780-156 Oeiras, Portugal.

The DNA damage response is a DNA repair surveillance pathway to ensure genome stability during cell division. In Drosophila, persistent meiotic double-strand breaks activate the checkpoint kinase ATR, causing loss of dorsal-ventral polarity in the eggshell. Mutations in the cohesion-related gene dPds5 lead to similar defects. By epistatic analysis we show that the dorsal-ventral defects observed in dPds5 mutants are not due to ATR activation, but ATM- and CHK2-dependent. Analysis of indirect active transcription markers, Poly-A Binding Protein 2 (PABP2) and H3K4me, reveal regions of active transcription in a generally silent wild-type karyosome. Moreover, dPds5 mutant germ line clones show the localization of these markers specifically affected in the oocyte. We show that dPds5 co-localizes with the conspicuous insulator protein CP190 and the insulator protein BEAF. These results suggest that dPds5 is necessary for insulator body formation in the oocyte and such function is under ATM and Chk2 surveillance. We discuss how ATM and Chk2 control gene transcription during proliferation as well as cell cycle exit and differentiation in the Drosophila midgut.


Rbf/dE2F complexes play a central role in regulating both cell cycle progression and cellular differentiation. However, how these diverse functions are coordinated when a cell progresses from a proliferating progenitor state towards a post-mitotic differentiated state remains poorly understood. This project aims to unravel how Drosophila Rbf/dE2F complexes control gene transcription during proliferation as well as cell cycle exit and differentiation in the Drosophila midgut. The midgut is a highly dynamic tissue in which intestinal stem cells (ISCs) continuously proliferate and give rise to all cell types that make up the intestine. ISCs self-renew and give rise to daughter cells called enteroblasts (EB) that differentiate into absorptive enterocytes (EC) or secretory enteroendocrine (EE) cells. To address the differences in Rbf/dE2F gene regulation between these cell types, we are developing a cell type-specific ChIP-Seq procedure that makes use of in vivo biotinylation. We used fosmid recombineering to tag all Rbf/dE2F members with an Avidin tag and express these under their native genomic environs. The Avi-tag is biotinylated by the expression of the biotinylation enzyme BirA. Cell type-specific Gal4 lines are used to drive expression of BirA in all cell types of the midgut: Di-Gal4 (expressed in ISC), Su(H)-GBE-Gal4 (EB), MyoA-Gal4 (EC) and Pros-Gal4 (EE). This tags Rbf/dE2F complexes only in the BirA-expressing tissues, allowing the isolation of protein/DNA complexes from quiescent, differentiated (EC and EE) or fast-cycling progenitor (ISC and EB) cells in a cell
type-specific manner. We will combine our ChIP-Seq data with cell type-specific mRNA profiling by Tu-tagging. Lastly, we are testing the effect of knocking down the function of Rbf/dE2F and their interacting components in the midgut in a targeted RNAi screen. This approach will generate a detailed picture of the genes controlled by Rbf/dE2F during the transition from a proliferative state towards a differentiated state as well as addressing tissue-specific differences in gene expression regulated by Rbf/dE2F.

264C Cell cycle regulation by Cul4/Cdt2 in the early Drosophila embryo. Christina I. Swanson1,2, Robert J. Duronio1. 1) Department of Biology, University of North Carolina, Chapel Hill, NC; 2) The SPIRE Program, University of North Carolina, Chapel Hill, NC.

Cell cycle progression is tightly controlled by multiple mechanisms. Recently, ubiquitin-mediated proteolysis has emerged as an important component of cell cycle regulation. In particular, the Cul4/Cdt2 E3 ubiquitin ligase has been shown to be essential for proper cell cycle progression. Cul4/Cdt2 recognizes and ubiquilates target proteins via a mechanism that is dependent on substrate interaction with chromatin-bound PCNA. This mechanism directly links substrate degradation to the beginning of S-phase, when PCNA is recruited to chromatin. Cul4/Cdt2 targets multiple proteins for degradation in this cell-cycle regulated manner, including Cdt1, p21, E2f1, and Set8. Because these substrates themselves are important regulators of cell cycle progression, their stabilization during S-phase can have dramatic consequences for the cell, including rereplication of DNA, cell cycle arrest, and increased apoptosis. Therefore, Cul4/Cdt2 function is hypothesized to be required for normal cell cycle progression. However, very little is known about its activity in different developmental contexts, particularly during non-canonical cell cycles. For example, E2f1 is stable during S-phase in the rapid cell cycles of the early Drosophila embryo, despite preliminary evidence suggesting that the Cul4/Cdt2 complex is intact at that developmental stage. This apparent contradiction suggests developmentally-specific regulation of either Cul4/Cdt2 complex activity or substrate availability. We are investigating the developmental regulation of Cul4/Cdt2 activity in order to better understand how this complex controls cell cycle progression in multiple developmental contexts.


DNA ligase 3 (LIG3) is one of three DNA ligases found in metazoans. In vertebrates, DNA ligase 3 interacts with the repair protein XRCC1 and is crucial for the ligation of single-strand DNA breaks during base excision repair (BER) and nucleotide excision repair (NER). In vitro experiments and studies using cultured mammalian cells suggest that LIG3 may play a role in alternative end-joining repair of double-strand breaks. Because LIG3 is an essential gene, its role(s) in the context of a metazoan remains to be defined. We have identified an ortholog of LIG3 in Drosophila melanogaster (Dm) and generated a null mutant by imprecise excision of a P element. Interestingly, we have observed that LIG3 is at least partially duplicated in several but not all Dm strains. The published, functional copy of the LIG3 gene is flanked by the cyprg2 gene upstream and the pseudogene for cyprgP1 downstream resulting in an ideal substrate for single strand annealing following P-element excision. Flies lacking LIG3 die at late-stage pupae. This lethality is rescued by expression of a LIG3 transgene driven by the endogenous promoter. Overexpression of LIG3 under the Gal4 promoter does not appear to be deleterious. We have utilized in vivo RNA interference to reduce LIG3 expression. Stocks with less than 25% of the normal expression of LIG3 are developmentally delayed with males exhibiting reduced viability and fertility. Knockdown strains are sensitive to methyl methanesulfonate, consistent with a role for LIG3 in BER. Sensitivity of knockdown strains to camptothecin and ionizing radiation, suggest a role for LIG3 in the repair of double-strand breaks. Strains lacking DNA ligase 4 and decreased LIG3 show synergistic decreases in viability but are not more sensitive to DNA damaging agents, suggesting that LIG3 is the dominant ligase for most types of DNA repair. Together, our data demonstrate an essential role for Dm LIG3 during larval and/or pupal development and lay the groundwork for further investigations regarding its role in alternative end-joining.

266B The structural and functional analysis of the Orc6 protein. Igor N. Chesnokov1, Maxim Balasov1, Shixuan Liu2, Hongfei Wang2, Lijie Wu2, Yingfang Liu2. 1) Dept Biochem & Molec Gen, Univ Alabama, Birmingham, AL; 2) National Laboratory of Biological Macromolecules, Institute of Biophysics, Beijing, China.

The Origin recognition complex (ORC) is a six-subunit protein important for the DNA replication in eukaryotic cells. ORC is also involved in other cell functions. The smallest metazoan ORC subunit, Orc6, consists of two functional domains. Larger N-terminal domain directly involved in DNA binding and is important for DNA replication. Smaller C-terminal domain is important for cytokinesis. The structural analysis revealed that the N-terminal domain of both Drosophila and human Orc6 has an overall fold similar to the transcription factor TFIIB. A model of Orc6 binding to DNA is produced. Amino acids of Orc6 which are directly involved in DNA binding are identified. Alterations of these amino acids abolish DNA binding ability of Orc6 and also result in reduced levels of DNA replication in vitro and in cultured cells. Furthermore, we have shown that the expression of human Orc6 in Drosophila Orc6 mutant cells rescued DNA replication. The ability of human Orc6 protein to support DNA replication in Drosophila cells indicates that two proteins are homologous in replication function and also provide an opportunity for further molecular dissection of human Orc6 in vivo, using Drosophila as a model system. We propose that Orc6 is a DNA binding subunit of ORC and may position ORC at the origin of DNA replication similar to the role of TFIIB in positioning transcription pre-initiation complex at the promoter.

267C Investigating the mitotic to endocycle transition in D. melanogaster. Christiane A. Hassel, Brian R. Calvi. Department of Biology, Indiana University, Bloomington, IN.

The endocycle is a variant cell cycle comprised of alternating G and S phases without mitosis. Repeated endocycle oscillations result in large polyploid cells in various Drosophila tissues. In addition to an altered cell cycle regulation, we have recently shown that endocycling cells in Drosophila also repress apoptosis (Mehrotra, et.al. 2008 G&D). Developmental signals induce cells to undergo a mitotic to endocycle transition (the M/E switch). The M/E switch can also be experimentally induced by knockdown of mitotic cyclins. We are investigating what other genetic alterations are sufficient to induce an M/E switch, and whether these experimentally-induced endocycles resemble developmental endocycles in their cell cycle regulation and repression of apoptosis. Our flow cytometry and fluorescent microscopy analysis suggest that knockdown or overexpression that compromises entry into mitosis is sufficient to induce cells in culture and developing tissues to undergo an M/E switch. Moreover, we have found that this experimental M/E switch also represses apoptosis. We are continuing to evaluate how similar these experimental endocycles are to those induced by developmental signals. We are also testing what other genetic perturbations are sufficient for the M/E switch and whether they also repress apoptosis. Answers to these questions should provide insight into the enigmatic endocycle and the relationship between polyploidy and cancer.

268A Characterization of DNA Polymerase δ using a combined in vivo and in vitro strategy. Chad M. Hunter1, Lena M. Keller1, Bonnie J. Bolkan2, Tim W. Christensen1. 1) Department of Biology, East Carolina University, Greenville, NC; 2) CROET, Oregon Hlth & Sci Univ, Portland, OR.

Cancer can be caused by defects in the regulation of DNA replication. Abnormal DNA replication can lead to unregulated growth, failure to differentiate, and aberrations in chromosome biology. Numerous different proteins are involved in DNA replication and understanding the role of these proteins is essential in understanding the nature of cancer. Of these different proteins, one of the most important is DNA Polymerase δ. DNA Polymerase δ is involved in elongation of the lagging strand of DNA during the S phase of the cell cycle. Interestingly, little work has been done in multicellular organisms with regards to DNA Polymerase δ. Using two novel mutant fly strains along with cell culture, we show that mutations in DNA Polymerase δ have effects on genome stability. The work employs a three-pronged approach consisting of an interaction analysis, a genetic analysis and a knockdown analysis in tissue culture. The interaction analysis was conducted using a yeast two hybrid system to confirm predicted interactions from humans. Interactions investigated include: DNA Polymerase α, members of the GINS complex, Mcm10, members of the MCM complex, and PCNA (mu209). The genetic analysis of DNA Polymerase δ consisted of using two novel mutant fly strains. Both homozygous lethal, missense mutations were found to be located in a conserved polymerase domain (G694N).
and a conserved exonuclease domain (C496Y). These lines were assayed for any abnormalities in cell cycle progression as well as for any chromosome aberrations. The knockdown of DNA Polymerase 6 in tissue culture was used to corroborate and extend the in vivo work. S2 cells were utilized in RNA interference studies to analyze effects on cell cycle progression as well as any morphological changes that might occur in cells or chromosomes. Understanding how DNA Polymerase 6 functions in vivo will help us to further understand the role of DNA replication in the context of a multicellular organism.

269B

The definition of size and pattern of an organ largely depends on the control of cell proliferation and differentiation. In order to better understand how these processes are regulated, it is critical to identify as many genes as possible involved in their regulation. Most of the screenings carried out for searching mutation that affect cell proliferation have been focused in the identification of loss of function alleles. One of the problems of this approach is that the lack of function of genes required for proliferation, usually induce cell death. In addition, it has been previously reported that, at least in Drosophila, the functions of some of the genes required for the control of cell proliferation are redundant. Both problems, the redundancy and the cell lethality effect, can be avoid in an over-expression screening. We have used a Gal4 line (GMR-Gal4) to drive the expression in the eye of a collection of about 500 P-UAS elements (EP) inserted randomly throughout the genome. Our screening is aimed to identify genes that affect the pattern of cell proliferation and neuronal differentiation in the second mitotic wave (SMW) during eye development. We have identified several genes that specifically affect one of these processes or both at the same time. The EP line M50.2 was selected for further analysis. This line encodes for ald, a checkpoint cellular kinase that is activated under hypoxia conditions. The over-expression of this kinase usually results in lethal phenotypes except in the case of SalPGal4. The ectopic expression of ald during imaginal disc development give rise to the lost of tissue integrity, marked by the disruption of E-caderine and other membrane markers. In these discs cell death is strongly increased. In addition, we find big round cells that have lost cell polarity and they seem to proliferate and invade adjacent tissues. This last effect is strongly enhances when oncogenes such as RasV12 or Notch intra (an active form) are co-expressed.

270C
CycA controls endoreplication dynamics in the Drosophila bristle cell lineage. Jérémy Sallé, Michel Gho, Agnès Audibert. UMR 7622, Developmental Biology Laboratory, CNRS-University Paris VI, 9, Quai Saint Bernard, 75005 Paris, France.

An endocyte is a variant of the canonical cell cycle characterized by repeated rounds of DNA replication without intervening mitosis and thus produces polyploid cells. Successive replication rounds are triggered by oscillations in the activity of the CycE/Cdk2 complex that in turn repress the APC/C-Fzr complex. APC/C-Fzr complex (Cdh1) sends proteins such as Orc1 and Geminin, both involved in pre-replication complex assembly, to the proteasome for degradation. This generates permissive windows for temporal firing of replication origins. During mitotic cycles, APC/C-Fzr also ensures the complete degradation of mitotic cyclins (CycA, B and B3). A similar effect of APC/C-Fzr on mitotic cyclins seems to occur during the endocyte since inhibition of APC/C-Fzr leads to an accumulation of these proteins. Based on this observation we hypothesized that mitotic cyclins are implicated in endocyte regulation. In order to study this hypothesis, we focused our analysis on the role of CycA during endocyte. We studied mechaanosensor organs in which two of the four terminal cells undergo several rounds of endocyte. By combining in vivo experiments to follow endocyte dynamics, clonal analysis and RNAi expression, we have shown that: (i) endocyte dynamics is regulated in a cell specific fashion; (ii) Cyclin A contains an cell cycle marker; (iii) cyclin A loss of function induces a delay in the late step of endocyte progression leading to a reduction in the resulting ploidy. Taken together, our results indicate that CycA regulates endoreplication dynamics.

271A
Deficiency screens reveal Pineapple eye, a predicted E3 ubiquitin-conjugating enzyme, as an essential regulator of Drosophila germline stem cell self-renewal. Yalan Xing, Irina Kurtz, Jillian Legard, Manisha Thuparani, Timothy Dosey, Hannele Ruohola-Baker. Department of Biochemistry, ISCRM, University of Washington, Seattle WA.

The germline stem cells (GSCs) of Drosophila ovary provide an excellent model system to study the molecular mechanisms of stem cell self-renewal. We perform a loss-of-function screen to reveal novel factors required for Drosophila female GSC maintenance and/or division. A group of mutations affecting various ubiquitin-conjugating enzymes are identified to cause defects in GSC self-renewal, including Plenty of SH3s (POSH), Ubiquitin conjugating enzyme 10 (UbcD10), and pineapple eye (pie). Ubiquitin-mediated protein degradation plays a variety of roles in the regulation of many developmental processes, including mediating stem cell division through degradation of cell cycle regulators. We demonstrate that pie, sharing highly conserved RING domains with human E3 ubiquitin ligase G2E3 that is critical for early embryonic development, is required for GSC maintenance and normal cell division. Despite the reported role in imaginal disc cell survival, pie mutant GSC loss cannot be rescued by overexpression of p35 in the germline, suggesting that pie acts in GSC by means other than cell death. Based on evidence from cell cycle markers, we propose that pie mutant GSCs are arrested at the G1/S phase transition, possibly dependent on the level of the p21/p27-like cyclin-dependent kinase inhibitor (CKI) Dacapo. Overexpression of pie in the germline, on the other hand, causes a high frequency of late-interphase GSCs, marked by bar-shape spectrosome, suggesting an elevated interphase/M phase duration ratio. Further exploration of pie, including the potential functions and targets in GSCs, will ultimately contribute to a better understanding of how the ubiquitin-conjugating enzymes regulate stem cell biology also in mammalian systems.

272B
The Regulation and Assembly of Histone Locus Bodies. Esteban Terzo, Anne White, Brandon Burch, Xiao-cui Yang, Pamela Gasdaska, Zhbigniew Dominski, William Marzluff, Robert Duronio. University of North Carolina, Chapel Hill, NC.

Histone proteins play crucial roles in the biological processes involving chromosomal DNA in eukaryotes. The replication-dependent histone genes rapidly express mRNAs at the beginning of, and throughout, S phase. Histone mRNA synthesis is a complex process that is highly regulated at both transcriptional and post-transcriptional levels. These gene expression events occur in association with a nuclear structure located at the histone locus known as the Histone Locus Body (HLB), which contains all of the known factors involved in histone transcription and pre-mRNA processing. Using RNAi and proteomics screens in drosophila S2 cells, we identified factors necessary for HLB assembly, including a homolog of the Cyclin E/Cdk2 substrate NAPF encoded by the homeotic gene multi sex combs (mxc). Mxc and the histone pre-mRNA processing factor FLASH co-localize to form the HLB prior to the onset of zygotic histone transcription, remain chromosome-associated during mitosis, and are continuously present in HLBs throughout development. The MPM-2 antibody, which recognizes Cyclin E/Cdk2 targets in the HLB, binds Mxc. FLASH and Mxc are necessary for the localization of all known HLB components and loss of Mxc reduces histone mRNA synthesis and is lethal. Thus, Mxc and FLASH provide a scaffold for the hierarchical assembly of HLBs during the cell cycle. We are currently determining which domains of Mxc promote localization to the HLB, which other molecular components of the HLB are Mxc binding partners, when these interactions take place, and what role they play in cell cycle progression.
Autophagy in Drosophila ovaries is induced by starvation and is required for oogenesis. Julia M.I. Barth1, Janos Szabad2, Ernst Hafen1, Katja Köhler1. 1) Department of Biology, Institute of Molecular Systems Biology, ETH Zurich, Zurich, Switzerland; 2) Department of Biology, Faculty of Medicine, University of Szeged, Szeged, Hungary.

In Drosophila, nutrient depletion induces autophagy in the fat body that is controlled by Insulin-Target of rapamycin (TOR) signaling. Interestingly, nutrient availability and Insulin/TOR signaling also influence the size and structure of Drosophila ovaries; however, the role of autophagy during this process remains to be elucidated. Here, we show that starvation induces autophagy in germline cells (GCs) and in follicle cells (FCs) in Drosophila ovaries. This process is mediated by the autophagy-related (ATG) machinery and involves the upregulation of ATG genes. Further, autophagy in GCs and FCs is controlled by Insulin/TOR signaling. The analysis of chimeric animals in which either the GCs or the FCs are mutant for ATG genes reveals that autophagy in GCs, but not in FCs, is required for proper oogenesis. Strikingly, when animals lack ATG gene function in both cell types, ovaries develop normally, suggesting that the incompatibility between autophagy-competent GCs and autophagy-deficient FCs disturbs egg development. We propose that autophagy is required for the communication between these two cell types, and provide evidence for signaling pathways involved in this process. Our data establish an important function for autophagy during oogenesis and contribute to the understanding of the role of autophagy in animal development.

Gradients of a Ubiquitin E3 Ligase Inhibitor and a Caspase Inhibitor Determine Differentiation or Death in Spermatids. Eli Arama, Yosef Kaplan, Liron Gibbs-Bar, Yossi Kalifa, Yael Feinstein-Rotkopf. Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel.

Caspases are executors of apoptosis, but also participate in a variety of vital cellular processes, including cell differentiation, cell signaling, and cellular remodeling. To date, more than two dozen vital cellular processes have been described in both insects and mammals. Nevertheless, very little is known about how caspases promote these vital processes and how some cells avoid death in the presence of these deadly proteases.

In Drosophila, spermatids terminally differentiate by removing their bulk cytoplasmic contents in a process that requires active caspases. We identified Soti, an inhibitor of the Cullin-3-based E3 ubiquitin ligase complex required for caspase activation during spermatid terminal differentiation (individualization). We further provide evidence that the giant inhibitor of apoptosis-like protein dBruce is a target for the Cullin-3-based complex, and that Soti competes with dBruce for binding to Klhl10, the E3 substrate recruitment subunit. We then demonstrate that Soti is expressed in a subcellular gradient within spermatids, and in turn promotes proper formation of a similar dBruce gradient. Consequently, caspase activation occurs in an inverse graded fashion, such that the regions of the developing spermatid that are the last to individualize experience the lowest levels of activated caspases. These findings elucidate how the spatial regulation of caspase activation can permit caspase-dependent differentiation while preventing full-blown apoptosis [1].


The role of apoptosis and JNK signaling in dpp-mediated ventral head development. Sung-Yeon Park, Brian Stultz, Deborah Hursh. Division of Cell and Gene Therapy, Center for Biologics Evaluation and Research, FDA, Bethesda, MD20892.

We are studying the role of decapentaplegic (dpp) in the formation of the adult head capsule. dppH6 mutations eliminate peripodial-specific dpp expression in the eye/antennal disc, thus affecting structures of the ventral head such as vibrissae, gena, rostral membrane, and maxillary palps. We find that these dppH6 mutations increase apoptotic cell death during third larval instar in both the peripodial and disc proper tissue layers of the eye/antennal disc. To determine how this apoptotic cell death is related to the mutant phenotype, we examined apoptotic pathway mutations to determine their requirements for the dppH6-induced pattern defects. Using the GAL4-UAS binary system, we observe that overexpressed anti-apoptotic gene products can suppress the vibrissae phenotype in mutant backgrounds. Furthermore, mutations in pro-apoptotic genes can rescue the vibrissae phenotype. This suggests that apoptosis is the main cause of the vibrissae phenotype seen in dppH6 mutations. We also show that the JNK pathway is activated in dppH6 mutations, both the PE, where dpp expression is lost, and in regions of apoptotic cells in the disc proper. Loss of function clones of the Dpp receptor thickveins also show ectopic JNK pathway activation, suggesting that Dpp negatively regulates JNK activity in the eye/antennal disc. While JNK activity in the disc proper is correlated with regions of apoptosis, we find no such association of JNK expression and cell death in the PE. This suggests that the JNK pathway may function differently in the PE, and this pathway may play different roles in the two tissue layers of the eye/antennal disc. Our goal is to elucidate how induction of JNK pathway relates to Dpp signaling during ventral head capsule formation.

276C

Signaling Pathways Underlying Metabolic Control of Apoptosis in Drosophila. Chih-Sheng Yang1, Thomas Merritt2, Sally Kornbluth1,3. 1) Molecular Cancer Biology Program, Duke University Medical Center, Durham, NC 27710 USA; 2) Dept of Chemistry and Biochemistry Laurentian University, Ontario P3E 2C6, Canada; 3) Dept of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC 27710, USA.

Apoptosis ensures tissue homeostasis in response to developmental cues or cellular damage. Recently reported genome-wide RNAi screens have suggested that several metabolic regulators can modulate caspase activation in Drosophila. Here we establish a previously unrecognized link between metabolism and Drosophila apoptosis by showing that cellular NADPH levels modulate the initiator caspase Drone (Ne) through its phosphorylation at Ser310. Depletion of NADPH removed this inhibitory phosphorylation, resulting in the activation of Drone and subsequent cell death. Conversely, upregulation of NADPH prevented Drone-mediated apoptosis upon DIAP1 RNAi or cycloheximide treatment. Furthermore, this CaMKII-mediated phosphorylation of Drone hindered Drone activation but not its catalytic activity. Blockade of NADPH production aggravated the death-inducing activity of Drone in specific neurons but not in the photoreceptor cells of the eyes of transgenic flies; similarly, non-phosphorylatable Drone was more potent than wild-type in triggering specific neuronal apoptosis. Notably, impairment of the pentose phosphate pathway, the primary NADPH-generating pathway, through overexpression of G6PDH (Zw) RNAi caused developmental neuronal defects. Our observations reveal a novel regulatory circuitry in Drosophila apoptosis, and, since NADPH levels are elevated in cancer cells, also provide a genetic model to understand aberrations in cancer cell apoptosis resulting from metabolic alterations.

277A

THE COMpanion OF REAPER Gene IS A POTENT INDUCER OF APOPTOSIS. Christian W. Antonio1, Zhou Lei2, Gina Chan3, Yanping Zhang2, Rong Yuan2, Bergmann Andreas4. 1) Biochem & Molec Biol, MD Anderson Cancer Ctr, Houston, TX; 2) Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida, Gainesville, FL.

The companion of reaper (corp) gene was identified as a gene whose expression is strongly induced in response to ionizing radiation in a p53 dependent manner. Although Corp does not have an IBM/AP binding motif domain, it shares a G153 homology domain with the H99 cell death inducing genes reaper, grim and sickle, so we tested its ability to induce cell death. We found that overexpression of Corp in the developing eye by the GMR promoter results in massive cell death and completely ablates the eye. This eye ablation can be almost completely suppressed by the coexpression of the caspase inhibitor P35 or in a background of dronc mutant clones, therefore cell killing is occurring via the canonical cell death pathway. Surprisingly, Corp-induced cell death is independent of the H99 genes, indicating that corp acts downstream or in parallel to the H99 genes. Corp overexpression leads to the degradation of Diap1. However, Diap1 degradation is dependent on Dronc indicating that Corp does not directly induce Diap1 degradation, consistent with the fact that Corp has no IBM domain. There may be feedback from Dronc or the effector caspases that causes the degradation of Diap1. This still leaves open, however, the question of where corp fits into the cell death pathway. It may act directly on Ark or Dronc. Our data shows a strong synergistic effect between Dark and Corp, i.e. much more cell death is seen when these 2 proteins are coexpressed as opposed to no synergistic effect between Corp and Dronc. This suggests direct interaction between Corp and Ark, which we
are currently testing biochemically. These data reveal a potentially novel cell death inducing mechanism is Drosophila.

278B

The Drosophila receptor tyrosine kinase tie interacts with the microRNA bantam to regulate organismal survival following exposure to ionizing radiation. Amber E. Bilak, Tin Tin Su. Molec Cell & Dev Bio, Univ Colorado Boulder, Boulder, CO.

Identifying genes that influence organismal survival following ionizing radiation (IR) exposure could be useful in developing better treatments for tumors, which are frequently mutated in genetic pathways that regulate radiation responses. To survive ionizing radiation exposure, organisms must strike a balance between inducing apoptosis to eliminate cells whose DNA has been irreparably damaged and limiting apoptosis to allow survival of the organism. The microRNA bantam has an established role in suppressing the apoptosis that occurs in response to double-stranded DNA breaks induced by ionizing radiation exposure (Jaklevic 2008), as well as apoptosis that occurs during development (Brennecke 2003). To identify upstream and downstream regulators of bantam, we performed a genetic screen for dosage-dependent modulators of radiation sensitivity of heterozygous bantam mutants. We identified a chromosomal deficiency containing tie that shows a significant interaction with bantam mutation to suppress survival. Three alleles of tie similarly interact with mutant bantam to decrease survival. tie has a known role in border cell migration in conjunction with PVR and Egfr (Wang 2006) but is otherwise uncharacterized. In other contexts, PVR and its receptor PVR play a role in survival. Similarly, Tie may interact with bantam to promote survival of cells following IR exposure.

279C

A Genetic Screen for Cell Death Regulators in Drosophila melanogaster Oogenesis. Michelle Gammill1, Victoria Jenkins1, Tatevik Keshishyan1, Jeremy Nguyen1, Jemma Taipan1, Sarah Durkin2, Luz Ceballos2, Aileen Leung2, Ruth Ranum2, Rebecca Chen1, Elizabeth Tanner1, Jeanne Peterson1, Kim McCall1. 1) Department of Biology, Boston University, Boston, MA; 2) Concordia College, Moorhead, MN; 3) University of California Berkeley, Berkeley, CA.

Programmed cell death mechanisms, especially apoptosis and autophagy, are essential for many processes in Drosophila, including oogenesis. Drosophila oogenesis is an excellent system for studying cell death, as it is self-contained and has several distinct stages where cell death occurs. Egg chambers contain a 16-cell germline cyst, where 15 nurse cells generate proteins and cellular components that are dumped to the oocyte during stages 11-12. Nurse cell (NC) death can be induced by starvation during stages 7-9 (mid-stage death), before vitellogenesis, but occurs developmentally during stages 11-13 (late-stage death). To identify novel regulators of cell death, we performed a misexpression screen of the second chromosome using lines containing a single randomly inserted stable EPgy2 (EY) P-element. The P-element insertions contain a UAS enhancer which drives misexpression of affected genes in the germline when crossed to flies carrying the germline driver NGT;nanos-GAL4. Of the 1200 lines we have screened so far, 21 show consistently abnormal cell death phenotypes. The affected genes function in processes such as DNA/RNA binding, cytoskeleton arrangement, cell signaling, and mitochondrial events. Phenotypes observed include persisting NC nuclei in late oogenesis, death-resistant NCs in mid-oogenesis, and excessive degeneration of egg chambers. Ongoing experiments include lysosomal staining, co-expression with the caspase inhibitor DIAP1, and analysis of loss-of-function mutants. Mechanisms for cell death in oogenesis, especially late oogenesis, are unique to the fly ovary and incompletely characterized. This screen has identified several potential new regulators of these cell death processes.

280A

buls, a novel regulator of apoptosis during metamorphosis. Yunsik Kang2, Anne Sapiro3, Arash Bashirullah1. 1) Sch Pharmacy, Univ Wisconsin, Madison, Madison, WI; 2) Lab Genetics, Univ Wisconsin, Madison, Madison, WI.

The steroid hormone ecysdrome triggers the rapid destruction of obsolete larval tissues during metamorphosis by inducing expression of death activators reaper (rpr) and head involution defective (hid), resulting in the activation of caspases. In a large-scale EMS mutagenesis screen for lethal mutations that die during metamorphosis, followed by a dominant modifier screen using GMR-rpr and GMR-hid, we identified 38 complementation groups that dominantly suppress the GMR-rpr and/or GMR-hid eye phenotype. We have already identified mutations in two core regulators of apoptosis (drong and diap1) and more importantly, we have identified a collection of new death regulators. The most frequently hit complementation group mapped to a novel gene, which we have named bulls for “immortal” in Korean. bulls mutant animals block the destruction of all tissues normally fated to die during metamorphosis, have a reduced ability to activate caspases and are impervious to death activators. Our preliminary data indicates that bulls acts by regulating transcription of apotosome components drong and dark (homologs of vertebrate caspase 9 and Apaf-1, respectively). bulls is one of several complementation groups identified in our screen with similar phenotypes, suggesting that we may have identified a new class of death regulators. We have also shown bulls is itself regulated by PI3K/Akt survival signals, thus may provide a link between signal survival signals and apoptosis. Thus, we present evidence that bulls defines a novel pathway that regulates the ability to activate caspases during development.

281B

Drosophila ASMase is required for apoptosis and photoreceptor homeostasis. Zhongua Liu1, Zhiguo Ma12, Xun Huang1. 1) Institute of Genetics and Developmental Biology, Beijing, Beijing, China; 2) Graduate School of Chinese Academy of Sciences, Beijing, China.

The sphingomyelin-ceramide pathway is a ubiquitous signal transduction system that regulates many cellular functions including apoptosis. Acid sphingomyelinase (ASMase), a key enzyme of sphingolipid metabolism, hydrolyzes sphingomyelin to ceramide. Mutations in human ASMase gene could lead to Niemann-Pick type A (NPA) disease, although the exact cause of disease pathology remains to be determined. In this study, we demonstrate that Drosophila ASMase promotes apoptosis and plays a critical role in maintaining photoreceptor homeostasis. In the absence of dASMase, the embryonic apoptotic cell death is greatly reduced. Overexpression of dASMase leads to retinal degeneration, which enhanced the small rough eyes caused by expressing the pre-apoptotic genes head involution defective (hid) and reaper (rpr) and could be suppressed by the expression of caspase inhibitor p35. Interestingly, overexpression of dASMase could also rescue the retinal degeneration in arrescin and norpA mutants, which affect the endocytic trafficking of photoreceptor Rhodopsin. Specifically, the expression of dASMase clears the high-molecular-weight Rhodopsin aggregates in norpA mutants. Our findings establish the roles for dASMase in the apoptosis and photoreceptor homeostasis.

282C

Identification of novel regulators of apoptosis required during metamorphosis. Yunsik Kang, Anne Sapiro, Sarah Ives, Yunsik Kang, Arash Bashirullah. Pharmaceutical Sciences & Laboratory of Genetics, UW-Madison, Madison, Madison, WI.

During metamorphosis, the steroid hormone ecysdrome induces apoptosis in obsolete larval tissues through expression of death activators reaper (rpr) and head involution defective (hid), overriding the inhibition of caspases by Drosophila inhibitor of apoptosis protein 1 (diap1) to trigger caspase activation. This destruction of obsolete tissues is essential for metamorphosis, and accordingly, mutations in known genes of the core apoptotic machinery, such as in the initiator caspase drong and caspase adaptor dark, result in lethality during metamorphosis. In order to identify new regulators of apoptosis, we performed a dominant modifier screen on a collection of 900 metamorphosis-specific EMS-induced lethal mutations on the third chromosome. We screened for mutations that dominantly suppress GMR-rpr and/or GMR-hid mediated ectopic death in the eye, and identified 38 complementation groups that appeared to act downstream of rpr and/or hid. To quickly map these mutations, we also screened the new Bloomington Deficiency Kit on the third chromosome, and found seventeen deficiencies that dominantly suppress GMR-rpr. Eleven of our complementation groups map to these deficiencies. Among these, we have mapped two loss-of-function alleles of drong and one gain-of-function allele of diap1. The isolation of mutations in both known components of the core apoptotic machinery on the third chromosome (dark is on the second chromosome) validates our genetic approach for identifying apoptotic regulators. We will present our progress in identifying the remaining 36 complementation groups, most of which appear to map to regions without known regulators of apoptosis.
283A


A major function of programmed cell death - apoptosis - is the removal of abnormal cells or cells damaged by stress events (irradiation, heat shock, etc). The apoptotic pathway in Drosophila is thought to be a linear cascade in which an initial stimulus induces the activity of pro-apoptotic genes (rpr and hid among others), whose function blocks the caspase inhibitor Diap1. The subsequent proteolytic activation of the caspases Drorne and Drice causes the death of cells. In stress-induced apoptosis the p53 gene and the JNK pathway initially activate rpr and hid. Here we demonstrate that p53 and JNK also function downstream of rpr and hid and that they establish a feedback loop that amplifies the initial apoptotic stimulus. This loop plays a key function in the apoptotic response because in its absence there is a dramatic reduction in the amount of cell death after triggering of apoptosis. Our results suggest that most of the apoptosis in Drosophila is mediated by the p53/JNK loop. Furthermore, they also demonstrate a mechanism of mutual activation of pro-apoptotic genes.

284B


The ability of ionizing radiation (IR) to induce apoptosis independently of p53 is crucial for successful therapy of cancers bearing p53 mutations. p53-independent apoptosis, however, remains poorly understood relative to p53-dependent apoptosis. IR induces both p53-dependent and p53-independent apoptosis in Drosophila melanogaster, making studies of both modes of cell death possible in a genetically tractable model (Wischnam et al., PNAS, 2006). So far, grapes (Chk1), JNK, and E2F1 and E2F2 are known to modulate the level of IR-induced p53-independent apoptosis, but none of these are absolutely required (McNamee & Bosdsky, Genetics, 2009; Wischnam et al., Dev Biol, 2010).

In order to identify genes that are required for p53-independent apoptosis, we are taking two approaches. 1. A forward genetic screen using UAS-dsRNA to knockdown p53 in a tissue-specific manner. This allows us to screen for dominant modifiers of IR-induced visible phenotypes in an F1 screen. 2. A genome-wide gene expression analysis for genes that are induced by IR in p53 mutants at the same time that p53-independent apoptosis is induced. Candidates thus identified are being tested for a direct role in apoptosis using classical mutants. Results from these studies will be presented.

285C

Cell type-specific control of apoptosis and cell survival in the developing Drosophila eye. Yun Fan, Andreas Bergmann. Dept Biochem & Molec Biol, UD MD Anderson Cancer Ctr, Houston, TX.

Despite our detailed knowledge of the biochemical mechanisms of the apoptotic execution machinery, a distinction about the control of apoptosis in cells of different developmental stages (for example proliferating, cell cycle-arrested, or differentiating) has rarely been made. The developing Drosophila eye imaginal disc provides a unique model system to address this in vivo as it is composed of mitotic cells, post-mitotic yet unspecified cells, and differentiating photoreceptor neurons. By expressing hid, a pro-apoptotic gene, we find that mitotic cells are extremely sensitive to hid-induced apoptosis. In contrast, cells that have exited the cell cycle become more resistant to apoptosis through distinct mechanisms. The survival of cell cycle-arrested yet unspecified cells depends on EGFR signaling. However, photoreceptor neurons become apoptosis-resistant through up-regulation of Diap1, the Drosophila inhibitor of apoptosis protein, as soon as they are specified. Mutant analysis indicates that Cullin-3-mediated protein degradation plays a critical role to determine the cellular level of Diap1. Moreover, as development proceeds, Diap1 is down-regulated in developmentally older photoreceptor neurons.

In summary, our study suggests that multiple mechanisms exist to regulate distinct apoptosis sensitivity and resistance of different cell types during development.

286A

Tumor suppressor Caliban coordinates with p53 and E2F1 to regulate DNA damage induced apoptosis and G1/S cell cycle transition. Xiaolin Bi1, Yajie Wang2, Zhe Wang2, Qing Yuan1, Peking Zhou1, Qm Wang2, Mark Mortin1, Deborah Hursht1. 1) CAS Key Laboratory for Biological Effects of Nanomaterials and Nanosafety, Chinese Academy of Sciences, Beijing,100049, China; 2) School of Life Sciences, Lanzhou University, Lanzhou, 730000, China; 3) Department of Radiation Toxicology and Oncology, Beijing Institute of Radiation Medicine, Beijing, 100050, China; 4) Laboratory of Molecular Genetics, NICHD, NIH, Bethesda, MD, 20892, USA; 5) Division of Cellular and Gene Therapies, CBER, FDA, Bethesda, MD, 20892, USA.

Apoptosis and cell cycle checkpoint are two of most important mechanisms to inhibit tumor. We previously identified Caliban, the Drosophila homolog of human tumor suppressor Sdceca1, as a bipartite nuclear export mediator. Here we investigate its roles at DNA damage response. Using genetic tractable model Drosophila, we found flies less sensitive than wild type. Caliban is sensitive to irradiation and tumor prone. Caliban is indispensable for DNA damage induced p53-dependent and -independent, yet E2F1-dependent apoptosis. To coordinate with p53 and E2F1 dependent apoptosis, Caliban shows a two stage activation after DNA damages, regulated by p53 and E2F1 respectively. Caliban has the pro-apoptotic function, Caliban induces apoptosis through regulating caspases activity. Moreover, Caliban is required for the establishment of G1/S checkpoint. Ecotopic expression of caliban suppresses tumor formation in athymic nude mice. We suggest Caliban is a downstream mediator between p53 and E2F1, to regulate DNA damage induced apoptosis and cell cycle progression.

287B

Mapping the genes that involved in cell death in cell competition. Abhijit T. Kale, Nicholas E. Baker. Dept Genetics, Albert Einstein Col Medicine, Bronx, NY.

Cell competition can be observed when some genetically different populations share the same developing compartment. One population (winner) progressively kills the other population (loser) and takes over the compartment. During competition between wildtype cells and Minute cells (heterozygous for a ribosomal protein gene), the Minute, loser cells are killed. By replacing the wildtype photoreceptor neurons with Minute cells, we can study cell competition. Unexpectedly, the Minute cells are not the only cells that are killed. Surprisingly, we find that the steady-state level of Diap1 in all cell types is negatively controlled by H99 genes, a group of major pro-apoptotic genes in Drosophila, although under these conditions the H99 genes do not trigger apoptosis. In summary, our study suggests that multiple mechanisms exist to regulate distinct apoptosis sensitivity and resistance of different cell types during development.

288C

Autophagy induced by mild ER stress protects against apoptosis in Drosophila photoreceptor cells. Clémence Levet, Antoine Fouillet, Marion Robin, Pierre Doourlen, Bertrand Molleireau. Laboratoire de Biologie Moléculaire de la Cellule, Ecole Normale Supérieure de Lyon, Lyon, France.

The unfolded protein response (UPR) is an evolutionarily conserved adaptive response to perturbations of normal endoplasmic reticulum (ER) physiology. Secreted and membrane-associated proteins are folded and assembled by chaperone proteins in the ER. In the event of an ER malfunction, unfolded proteins accumulate and generate an ER stress. ER stress is commonly associated with degenerative diseases, such as Parkinson’s and Alzheimer Disease, but its role in disease progression remains to be established. We use the drosophila eye to study the role of ER stress in apoptosis regulation. We first used a cell-death model by ectopically expressing pro-apoptotic genes such as reaper or diap3 in the photoreceptors (PRs). Next, to induce mild ER stress in the dying photoreceptor neurons, we incorporated a mutant for the ER-resident chaperone protein NinaA in the PR cells overexpressing Reaper. We observed PRs bearing ninaA mutation were protected from the expression of Reaper, indicating that mild ER stress protects PRs against apoptotic stimuli. We have demonstrated that an ER stress mediated signal inhibits caspases activation. Interestingly, we now found that an autophagic response was triggered in cells subjected to both an apoptotic signal and an ER stress signal. This result leads us to further investigate the role of autophagy in the control of cell death. We found...
that impairing autophagy abrogates the ER stress-mediated protective effect against the apoptotic stimulus. We also demonstrate that the activation of autophagy blocks caspases activation hence resulting in the inhibition of apoptosis. In conclusion, we demonstrate that moderate ER stress protects not only against the deleterious effects of unfolded protein accumulation, but also against external apoptotic stimuli through the activation of autophagy.

289A Direct and indirect roles of the mitochondria in caspase activation during Drosophila spermatogenesis. Liat Ravid, Eli Arama. Weizmann institute of science, Rehovot, Israel.

Being at the core of the intrinsic apoptosis pathway, mitochondria have emerged as the central regulators of the apoptotic program in mammalian cells, providing a reservoir for protein factors, such as cytochrome C, which induce caspase activation upon their release to the cytosol. Recent studies suggest that the release of pro-apoptotic factors from the mitochondria is regulated by mitochondrial dynamics and cristae structures. Whereas a role for cytosplasmic cytochrome C in the activation of the apoptosome complex and apoptosis has been well established, the mechanisms by which mitochondrial dynamics promote apoptosis are still poorly understood. The Drosophila sperm system is an ideal model to study the significance of the mitochondria for caspase activation, as we have previously shown that spermatids are the only known cells in Drosophila that absolutely require the mitochondrial pathway for caspase activation. Furthermore, we have identified eight different mutants in a genetic screen, which in addition to blocking caspase activation, also display severe defects in mitochondrial morphology. We mapped three of these mitochondrial mutants, one of which corresponds to one of the two Drosophila cytochrome c (cyc1) genes. Cyc1 is a component of the respiratory chain complex III, which passes electrons to cytochrome C, and from there to complex IV. As opposed to cyc1 mutant spermatids, which block caspase activation in spermatids, but still display an overall normal mitochondrial structural organization, fluorescent and electron microscopy analyses of cyc1 mutants revealed severe defects in the exit from the fused state, and hence the abnormal unfurling and elongation of the mitochondria. Since a role for Cyc1 in the regulation of caspase activation has never been demonstrated, we started investigating possible mechanisms. Collectively, our findings suggest that Cyc1 indirectly regulates caspase activation, independently of cytochrome C, by affecting energy metabolism and the structural organization of the mitochondria.

290B GENETIC SCREENS TO ISOLATE REGULATORS OF APOPTOSIS-INDUCED COMPENSATORY PROLIFERATION IN PROLIFERATING DROSOPHILA TISSUES. Shiuian Wang1,2, Yun Fan1, Andreas Bergmann1,2. 1) Biochemistry and Molecular Biology, University of Texas M.D. Anderson Cancer Center, Houston, TX; 2) Program in Developmental Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX.

In multi-cellular organisms, apoptotic cells are able to induce compensatory proliferation (CP) of neighboring cells for tissue homeostasis. By simultaneously expressing the proapoptotic gene head involution defective (hid) and the effector caspase inhibitor p35 under the control of eyeless-GAL4 (ey-GAL4) to keep cells undead, CP can be induced in the proliferating Drosophila eye tissue, leading to the overgrown head capsule. Notably, the overgrowth phenotype can be largely suppressed by loss of one copy of the initiator caspase dronc or its adaptor ark. We are therefore carrying out a modifier screen by using deficiency stocks. In a preliminary screen, 270 overlapping deficiencies spanning the whole second chromosome are screened. Among these, 22 deficiencies covering 10 separate genomic regions were identified to contain CP suppressors. To identify potential CP suppressors from the deficiency screen, we are subsequently conducting a gene-based screen by using UAS-RNAi lines. Given that CP may be a non-cell autonomous event, a more definitive assay to distinguish regulators in the dying (signal producing) cells from those in the neighboring cells is in need. Taking advantage of Dorsal-Eye GAL4 (DE-GAL4), which is specifically expressed in the dorsal part of the eye discs, in combination with a temperature sensitive GAL80 variant driven by tubulin promoter (tub-GAL80ts), we are generating a follow-up temperature shift assay to investigate CP suppressor candidates we obtained. In the temperature shift DE-GAL4-tub-GAL80ts assay, promising CP suppressors can be specifically characterized in dying cells. Further analyses of the DE-GAL4-tub-GAL80ts assay are currently ongoing.


Apoptosis, also referred to as type I cell death, is the major form of programmed cell death (PCD). Apoptosis depends on the action of proteases called caspases and is characterized by distinct morphological features. However, alternative cell death pathways have been also reported. Type II death displays the molecular and morphological features of autophagy, while necrosis (type III death) exhibits a necrotic morphotype manifested by a pronounced swelling of cytoplasmic organelles. Cell death pathways that exhibit mix morphologies were also reported and recent analyses of molecular networks point to many cross-talks among the different types of death pathways. Nevertheless, relatively little is known about the molecular mechanisms, constituents and physiological significance of these alternative death pathways. One reason for the slow progress in this field of research is the extremely few physiological paradigms available for studying alternative death pathways. We discovered and characterized a new noncanonical cell death pathway acting to normally execute about 20% of premeiotic germ cells (spermatogonia) in the adult Drosophila male. Using mutants in several key components of the apoptotic pathway, including caspases, we demonstrate that spermatogonial death is independent of the core apoptotic machinery. On the other hand, dying spermatogonia display high lysisosomal activity, and specific mutations in the lysisosomal pathway attenuate this death. Additional mechanistic studies and ultrastructural analysis are consistent with the idea that spermatogonial death is highly parallel to type I death pathway. Importantly, the mitochondrial sertine protease HhrA2/Omi was identified in a genetic screen as a positive regulator of this death pathway, providing a first physiological paradigm for the involvement of this protease in any type of cell death.

292A How Do Endocycling Cells Block Apoptosis? Bingqin Zhang1, Sonam Mehrotra2, Jhomar E. Marquez3, Brian R. Calvi1. 1) Biology, Indiana University, Bloomington, IN; 2) Department of Radiation Oncology, CINJ-UMDNI, New Brunswick, NJ; 3) Department of Biochemistry, Emory University School of Medicine, Atlanta, GA.

Eukaryotic cells have evolved multiple mechanisms to preserve the integrity of the genome including DNA damage checkpoints, cell senescence, and apoptosis. These mechanisms are major barriers against genome instability and cancer. Using Drosophila melanogaster as a model system, we seek to determine if cells in different developmental contexts have distinct responses to DNA damage. We have recently focused on cells that enter an endocycle, which is a variation of the classic mitotic cell cycle and consists of only G and S phases. We have shown that, unlike mitotic cycling cells, endocycling cells do not apoptose in response to DNA damage caused by replication stress or ionizing radiation. We are currently investigating the mechanism(s) by which endocycling cells block apoptosis using a combination of genetic, molecular, and biochemical approaches. We find that endocycling cells of the larval salivary gland have an enrichment of epigenetic silencing marks at the h99 locus that encodes the pro-apoptotic genes reaper, hid, grim and sicle. These endocycling cells also repress reaper and hid promoter reporters. These data favor a model wherein the pro-apoptotic genes at the h99 locus are epigenetically silenced in endocycling cells. We will present our results towards our ultimate goal of defining the mechanism for how the endocycle program is linked to the repression of apoptosis. This study will provide insights into the developmental regulation of the cellular response to stress and the decision to activate the apoptotic pathway. Discovery of conserved mechanisms that also regulate apoptosis in humans would have important implications for the predisposition of different tissues to cancer.


Apoptosis is used during development to eliminate specific cells for strict control of cell number. In developing organisms, cells are embedded in complex and fast-changing environments and understanding how supernumerary cells destined to die are chosen is thus a great challenge. During Drosophila oogenesis, polar cells, which act as organizing centers controlling many aspects of egg chamber development are produced in excess and are subsequently reduced to a pair of cells per egg chamber extremity. Reduction in polar
Basket and Draper regulate the engulfment of nurse cells by follicle cells during starvation induced mid-oogenesis cell death. Allison Timmons¹, Jon Iker Etchebarry¹, Tracy Pritchett¹, Elaine Welch², Kim Calle³. 1) Biology, Boston University, Boston, MA; 2) Albany State University, Albany, GA.

Programmed cell death and the subsequent removal of cell corpses is an important process in animal development and tissue homeostasis. Failure to engulf cell corpses can lead to leakage of cellular contents, secondary necrosis, and ultimately disease caused by inflammation. The engulfment of apoptotic cells is typically performed by professional phagocytes, such as macrophages. However, in tissues with limited accessibility to circulating cells, engulfment is performed by neighboring non-professional phagocytes, such as epithelial cells. The Drosophila ovary provides an excellent system to study engulfment because the germline can be induced to die by starvation and their remnants are subsequently engulfed by the surrounding epithelial follicle cells. Interestingly, JNK (basket) activity is induced in engulfing follicle cells, as visualized by the expression of a lacZ enhancer trap in puckered, a basket target. Furthermore, expression of a dominant-negative version of basket disrupts engulfment, which is evident by reduced enlargement of the follicle cells. Moreover, there is premature death of the follicle cells, as well as lingering nurse cell remnants. Additionally, draper, a known regulator of engulfment, is required for engulfment by the follicle cells in the ovary. Together, these data suggest a requirement for JNK signaling and Draper in the follicle cell’s transition from an epithelial cell to a phagocytic cell responsible for engulfing nurse cell corpses. Further investigations are currently in progress to identify additional genes involved in the control of engulfment in the Drosophila ovary.

Necrotic cell death is transcriptionally regulated in Drosophila. Kai Liu, Xinping Chen, Yuhong Li, Tao Wang, Lei Liu. School of Life Sciences, Peking University, Beijing, China.

Three major types of cell death in development and diseases have been identified, including apoptosis, autophagy and necrosis. In human, necrosis frequently occurs in acute-onset diseases such as stroke and traumatic brain injury. It requires specific enzymes, such as calpains and cathepsins. However, whether necrosis is regulated at transcriptional level is still unknown. By heat shock-induced expression of a constitutively opened ion channel (human Brain Na⁺ Channel 1, nscn), we named it “C16”, we generated a transient model of necrosis in Drosophila. We found that C16 caused the fly lethality, which was not rescued by genetic manipulation to block apoptosis or autophagy. Instead, it could be rescued by loss-of-function of calreticulin, calpain and cathepsin, which have been reported to involve in the necrosis in C. elegans. To understand possible pathways, we studied gene expression profile during necrosis. Expression levels of 707 genes were changed, including 40 genes involving in transcription. By genetic screen, we found loss-of-function regulation of necrosis, in a drug screen, we found a histone deacetylase (HDAC) inhibitor, tricostatin A (TSA), rescued the C16 lethality. To understand the mechanism, we screened the transcriptional targets of TSA. We found that the transcript of HDAC1 is rescued. Furthermore, the transcript of shn and Ncs increased in the C16 flies under the C16 mutant background. These results suggest necrotic cell death may be a regulated process requiring activation of specific transcription factors. Consistent with transcriptional regulation of necrosis, in a drug screen, we found a histone deacetylase (HDAC) inhibitor, tricostatin A (TSA), rescued the C16 lethality. To understand the mechanism, we screened the transcriptional targets of TSA. We found that the transcript of HDAC1 is rescued. Furthermore, the transcript of shn and Ncs increased in the C16 flies under the C16 mutant background. These results suggest TSA may protect from cell death through targeting on HDAC1 to down-regulate shn and Ncs expression. In conclusion, we found necrosis is a programmed process and requires transcriptional regulation. Manipulation of transcription by HDAC inhibitors may be an effective way to cure necrosis-related diseases in human.

Uncovering a novel cancer prevention mechanism in Drosophila through the analysis of a Hox responsive enhancer. Zongzhao Zhai¹, Nati Ha², Daniela Bezdan³, Ingrid Lohmann¹. 1) CellNetworks - Cluster of Excellence and BIOQUANT Center, University of Heidelberg, D-69120 Heidelberg, Germany; 2) Max Planck Institute for Developmental Biology, D-72076 Tübingen, Germany.

Cancer is a genetic disease where the coordination of cell differentiation, cell proliferation, and cell death is disrupted. Over the last several decades, many oncomgenes and tumor suppressor genes have been identified and the study of these genes shed light on the mechanisms of tumorgenesis. However, since cancer happens rarely in organisms, there could be previously uncharacterized mechanism existed that allows the organism to prevent cancer. HOX proteins, as master regulators of segmental identities, have not yet been linked to tumorgenesis in Drosophila. With bioinformatic and experimental approaches, we identified a HOX protein Abd-B dependent enhancer of the pro-apoptotic gene reaper (rpr), termed rpr-HRE-571. By sub-dissecting and further analyzing rpr-HRE-571, we found that transcription factor Cdt (Ct) represses endogenous rpr expression directly through the Ct binding sites on rpr-HRE-571. The repression of apoptosis allows the formation of filzkörper. Interestingly, Ct, the Drosophila homolog of human differentiation-specific factor CDP, was also found to be actively repressing apoptosis in many other tissues examined, thus allowing the formation of respective structures. Since the undifferentiated cells are eliminated by apoptosis when Ct function is disrupted, the undifferentiated undead cells generated by simultaneously reducing Ct function and inhibiting apoptosis could possess a risk of developing cancer. To test this hypothesis, we studied Ct function in a well-established eye cancer model. Generating undifferentiated undead cells in the eye cancer background significantly increased the severity of tumor phenotype and the occurrence of tumor metastatic event. Considering our previous observations, we propose that the active repression of apoptosis by a differentiation factor is pervasively used as a novel cancer prevention mechanism in Drosophila.
Cell competition is a cell-cell interaction observed in Drosophila melanogaster that results in the removal of cells from a developing tissue. "Loser" cells can be eliminated through apoptosis and engulfment by "winner" cells. Since loser cells on their own are not eliminated, the competitive ability of a cell must be determined by its interaction with neighboring cells. The molecular mechanism of cell competition is not well understood and requires identifying novel molecules that allow cells to detect and respond to competitive ability. Using a sensitized genetic assay in the eye imaginal disc, we identified two novel mutations that cause mutant cells to act as supercompetitors by reducing neighboring wildtype tissue as observed in the adult eye. One mutant, named jabsa, is homozygous viable and exhibits no obvious adult phenotypes, therefore may be specific to cell competition. In addition, preliminary experiments suggest jabsa mutant clones do not obviously perturb Mtor or Hippo signaling, two pathways previously reported to induce supercompetition. Meiotic mapping and complementation tests suggest these two mutations do not correspond to genes known to cause supercompetition, therefore identification of the underlying lesion may contribute to a better understanding of the genetic players that normally coordinate cell competition.

During cell competition in developing imaginal epithelia, cells with slower proliferation or growth rate are outcompeted by the adjacent faster-growing ones through c-Jun N-terminal kinase (JNK) pathway-mediated apoptosis, but how cells determine winners and losers and how apoptosis is induced in loser cells remain unclear. We recently identified mahjong as a novel binding partner of Lethal Giant Larvae (Lgl), a tumor suppressor protein. Homozygous terminal kinase (JNK) pathway-mediated apoptosis, but how cells determine winners and losers and how apoptosis is induced in loser cells remain unclear. We recently identified mahjong as a novel binding partner of Lethal Giant Larvae (Lgl), a tumor suppressor protein. Homozygous mahjong mutant (mahj-/-) flies do not have obvious pattern defects during embryonic and larval development but develop more slowly than wild-type flies. In the mosaic wing disc, however, mahj-/- cells show hallmarks of the loser cell behavior in cell competition: the mutant cells undergo apoptosis at the clone boundary but not at the center of the clone. Phosphorylation of JNK in the mutant cells is cell-autonomously increased, and inhibition of JNK activation suppresses apoptosis in these cells. To determine whether compensatory proliferation of the winner cells is essential for cell competition, we studied mahj+/- cells in mosaic follicular epithelia. Drosophila follicle cells undergo mitotic divisions up to stage 6. Afterward, they switch off the mitotic cycle and undergo endoreplication during stages 7-10, during which cells duplicate their genomic DNA without cell division. In the mosaic follicular epithelia of stage-10 egg chambers, mahj+/- cells are still outcompeted by the adjacent wild-type or mahj+/- cells, and no cell division was detected in the neighboring winner cells. These results suggest that cell competition can occur in nondividing cells and that the proliferation rate is not the primary determinant of winners and losers. In the follicular mosaic, intriguingly, the JNK pathway is down-regulated in the mahj+/- cells but activated specifically in the wild-type or mahj+/- cells adjacent to the mahj-/- cells. These findings indicate that the mechanism for inducing apoptosis in loser follicle cells differs from the one described in imaginal epithelia.

**Identification of novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

**Identifying novel genes controlling cell competition.** Justin A. Bosch, Yassi Hafezi, Kevin Gandhi, Iswar Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.
Characterization of \(l(2)37C\), the Drosophila ortholog of Prohibitin1. Stephanie Toering Peters. Biology Dept, Warburg College, Waverry, IA.

Prohibitlin (PHB1) is highly conserved among all eukaryotes, and has been identified among multiple diverse molecular functions including mitochondrial chaperone, cell signaling protein, and transcriptional repressor. The Drosophila ortholog of PHB1 is \(l(2)37C\), a gene first identified in 1981 based on its location next to \(dopa decarboxylase\) on the second chromosome. Multiple alleles of \(l(2)37C\) were generated in screens looking for \(ddc\) mutants, and are the only published mutant alleles of a PHB1 ortholog in a multicellular organism. The existence of these alleles combined with the wealth of genetic and molecular tools available in Drosophila makes Drosophila the ideal system to learn more about the function of this highly conserved protein. Characterization of flies heterozygous for a lethal and hypomorphic allele of the Drosophila ortholog of PHB1 is important for the control of overall organ size, and has implications in other growth regulatory interactions. Here we present evidence suggesting that cell death induced by Hippo activation depends on the activity of pro-apoptotic genes indicating that Hippo pathway controls organ size by coordinately regulating cell proliferation and cell survival. Current work is focused on the molecular characterization of the \(l(2)37C\) alleles and the localization of the \(Cc\) protein in embryos.

**POSTER: Cell Division and Growth Control**

See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.
In the adult thorax, external sensory organs develop from a single sensory organ precursor (SOP) that divides asymmetrically to generate daughter cells (pIIb and pIIa) whose dephosphorylates PI(4,5)P2 on internal membranes to restrict this phosphoinositide at the plasma membrane and thereby regulates cleavage furrow stability. Identification of localized on giant cytoplasmic vaccuoles rich in PI(4,5)P2 and in endosomal markers. We demonstrate that dOCRL is associated with endosomes and we propose that it (PIP) cycle in Drosophila. We show that depletion of the drosophila ortholog of human OCRL1, an enzyme mutated in the X-linked disorder oculocerebrorenal Lowe syndrome, furrowing and binucleation. To understand how PI(4,5)P2 is regulated during cytokinesis, we individually knocked-down each of the enzymes controlling the phosphoinositide important role by recruiting essential proteins of the cytokinesis machinery. Consistent with a role during cytokinesis, perturbation of PI(4,5)P2 regulation leads to abortive During cytokinesis, constriction of an equatorial acto-myosin ring physically separates the two daughter cells. At the cleavage furrow, the phosphoinositide PI(4,5)P2 plays an important role by recruiting essential proteins of the cytokinesis machinery. Consistent with a role during cytokinesis, perturbation of PI(4,5)P2 regulation leads to abortive furrowing and binucleation. To understand how PI(4,5)P2 is regulated during cytokinesis, we individually knocked-down each of the enzymes controlling the phosphoinositide (PIP) cycle in Drosophila. We show that depletion of the drosophila ortholog of human OCRL1, an enzyme mutated in the X-linked disorder oculocerebrorenal Lowe syndrome, triggers high rate of cytokinesis failure. In absence of the inositol 5-phosphatase dOCRL, several essential components of the cleavage furrow were found to be incorrectly localized on giant cytoplasmic vacuoles rich in PI(4,5)P2 and in endosomal markers. We demonstrate that dOCRL is associated with endosomes and we propose that it dephosphorylates PI(4,5)P2 on internal membranes to restrict this phosphoinositide at the plasma membrane and thereby regulates cleavage furrow stability. Identification of dOCRL as essential for cell division may help explain why mutations in any of their orthologs results in a common human disorder, microcephaly.

309C

Differences in Astral vs Anastral Spindle Assembly Using Genetic and Proteomic Approaches. Oscar A Cabrera1, Violeane Mottier-Pavé2, Timothy L Megraw1 . 1) Biomedical Sciences, Florida State University College of Medicine, Tallahassee, FL; 2) Pharmacology, University of Texas Southwestern Medical Center, Dallas, TX.

Centrosomes are analogous to centrioles prior to PCM recruitment. Importantly, we find that purified cytoplasmic PCM complexes directly bind to stripped-centrosomes. Accordingly, Sas-4 appears to tether Sas-4-PCM complexes to a centriole via its C-terminal domain. The fact that these PCM proteins reassemble into cytoplasmic complexes may help explain why mutations in any of their orthologs results in a common human disorder, microcephaly.

310A

A novel Centrosomin partner regulates spindle orientation and cell polarity in neural stem cells. Jieyan Chen. Biomedical Sciences, Florida State University, Tallahassee, FL.

Asymmetric division is a crucial aspect of stem cell division to regulate cell differentiation during development. During neurogenesis, asymmetric mitosis of the neuroblast gives rise to a renewed daughter neuroblast and one differentiated ganglion mother cell (GMC), while two rounds of asymmetric division in sensory organ precursor cells (SOPs) produce specified cells of the external sensory organ. To ensure proper asymmetric division, cell fate determinants in the cell must be appropriately polarized, and then the mitotic spindle must orient correctly along the polar axis in order for polar division to be accomplished. Fate determinants such as aPKC, Par-6 localize to the apical cell cortex to specify the stem cell fate, while Miranda, numb etc. are enriched basally to specify the differentiating cells. We have identified a novel protein that is required for proper spindle orientation and cell polarization. Homozygous mutants exhibit extra (doubled) bristles and sockets on the notum, indicating defects in SOP asymmetric division. Additionally, cell fate determinants were mislocalized in asymmetrically dividing mutant larval neuroblasts, and there is a delay in spindle orientation along the apical-basal axis. These effects on asymmetric division may involve the centrosome as we have identified a physical interaction between this novel protein and Centrosomin, a centrosome core component. However, astral microtubules, which anchor the spindle to the cortex, are not overtly disrupted in mutant neuroblasts. Other phenotypes include wing defects and maternal effect lethality. We will present a model for how this novel molecule links coordination of activities at the centrosome to polarization of stem cells.

311B

Design of new Drosophila acentriolar cell lines for studying mitosis in absence of centrosome. Alain Debec1, Nicolas Lecland1, Nicolas Malmanche2, Sara Pereira2, Helder Maia2, Antoine Guichet1 . 1) IJM, UMR 7592, Paris, France; 2) CID lab, IBMC, Porto, Portugal.

In animal cells the centrosome is the assembly mechanism of the mitotic spindle. However an alternative pathway allows spindle establishment in absence of centrosomes, the chromatin pathway, which remains poorly understood. In order to better characterize this process, we have established new Drosophila cell lines able to divide without centrioles. In Drosophila, the protein DSas-4 is essential for the duplication of centrioles. We established 11 cell lines from embryos derived from flies heterozygous for the loss of function mutation DSas-4-6, 6 homozygous mutant lines were retained. These lines present characteristics of acentriolar cells. The pericentriolar material proteins D-PLP, centrosomin, γ-tubulin are not gathered in a centrosome. The mitotic spindle does not show any acentriolar microtubules. The spindle poles are poorly focussed and generally reach the plasma membrane. Preliminary ultrastructural analysis confirms an absence of centrosomes in these cells. An in vivo study of the formation of the acentriolar spindle was carried out using spinning disc confocal microscopy to follow the dynamics of microtubules with Jupiter-GFP. In prophase, acentriolar mitoses show microtubules nucleated from multiple points close to the chromosomes. These nascent microtubules are then captured by kinetochores allowing the expansion of kinetochore fibers which will constitute the scaffold of the future acentriolar spindle. Each chromosome seems to build its own mini spindle independently, and these structures then fuse into a single edifice. Microtubules depolymerization / repolymerization assays show clearly the formation of acentriolar spindle upon an "inside out" process, contrarily to the wild type cells. These cells keep a spindle assembly checkpoint, they are transf ectable, respond to RNAi and thus constitute a simple biological model to dissect the chromatin pathway. They will also represent an excellent system to study centrosome biogenesis.

312C

dOCRL controls P(4,5)P2 homeostasis and is necessary for cleavage furrow stability during cytokinesis. Khaled Ben El Kadhi, Chantal Roubinet, Sara Solinet, Grégory Emery, Sébastien Carréno. ICR - University of Montreal, Montreal, Canada.

During cytokinesis, constriction of an equatorial acto-myosin ring physically separates the two daughter cells. At the cleavage furrow, the phosphoinositide P(4,5)P2 plays an important role by recruiting essential proteins of the cytokinesis machinery. Consistent with a role during cytokinesis, perturbation of P(4,5)P2 regulation leads to abortive furrowing and binucleation. To understand how P(4,5)P2 is regulated during cytokinesis, we individually knocked-down each of the enzymes controlling the phosphoinositide (PIP) cycle in Drosophila. We show that depletion of the drosophila ortholog of human OCRL1, an enzyme mutated in the X-linked disorder oculocerebrorenal Lowe syndrome, triggers high rate of cytokinesis failure. In absence of the inositol 5-phosphatase dOCRL, several essential components of the cleavage furrow were found to be incorrectly localized on giant cytoplasmic vacuoles rich in P(4,5)P2 and in endosomal markers. We demonstrate that dOCRL is associated with endosomes and we propose that it dephosphorylates P(4,5)P2 on internal membranes to restrict this phosphoinositide at the plasma membrane and thereby regulates cleavage furrow stability. Identification of dOCRL as essential for cell division may help explain why mutations in any of their orthologs results in the molecular basis of the phenotypic manifestations of the Lowe syndrome.

313A

The role of Septins during asymmetric cell divisions of sensory organ cells. Nabila Fournounou, Roland Le Borgne. CNRS UMR 6061-IGDR Université de Rennes 1.

In the adult thorax, external sensory organs develop from a single sensory organ precursor (SOP) that divides asymmetrically to generate daughter cells (pIIb and pIIa) whose fate is governed by differential Notch activation. To identify new regulators of binary cell fate decisions, we performed a tissue-specific dsRNA screen and report that silencing of four out of the five Drosophila septins, including peanut (pnut), leads to a Notch loss-of-function phenotype. These results were further confirmed by a null allele of pnut, indicating that Septins are positive regulators of Notch dependent decisions. By investigating Sep2::GFP dynamics, we observed a rapid enrichment at the presumptive cleavage furrow during metaphase-to-anaphase transition, where it colocalizes with endogenous Pnut. Sep2::GFP staining is lost in pnut mutant cells, indicating that Septins form hetero-oligomers. Septins are small GTPases acting mainly as a diffusion barrier during cytokinesis. Interestingly, in our dsRNA screen we also identified Orc6, previously reported to
regulate Septins’s GTPase activity. We report that loss of Orc6 phenocopies pnut loss-of-function phenotype, and that in orc6 mutant cells Sep2::GFP and Pnut are no longer restricted to the cleavage furrow. These results prompted us to follow asymmetric cell divisions of pnut mutant sensory organs expressing Partner of Numb::GFP (Pom::GFP) by MARCM technique. In the absence of Pnut, SOP specification and unequal segregation of Pom::GFP occurs properly during SOP mitosis. However, SOP abscission failed giving rise to a binucleated cell, which adopts the pbl-like identity. The pbl-like cell then undergoes two rounds of division but before the completion of cytokinesis, Pom::GFP that was initially asymmetrically localized, partitioned equally in the two daughter cells. FRAP analysis indicate that Pom::GFP is leaking from one cell to the other suggesting that Septins act as a diffusion barrier at this stage. Together our data point to the mechanistic role of Septins and Orc6 during mitosis in asymmetrically dividing cells.

314B Exploring the role for FoxO in body size regulation by the integration of insulin and ecdysone signaling. Takashi Kuyama, Christen Mirth. Instituto Gulbenkian de Ciência, Oeiras, Portugal.

Body size regulation, a conspicuous and fundamental process in any organism, is best understood in insects. In the fruit fly Drosophila melanogaster, larvae regulate their final body size by controlling both growth, through insulin/insulin-like growth factor signaling (IIS) and target of rapamycin signaling, and regulating the duration of the growth period, via the steroid hormone ecdysone. Recent studies have shown that these two processes interact; IIS stimulates ecdysone synthesis from the prothoracic gland by up-regulating ecdysone biosynthesis genes. How this occurs is still unclear. In mammalian and nematode cells, the IIS modulates steroid hormone signaling through the interaction between a downstream component of the IIS, forhead box type O (FoxO), and nuclear hormone receptor superfamily members. Given this, we have hypothesized that the IIS modulates ecdysone signaling via interactions between FoxO and the functional ecdysone receptor, Ecdysone Receptor (EcR) and Ultraspiracle (Usp) heterodimer complex. To examine this hypothesis, we have explored whether FoxO is involved in ecdysone synthesis in the prothoracic gland (PG), which produces ecdysone, and whether FoxO and EcR and/or Usp associate in Drosophila. We report that overexpression of FoxO RNAi in the PG induces premature patterning of the imaginal discs in early third instar larval fed on sucrose alone, suggesting ecdysone synthesis occurs prematurely. Furthermore, we found that FoxO binds to Usp directly and not to EcR. This FoxO-Usp association occurs independently of 20-hydroxyecdysone, suggesting that the complex may potentially regulate the ecdysone signaling in nutrient-dependent but ecdysone-independent manner. These exciting discoveries suggest that direct interaction between the ecdysone and insulin pathways could control ecdysone synthesis, thereby regulating the duration of the growth period.

315C Homolog Coorientation at Metaphase Arrest in Female Meiosis. William D. Gilliland, Shane Gillies, Klateriaa Pyrtel, Wonbeom Paik, Adrian Segura, Maria Uhler, Nneka Wallace. Department of Biological Sciences, DePaul University, Chicago, IL.

During female meiosis I, homologous chromosomes must coorient to attach to opposite spindle poles. The recent discovery that chromosomes congress prior to metaphase I arrest in Drosophila requires a reexamination of the mechanisms behind chromosome nondisjunction. To examine this, we have studied a series of altered disjunction (ald) mutant alleles generated by imprecise excision of a P element in the 5’ UTR. These mutants are still competent to complete congression, but produce varying levels of wildtype Ald protein. The change in Ald dosage results in varying nondisjunction rates, ranging from wildtype to near-random segregation, all within an otherwise identical genetic background. The nondisjunction rate for these alleles will be measured genetically, and chromosome congression rates for these alleles will also be measured cytologically using chromosome-specific fluorescent in situ hybridization (FISH). If nondisjunction is caused by congression errors that result in maloriented homologs at metaphase arrest, then these two rates should be equal.

316A Gamma-tubulin localizes to the anasterial meiosis I spindle in Drosophila oocytes. Stacie E. Hughes1, J. Scott Beeler1, Brian Slaughter1, Angela Seale2, R. Scott Hawley2,3. 1) Stowers Inst Med Res, Kansas City, MO; 2) Department of Physiology, University of Kansas Medical Center, Kansas City, Kansas, United States of America.

The oocyte meiosis I spindle in many species, including Drosophila melanogaster, lacks centrosomes. Previous studies by multiple labs had failed to detect localization of the centrosome component gamma-tubulin on meiosis I spindles in Drosophila oocytes, suggesting that gamma-tubulin did not function directly in bipolar spindle formation. Examination of oocytes containing a point mutation in the oocyte enriched gamma-tubulin, ytab37C, using both live and fixed methods suggest a role for gamma-tubulin in the efficient formation and maintenance of bipolar spindles and in the proper alignment of chromosomes on the metaphase plate during prometaphase I. Organized spindle were absent in some mutant oocytes. In many other mutant oocytes the metaphase I spindles displayed wide, non-tapered spindle poles and abnormal chromosome configurations. The majority of metaphase I mutant oocytes showed relatively normal chromosome configurations in short bipolar spindles. Up-to-date careful examination, gamma-tubulin was detected on the meiosis I spindle in wild-type oocytes. Gamma-tubulin was found along the length of the spindle, overlapping with alpha-tubulin rather than localizing exclusively to spindle poles. D-TACC localization was aberrant in both prometaphase I and metaphase I spindles in ytab37C mutant oocytes. These data suggest a direct role for gamma-tubulin in the mechanism to form and maintain a bipolar spindle during prometaphase, though additional mechanisms appear to exist that can eventually taper the spindle poles when yTab37C fails to function properly.


Ca2+/CaM-dependent phosphatase calcineurin is essential for exit from meiotic arrest at metaphase I or II in Drosophila and vertebrate oocytes. We previously found that Sra, the Drosophila RCAN (regulator of calcineurin), acts as a positive regulator of calcineurin and functions to complete female meiosis. However, the mechanism by which Sra regulates calcineurin and the upstream signaling pathway that regulates both proteins are unknown. Here, we demonstrate that phosphorylation of Sra plays an important role in calcineurin regulation. A search for phosphorylated residues by the post-translational modification (PTM) analysis revealed that Sra is highly phosphorylated at Ser448, Thr451 and Ser519 in the ovary. Functional analyses using mutant forms of Sra showed that Ser448, GSK-3p-phosphorylatable residue and its priming kinase site by MAPK, Ser519 are essential for Sra function. Furthermore, genetic clones lacking shogg (Drosophila GSK-3p) fail to complete meiosis and show a meiotic arrested anaphase I chromosome configuration, which is identical to those seen in calcineurin and sra mutants. These results suggest a novel role of GSK-3p in calcineurin activation via Sra phosphorylation and completion of meiosis upon egg activation in Drosophila.

318C A role for Deco in meiotic cohesion rejuvenation and chiasma maintenance. Katherine A Weng, Charlotte A Jeffreys, Sharon E Bickel. Department of Biological Sciences, Dartmouth College, Hanover, NH.

Human meiosis has high error rates and most aneuploid gametes arise from chromosome segregation errors during female meiosis I. These errors are the leading cause of birth defects and miscarriages, and as women age, the risk of aneuploid pregnancy increases exponentially. Accurate chromosome segregation in human oocytes requires that meiotic cohesion remain intact for decades and work in model organisms indicates that deterioration of meiotic cohesion over time may be a major determinant of age related aneuploidy. One unresolved question is whether oocytes rely exclusively on cohesive linkages that are established during meiotic S phase or if maintenance of meiotic cohesion is an active process that requires re-establishment of cohesive linkages throughout the extended period of prophase I. We are using Drosophila oocytes to unravel the normal mechanisms that facilitate cohesion maintenance during meiotic prophase. Deco is the Drosophila homolog of the yeast cohesin establishment factor, Eco1, which is required to establish cohesive linkages during S phase. To investigate the role of Deco in meiotic cohesion maintenance, we knocked down Deco after S phase using a Gal4/UAS inducible system to drive the expression of a deco RNAi hairpin during prophase I in the female germ-line of deco/+/ flies. Because the synaptonemal complex (SC) depends on normal meiotic cohesion, we
used the SC protein C(3)G as a cytological marker to monitor cohesion and find that SC defects arise during mid prophase, after the RNAi driver is turned on. Moreover, we observe increased levels of meiotic nondisjunction (NDJ) when Deco is knocked down during prophase I. Both the SC and NDJ phenotypes are enhanced when Dicer-2 overexpression is used to increase RNAi efficiency. These results argue that Deco is required for cohesion maintenance and support the hypothesis that active rejuvenation of meiotic cohesion during prophase I is required for chiasma maintenance. We are currently using both genetic and cytological assays to monitor chiasma maintenance directly in Deco knockdown oocytes.

319A
Meiosis-Specific Control of the Anaphase Promoting Complex by Cortex. Zachary J. Whitfield, Terry L. Orr-Weaver. Whitehead Institute and Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA.

Regulation of protein degradation plays a pivotal role in progression of the cell cycle. The Anaphase Promoting Complex/Cyclosome, a large multi-subunit protein complex, targets specific proteins to the proteasome via ubiquitination. While canonical targets such as Securin, Cyclin A and Cyclin B must be degraded to permit exit from both mitosis and meiosis, there are key differences in the proteolysis pathway between these two division cycles. To further explore the unique process of meiosis, we are investigating a meiosis-specific form of the APC/C. During Drosophila female meiosis, two APC/C forms are active, with both APC/C(Cdc20) and APC/C(Centr) playing necessary roles in the completion of meiosis. The non-redundancy between APC/C(Cdc20) and APC/C(Centr) suggests differential regulation and/or unique substrate recognition.

We are taking numerous approaches to identify potential substrates and regulators of Cortex. First, mass spectrometry was used to identify proteins that bind to a functional Myc-Cort fusion protein expressed in the ovary. In addition to known interactors such as the APC/C subunits, numerous other classes of proteins were found. Proteins involved in processes such as kinetochore binding, microtubule binding/motors, chromosome segregation and mitosis/meiosis were preferentially immunoprecipitated by Myc-Cort over a control. These proteins are candidate substrates and regulators of Cortex. Secondly, Cortex was found to be phosphorylated using gel shift assays combined with the Phos-tag compound. Potential phosphorylation sites were then localized to the N-terminal 50 amino acids by mass spectrometry. These sites may play an important role in the regulation of Cortex and could shed light on potential differences in regulation between Cortex and Cdc20.

320B
SOLO associates with SMC1 in vivo and is required for various aspects of Drosophila female meiosis. Rihui Yan, Bruce McKee. Dept Biochem, Cell, Molec Biol, Univ Tennessee, Knoxville, TN.

Recombination between homologous chromosomes and cohesion between sister chromatids are jointly required for chromosome segregation in meiosis in many organisms. However, the role and mechanism of cohesion in Drosophila meiosis are not well understood as yet. Mutations of solo, which encodes a cohesion protein previously found to be involved in Drosophila male meiosis, cause severely reduced fertility and homologue and sister chromatid nondisjunction of both sex chromosomes and autosomes in female meiosis. Reduced frequencies of homologous recombination and the loss of inhibition of sister chromatid recombination in solo females are probably due to the delayed repair of double-stranded DNA breaks. Synaptosomal complex assembly and SMC1 localization to chromosomes are defective in early meiotic stages in solo ovaries. SOLO appears on chromosomes, at centromeres and on synaptonemal complexes, prior to meiosis I and colocalizes with the cohesion component SMC1 and the synaptosomal complex (SC) component C(3)G in meiosis. Moreover, SOLO physically interacts with SMC1 in vivo. Our studies about SOLO in Drosophila suggest that SOLO is a novel cohesion protein in Drosophila and is required for DSBR, homologous recombination and chromosome segregation during Drosophila meiosis. Furthermore, the timing of SOLO expression and defects of SC in solo mutants suggest SOLO may be required for the assembly of SC.

321C
Dynamics of Moe activation creates a pattern of differential cortical stability to control cell division. Sébastien Carrero1, Barbara Decelle1, Gaetan Chicanne2, Bernard Payrastré, François Payre2, Chantal Roubinet1,2. 1) IRIC - University of Montréal, Montréal, Canada; 2) Université de Toulouse UPS, Centre de Biologie du Développement, Toulouse, France; 3) Université de Toulouse UPS, Centre de Physiopathologie de Toulouse , France.

Cell division involves a stereotyped series of cell shape transformations. The molecular mechanisms orchestrating the temporal and spatial control of cortical organization that underlies these transformations remain, however, poorly understood. Using functional and live imaging approaches in Drosophila cells, we show here the existence of two regulatory mechanisms that coordinate cortical reorganization through the localized activation of the actin/membrane linker Moesin. A balance between the Slk kinase and the PI(4,5)P2 phosphatase activities provides a temporal control of Moes phosphorylation, and restricts high levels of Moes activity after the onset of mitosis. In addition, we show that dynamics of PI(4,5)P2 cortical distribution spatially control Moe activation during the late steps of mitosis. Through a systematic analysis of Drosophila enzymes implicated in PI(4,5)P2 production, we identify two complementary pathways relying on the PI(4)P-5 kinase Skittles and the tumor suppressor Pten. These pathways collectively regulate local PI(4,5)P2 levels at the cortex of dividing cells. This interplay between membrane phosphoinositides and Moesin plays a key role in regulating stiffness and dynamic organization of the mitotic actin cortex, which in turn is essential for proper cell division.

322A
Stretched lagging chromosomes during anaphase increase spindle length and cell size. Shaila R. Kotadia1, Anne Royou2, William Sullivan1. 1) University of California Santa Cruz, Santa Cruz, CA; 2) Institut Jacques Monod, Paris, France.

Acentric chromosome fragments, generated from I-CreI induced double-strand breaks, form chromatin-based tethers, facilitating their faithful segregation during anaphase. Tethers stretch and thereby create very long chromosomes due to the centric fragment moving towards the pole, while the acentric fragment lags at the metaphase plate. By live-imaging acentric chromosomes in dividing neuroblast cells, we discovered that the abnormally long, tethered chromosomes induce dramatic increases in spindle length and cell size. Additional insight into this adaptive response comes from dicentric chromosomes. Dicentric chromosomes either stretch and break, or remain intact, spanning the metaphase plate. We find that only dicentrics that stretch and break produce increases in cell size. We have also discovered that another unusually long chromosome, the compound chromosome 2, stretches, but does not break, during anaphase. Significantly, the compound chromosome 2 induces increases in spindle length and cell size, similar to the acentrics and broken dicentrics. Given these results, in some instances, chromatin remaining on the plate during anaphase induces increases in spindle length and cell size. We speculate that only stretched chromatin under excess tension is capable of inducing these cellular responses. Finally, we have identified central spindle components mediating these responses, giving us insight into the structural changes required for increases in spindle length and cell size.

323B
Drosophila aPKC is required for mitotic spindle orientation in larval wing disc epithelium. Rui G. Martinho1, Leonardo Guilgur1, Pedro Prudencia1, Andre Ros2, Tania Ferreira1, Ana Pimenta-Maques1, Buzz Baum2, Rui G. Martinho1. 1) Instituto Gulbenkian de Ciencia, Oeiras, Portugal; 2) MRC-Laboratory of Molecular Cell Biology, UCL, London, UK.

Loss-of-function alleles of atypical protein kinase C (apkc) show a complete loss of epithelial integrity in embryonic, follicular, and larval wing disc epithelia, which is likely to make a detailed analysis of the epithelial functions of apkc difficult. We took advantage of a temperature-sensitive allele of apkc (apkc<sup>ts</sup>) to generate graded reductions of apkc activity and analyze the responses to sub-optimal levels of apkc activity. From this work we concluded that 1) epithelial tissues showed dramatically different responses to sub-optimal levels of apkc activity, 2) apkc was required (in an otherwise apparently normal epithelium) for the correct planar orientation of the mitotic spindle during larval wing disc growth. Misorientation of the mitotic spindle along the apicobasal axis was associated with cell extrusion and apoptosis. 3) apkc was required for apical exclusion of Pins in dividing epithelial cells and mitotic cell rounding. These functions were likely to be important for mitotic spindle orientation. 4) Although viable and morphologically normal at
the permissive temperature, zygotic mutants of apc
d were nevertheless extremely sensitive to apoptosis inhibition, which induced the development of tumor-like tissues in the larval wing discs.

324C
Determining the molecular mechanisms of APC2’s cytoskeletal functions. John Poulton, Dave Roberts, Frank Mu, Mark Peifer. Dept. of Biology, University of North Carolina, Chapel Hill, NC.

Approximately 80% of colon cancers are caused by mutations in Adenomatous Polyposis Coli (APC). APC proteins are negative regulators of Wnt signaling, acting as part of the destruction complex that degrades the Wnt effector β-catenin (β-cat). APC proteins also have important cytoskeletal functions, but the mechanisms by which APC regulates the cytoskeleton remain unclear. In the syncytial stages of Drosophila embryogenesis, APC2 is hypothesized to mediate anchoring of cortical nuclei by tethering astral microtubules of mitotic spindles to cortical actin via a complex of β-cat and n-cat. This model predicts that APC2’s cortical localization and ability to bind β-cat are necessary for its cytoskeletal function. Consistent with this, we found that transgenic expression of APC2 lacking β-cat binding sites is unable to rescue the APC2 mutant phenotype. Full-length APC2 mislocalized to mitochondria is also unable to perform the cytoskeletal functions of APC2, suggesting cortical localization of APC2 is critical; importantly, this same mislocalized form of APC2 can rescue the Wnt signaling functions of APC2. Surprisingly, we found that APC2’s cytoskeletal functions require its ability to bind the destruction complex scaffolding protein Axin. We also found that Axin itself is required for tethering of cortical nuclei in the embryo. It is uncertain how Axin functions here, but it does not appear essential for cortical localization of APC2. Using live imaging of APC2 mutant embryos we discovered that loss of cortical nuclei does not appear to be solely based on defects in spindle attachment. Instead we observed complex misregulation of actin dynamics that appear correlated with loss of cortical nuclei, suggesting APC2 plays multiple roles in maintaining nuclei at the embryonic cortex. We are therefore testing a new model, in which APC2’s known role in centrosome separation promotes attachment of cortical nuclei by facilitating proper cycling of actin reorganization during mitosis.

325A
Characterization of the novel growth-regulating gene lig. Roland Baumgartner1, Carmen Rottig1, Alexander Wepf1, Matthias Gstaiger1, Hugo Stocker1, Ernst Hafen1. 1) ETHZ, Institute of Molecular Systems Biology (IMSB), Zürich, Switzerland; 2) Recipient of a DOC-fellowship of the Austrian Academy of Sciences at the Institute of Molecular Systems Biology.

Growth is a tightly regulated and essential process during animal development. Deregulated growth can result in overproliferation or size reduction, manifested in abnormal organ and body sizes or diseases like cancer. Despite extensive investigations on growth and size-controlling mechanisms, the picture of the growth-regulatory genetic network has remained incomplete. We performed an unbiased genetic forward screen based on the ey-FLP/FRT technique to identify new growth-suppressing genes. In addition to components of the insulin receptor/TOR and Hippo signaling pathways, our screen revealed mutations in lingerer (lig), which was originally identified in a screen for genes involved in copulation. Whereas lig mutants displayed moderate overgrowth of the eye and head structures, an excess of Lig protein induced apoptosis in eye and wing imaginal discs of third instar larvae. Using an affinity purification/MS approach, we identified two non-essential RNA binding proteins, Raspuin (Rin) and Fragile X mental retardation protein 1 (FMR1), as binding partners of Lig. Co-localization studies as well as genetic analysis confirmed the interaction of Lig with Rin and FMR1. Furthermore, rin and FMR1 double mutants were lethal and displayed a lig mutant phenotype. Thus, we propose that Lig is involved in a complex with Rin and FMR1 that probably contributes to translational control.

326B

To maintain tissue homeostasis, some organs are able to replace dying cells with additional proliferation of surviving cells. This process is referred to as compensatory or regenerative growth. Compensatory growth is particularly important in developing tissues and for liver regeneration following partial hepectectomy or chemical-induced injury. During Drosophila larval development, imaginal discs can recover from extensive damage induced by irradiation or genetic ablation, producing normally sized and patterned adult organs. The recovery of normal adult structures suggests that apoptotic cells are cleared from the epithelium and replaced by the compensatory growth of their neighbors. Relative little is known about how this type of growth is regulated or produced. In particular, whether compensatory growth uses novel growth pathways or is rather a reiteration of developmental growth remains largely unanswered. We have developed a genetic system that allows us to conditionally ablate significant portions of developing imaginal discs. Using this system, we have conducted an unbiased screen of chromosome 2L for genes involved in compensatory growth. To identify affected genes we have used a combination of traditional deficiency mapping and whole genome sequencing (WGS). Interestingly, two of the genes identified so far are chemotherapeutic targets. In addition, our WGS approach and analysis will be presented. While published reports have identified EMS-induced mutations from homoyguous individuals, we have adapted this technique to identify three of our mutations in heterozygous backgrounds.

327C
Jabba mediates sequestration of histones on embryonic lipid droplets. Zhihuan Li, Michael A Welte. Department of Biology, University of Rochester, Rochester, NY.

The eggs of many animal species contain surplus building materials thought to be crucial for embryogenesis. Newly fertilized Drosophila embryos, for example, contain enough histones to package the DNA of thousands of nuclei. Maternal histone deposits were proposed to support the extremely rapid mitoses of cleavage stages. Interestingly, a subset of these histones (H2A, H2B, and H2Av) is bound to lipid droplets; in fact, they are among the most abundant proteins on embryonic droplets. To find potential binding partners for histones, we biochemically purified droplets and identified the major proteins by SDS PAGE and mass spectrometry. Among the few candidates, we focused on the novel protein Jabba. As shown by immunolocalization and Western analysis, Jabba resides exclusively on lipid droplets. Embryos with reduced Jabba dosage have lower histone levels on droplets and lower embryonic histone stores. In embryos without Jabba, H2A, H2B and H2Av are absent from droplets and their maternal deposits are greatly diminished; yet, the embryos have normal levels of H3, a histone not sequestered on droplets. We propose that Jabba recruits histones to droplets and protects them from degradation. Surprisingly, Jabba mutant embryos develop grossly normally, demonstrating that the massive histone deposits in wild-type embryos are not essential for life. Histones might serve as a moonlighting function on droplets since in Jabba mutants lipid droplets are bigger. Yet, multiple lines of evidence suggest that histones can be transferred from droplets to nuclei, consistent with the storage model. In Jabba mutants, translation of maternal provided histone messages may compensate for the lack of histone protein stores, or this histone pool may be a contingency mechanism important under stressful conditions. Transient interactions between lipid droplets and proteins from other cellular compartments are widespread, but remain largely mysterious. The analysis of Jabba should provide a general paradigm how such “refugee” proteins are targeted to lipid droplets and how their sequestration is regulated.

328A
Developmental Timing and Regulation of Size. Marisa M Oliveira, Christen K Mirth. Instituto Gulbenkian de Ciência, Oeiras, Portugal.

The essential question of how size is regulated, from organelles to organisms, remains unsolved. A fundamental developmental process, if size regulation fails it can affect organ function as well as the whole body integrity and fitness. We aim to determine how mechanisms regulating nutrition-dependent development, developmental timing and tissue patterning are integrated to produce an organism of the appropriate size and proportions. To achieve this, we will first establish a temporal patterning map which will be used both as a measure for developmental timing and to better understand growth termination. This map will include the description of gene expression patterns representative of all of the major patterning cascades in the imaginal disc throughout the third instar larva, the stage at which most growth occurs. Next, we will examine the progression of this patterning...
map in larvae expressing a variety of growth suppressors/enhancers (ecdysone, DMyC, cyclin D/cdk 4, Insulin, and TOR) in the wing disc. This will allow us to address how the various growth pathways interact with the patterning of the imaginal discs. In addition, we will assess the effects of these growth suppressors/enhancers on developmental timing. Third, we will explore how different components of nutrition influence developmental timing and patterning of the imaginal discs. Using this approach, we hope to generate a more comprehensive view of growth control.

329B

PLC-γ is a regulator of growth and differentiation in animal cells. It is over-expressed in several human tumors and has recently been implicated as a key player in the transition to a metastatic state. There has therefore been interest in identifying inhibitors of PLC-γ as a potential anti-cancer therapeutic. Screens to identify novel PLC-γ inhibitors are made more difficult by the fact that loss of PLC-γ function is lethal in mouse. By contrast, Drosophila lacking PLC-γ function are viable and fertile, showing only a mild shortening of the wing and roughness in the eye - the small wing mutant phenotype. We have designed a screen to identify PLC-γ inhibitors among the 100,000+ molecules in the NCI DTP repository, looking for phenocopies of the small wing mutation. We are at the early stages of this screen and will present current progress towards identifying a novel PLC-γ inhibitor.

330C
Identification and characterization of a mutant allele of DNApol-c, DNApol-c<sup>DNApol-c<sup>Rpl10R</sup></sup>, gene in Drosophila melanogaster. Akanksha Verma<sup>1</sup>, Sonali Sengupta<sup>2</sup>, Subhash Lakhotia<sup>1</sup>.
1) Zoology, Banaras Hindu University, Varanasi, Uttar Pradesh, India; 2) School of Biosciences and Technology, Vellore Institute of Technology, Vellore, India.

DNApol-c is one of the members of DNA replication and repair machinery. In the absence of a conventional mutant allele of DNApol-c, there have been very few functional studies on this important enzyme. Using genetic and molecular approaches, we identified a novel mutant allele (DNApol-c<sup>DNApol-c<sup>Rpl10R</sup></sup>) of DNApol-c. During an earlier P transposon mutagenesis screen, a new recessive mutation (without any P transposon insertion) resulting in extended 3rd instar larval stage and culminating in 100% late larval or early pupal lethality, was isolated in our laboratory and was named as l(3)pl10R. Using recombination and deletion mapping, this mutant allele was placed within a 39kb region in the 94E13 to 94F1 interval, where seven genes, pointed, DNApol-c, ATPsyn-Cf6, sec13, RpS3, CG4408 and CG6784 are reported to exist. Available mutant alleles for all of these genes, viz., pointed, ATPsyn-Cf6, sec13, RpS3, CG4408 and CG6784 complemented the l(3)pl10R mutation, while Act5C-GAL4 driven DNApol-c<sup>Rnai</sup> expression did not complement the l(3)pl10R phenotype. PCR amplification and sequencing of the ~39kb interval revealed a base pair deletion in the promoter and other synonymous mutations in the coding region of the DNApol-c gene in the l(3)pl10R chromosome. RNA-RNA in situ hybridization revealed reduction in the levels of DNApol-c transcripts in l(3)pl10R homoygotes. In view of these results, we believe this mutant to be an allele of DNApol-c and have, therefore, renamed it as DNApol-c<sup>DNApol-c<sup>Rpl10R</sup></sup>. Homozygous DNApol-c<sup>DNApol-c<sup>Rpl10R</sup></sup> 3rd instar larva show small imaginal discs with fewer cells and reduced polyteny in salivary glands and other endo-derivating tissues. Such defects in cell growth and proliferation also suggest a compromised DNA polymerase function. Analysis of mosaic clones suggests that the DNApol-c<sup>DNApol-c<sup>Rpl10R</sup></sup> phenotype is cell autonomous. Availability of a mutant allele of the DNApol-c gene will help in functional characterization of its activity.

331A
Incorporating a model of chemical signalling factors into a cell-based model of growing epithelial tissues. Ruth E Baker<sup>1</sup>, Aaron Smith<sup>1</sup>, David Kay<sup>2</sup>, Philip K Maini<sup>2</sup>, 1) Centre for Mathematical Biology, Mathematical Institute, University of Oxford, 24-29 St Giles', Oxford, OX1 3LB, UK; 2) Oxford Centre for Integrative Systems Biology, University of Oxford, Department of Biochemistry, South Parks Road, Oxford, OX1 3QU, UK; 3) Oxford University Computing Laboratory, University of Oxford, Wolfson Building, Parks Road, Oxford, OX1 3QD, UK.

In this talk a comprehensive computational framework will be presented, within which the effects of chemical signalling factors on growing epithelial tissues can be studied. The method incorporates a vertex-based cell model, in conjunction with a solver for the governing chemical equations. The vertex model provides a natural mesh for the finite element method, with node movements determined by simple force laws. The arbitrary Lagrangian-Eulerian formulation is adopted to account for domain movement between iterations. The effects of cell proliferation and junctional rearrangements on the mesh are also examined. It is shown that in the case of the diffusion equation, mass is conserved and mesh quality does not deteriorate. The potential utility of the system is demonstrated in the context of Decapentaplegic (Dpp), a morphogen which plays a crucial role in the development of Drosophila imaginal wing discs. It is shown that by making the growth rate of cells dependent on Dpp concentration, the number of proliferation events increases in regions of high concentration. The method we describe may be adapted to a range of potential application areas, and even to other cell-based models with designated node movements, to accurately probe the role of morphogens in epithelial tissues.

332B

Tissue morphogenesis is fundamental to the formation of functional organs. To understand how tissues acquire size and shape we use long-term live imaging (>24 hr) and in house developed software to study the morphogenesis of the Drosophila pupal dorsal thorax epithelium. Particle image velocimetry (PIV) and segmentation based image analysis allows us to quantify tissue morphogenesis from the cell level to the tissue level. Here we describe both global tissue as well as individual cellular dynamics within the Drosophila dorsal thorax epithelium during metamorphosis. We aim at analyzing how mutations that are known to affect tissue size and shape impair tissue and cell morphogenesis.

333C
Homeodomain-interacting protein kinase inhibits Hippo signaling to promote growth during Drosophila development. Joanna Chen, Esther Verheyen. Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, Canada.

The Hippo (Hpo) pathway is the central mechanism that regulates tissue size by controlling both cell proliferation and apoptosis. The core components of the Hpo pathway, Hpo, Salvador, Warts (Wts) and Mats, form a kinase cascade to inhibit activity of Yorkie (Yki), the transcription regulator of the pathway. Inhibition or loss of Hpo signaling results in massive overgrowth due to unregulated target gene expression. From our previous studies, we demonstrated that homeodomain-interacting protein kinase (hikp) overexpression using wing-specific Ga4 drivers leads to wing overgrowth phenotypes. These overgrowth phenotypes suggest that Hikp may play a role in the Hpo pathway to control tissue growth. hikp misexpression in the Drosophila wing imaginal disc results in enhancement of phospho-Histone 3 staining and BrdU incorporation, suggesting that Hikp promotes cell proliferation. The wing overgrowth caused by hikp misexpression is enhanced by heterozygosity for hpo or wts, as well as in the background of ex and mats knock down by RNAi. In contrast, the hikp overexpression phenotype is suppressed by losing one copy of yki. In addition, we have found that overexpressing hikp induces expression of the Hpo target reporters, such as ex-lacZ, diap1-lacZ and cyclin E. These findings suggest that Hikp promotes growth by inhibiting Hpo signaling during development. Further studies will uncover the mechanism(s) of Hikp action in regulating Hpo signaling to advance our current understanding of growth and development.

334A

The conserved Hippo tumor suppressor pathway is a key kinase cascade that controls tissue growth by regulating the nuclear import and activity of the transcription co-activator Yorkie. Here, we report that the actin-Capping Protein αα heterodimer, which regulates actin polymerization, also functions to suppress inappropriate tissue growth by inhibiting

203
Molecular and phenotypic characterization of fried mutants. Carlisdania Mendoza1, Alison Lindsay1, Gaurav Saharia1, Lillian Chiu1, Jason Morris1. 1) Department of Natural Sciences, Fordham University, New York, NY; 2) Cognitive Science Program, McGill University, Montreal, Quebec, Canada.

fried loss-of-function mutants were isolated in a clonal screen for oogenesis defects. These mutants also show larval growth defects and developmental arrest phenotypes. The goal of this study is to characterize the gene disrupted in fried mutants and to further characterize the fried phenotype. By using a combination of genome sequence analysis and functional genomics, we have cloned a 300 kb region of the right arm of chromosome 3 (3R). We have carried out further deficiency mapping, complementation testing, and sequencing of candidate genes to rule out most of the original fried candidates. We have also begun characterizing the fried mutant growth defects. fried307 mutants die as small larvae on day three after egg deposition. fried47 mutants show a range of growth defects and can live until the pupal stage. We present further characterization of the fried phenotypes and progress in identifying the gene disrupted in fried mutants.

The Hippo signaling pathway regulates organ size homeostasis while its inactivation leads to severe hyperplasia in flies and mammals. The transcriptional co-activator Yorkie (Yki) mediates transcriptional output of the Hippo signaling. Yki lacks a DNA binding domain and is recruited to its target promoters as a complex with DNA binding proteins that promote cell survival and proliferation. Interestingly, F-actin also accumulates abnormally when Hippo pathway activity is reduced or abolished, independently of Yorkie activity, whereas overexpression of the Hippo pathway component expanded can partially reverse the abnormal accumulation of F-actin in cells depleted for Capping Protein. Finally, reduction in two other actin-regulatory proteins, the Cyclase associated protein Capulet and Cofflin, causes abnormal F-actin accumulation, but only the loss of Capulet, like that of Capping Protein, induces ectopic Yorkie activity. Taken together, these findings indicate a novel interplay between Hippo pathway activity and actin filament dynamics that is essential for normal growth control.
340A  
**Tumor induction in Drosophila by DNA minor groove binding ligands.** Kirill I. Kirsanov, Ekaterina A. Lesovaya, Olga Yu. Susova, Gemmadiy A. Belitsky, Marianna G. Yukovskaya. Dept. of Chemical Carcinogenesis, Blokhin CRC RAMS, Moscow, Russia.

Widely used in molecular biology DNA minor groove binding ligands (MGBLs) Hoechst33258 and Hoechst33342 represent fluorochromes quantitatively binding DNA in living cells. Evaluation of their genotoxicity in short-term assays is complex due to the mode of its action. They neither alkylate DNA nor form interstrand-crosslinks or DNA adducts. Hoechst33258 and Hoechst33342 bind DNA via electrostatic, hydrogen and van der Waals bonds and inhibit some enzymes, causing genetic damage in an indirect way. On account of that they are negative in the main Salmonella/mammalian microsome reverse mutation assay (Ames test) using TA98 and TA100 strains. Marginal mutagenic activity was detected only in a special TA102 strain. In mammalian cells in the conventional 6-thiouguanine resistance assay on Chinese hamster V79 cells Hoechst 33342 harbors mutagenic activity at highly toxic concentrations only. We evaluated blastomogenic activity of these compounds in Somatic Mutation and Recombination test (SMART) in Drosophila using wtsP4/wtsWT heterozygous larvae. The assay is based on the manifestation of the recessive wtsP4 mutant allele in mosaic clone due to inactivation of wild allele because of point mutation, chromosomal deletion or loss of heterozygocity through homologous recombination. Wts gene is involved in the proliferation and cell cycle regulation and its inactivation leads to overgrowth of imaginal discs cells. Larvae were fed with non-toxic concentrations of bisbenzimidazols. Both compounds produced significant increase wtsP4 clone frequency. These dyes exhibited dose-dependent blastomogenic effect. Moreover, Hoechst33342 showed twice higher clone frequency than Hoechst33258 and we have demonstrated that it was associated with different cell-membrane penetration of the agents. It was estimated by salivary gland DNA staining. Thus, investigation of MGBLs mutagenic properties needs to include the tests for both point mutagenesis and recombinogenic activity determination. In our research we showed that SMART allows revealing the blastomogenic effect of the compounds belonging to this class.

341B  
**Zw3 phosphorylation of Mad restricts mitosis in the sensory organ lineage during wing development.** Stuart J. Newfeld, Janine Quijano, Michael Stinchfield. Sch Life Sci, Arizona State Univ, Tempe, AZ.

There are many instances during development when interactions between the Dpp and Wg signaling pathways are required. Frequently the mechanism underlying these interactions is the formation of heteromeric complexes containing the Dpp signal transducer Mad and the Wg signal transducers Arm and/or dTCF that activate target genes via bipartite enhancer sequences. Here we report that we recently discovered cytoplasmic mechanism for pathway crosstalk, the phosphorylation of Mad by the Wg antagonist Zw3 (a serine-threonine kinase and homolog of mammalian Gsk3-beta), regulates the development of sensory organs in the wing. First, we determined that mutating the Zw3 phosphorylation sites in Mad (creating UAS.MGM) results in a loss of function allele because side by side studies yielded a common phenotype for MGM and Mad-RNAi - ectopic sensilla and bristles on the wing blade. Then employing an antibody recognizing only Zw3 phosphorylated Mad (pMad-Gsk), we analyzed third instar wing disks. We found that pMad-Gsk is only expressed in a subset of sensory organ precursor cells, that Zw3 phosphorylation of Mad is Wg dependent and that it is independent of Dpp. Examination of pMad-Gsk and a number of sensory organ lineage cell-type specific markers in pupal wings revealed that pMad-Gsk is expressed in every cell type but only during mitosis. We confirmed the restriction of pMad-Gsk expression to mitotic cells by double labeling pupal wings with pMad-Gsk and the mitotic marker pH3. These studies also revealed that pMad-Gsk is cytoplasmic in pH3 positive cells that are not visibly dividing but associates with chromatin during active cell division. In pupal disks side by side expression of Mad, Mad-RNAi and MGM had no effect on pH3 expression but in third instar disks significantly more pH3 expressing cells were present with UAS.Mad-RNAi and UAS.MGM than with UAS.Mad. We conclude that phosphorylation of Mad by Zw3 is not an example of pathway crosstalk but instead accomplishes a Wg dependent function - the limitation of mitosis in the sensory organ lineage during wing development.

342C  
**Loss of PTEN confers growth advantage to imaginal disc cells under starvation in Drosophila.** Katarzyna Nowak, Gerhard Seisenbacher, Hugo Stocker, Ernst Hafen. Institite of Molecular Systems Biology, ETH Zürich, Zürich, Hoengerberg, Switzerland.

The rapid growth of tumor cells leads to a microenvironment that is characterized by a limited access to nutrients and oxygen. Recently, it was shown that overactivation of PI3K pathway renders tumor cells resistant to dietary restriction and thus gives them a potential growth advantage. Nevertheless, the underlying mechanisms are poorly defined. Using the mitotic recombination system in developing Drosophila imaginal tissues, we induced site-specific loss of DPTEN, a negative regulator of PI3K signaling, to mimic the tumor microenvironment. Our results demonstrate that cells lacking PTEN overgrow more in starved larvae as compared to fed ones, with concomitantly increased starvation sensitivity and growth disadvantage of the wild-type cells. Cells devoid of PTEN proliferate faster than the wild-type tissue but apoptosis is exclusively induced in the mutant tissue, indicating that the wild-type cells are not eliminated by competition. Our findings will contribute to a better understanding of the overgrowth mechanism of mutant cells with PI3K activation in tumor-mimicked microenvironment.
Eukaryotic genomes are organized into domains of distinct chromatin structure that differ in DNA sequence content, histone modifications and non-histone chromosomal proteins. Heterochromatin Protein 1 (HP1) proteins are enriched in centric and telomeric regions of eukaryotic genomes. HP1 proteins possess a conserved chromo domain (CD) that interacts with methylated lysine 9 of histone H3 (H3K9me3) and a chromo shadow domain (CSD) that dimerizes. A multifaceted approach is being taken to understand the mechanisms by which HP1 forms heterochromatin. Atomic-scale molecular in silico modeling is being used to make predictions that are tested by in vitro biochemical and in vivo Drosophila experimentation. In silico modeling predicted that a dimer of HP1 can interact with a mono-nucleosome; EMSA analyses confirmed that HP1 interacts with mono-nucleosomes in a methylation- and CSD-dependent manner. In vivo, the bulk of the genome is packaged into extensive arrays of nucleosomes. To better mimic this arrangement in silico, modeling was performed using a 12-nucleosome array. Multiple HP1 binding configurations are predicted to be possible. EMSA experiments confirmed an interaction between HP1 and nucleosomes, showing different relative to mono-nucleosomes. In vitro studies showed that HP1 facilitated interchromosomal interactions, which were dependent upon histone methylation, the CD and the CSD. The role these interactions play in development was tested in Drosophila, where loss of HP1 is lethal. A transgene encoding wild-type HP1 rescued lethality. For the mutant HP1 constructs, only HP1 lacking the hinge region was able to rescue. Taken together, this multifaceted approach has revealed a myriad of possible interactions between HP1 and the chromatin template that are likely to contribute to heterochromatin formation.

344B
Assembly and function of Drosophila melanogaster centromeric chromatin in meiosis and development. Elaine Dunleavy, Gary Karpen. Department of Genome Dynamics, Life Sciences Division, Lawrence Berkeley National Laboratory and Department of Molecular Cell Biology, UC Berkeley, Berkeley, CA.
Centromeres are key regions of eukaryotic chromosomes that ensure proper chromosome segregation at cell division. In most eukaryotes, centromere identity is defined epigenetically by the presence of a centromere-specific histone variant CenH3 (called CID in flies). How CenH3 is incorporated and reproducibly propagated during the cell cycle is key to understanding this essential epigenetic mechanism. Much is known about the CenH3 function and assembly in mitosis, but its roles and regulation in meiosis are largely unknown. Meiosis is an essential part of the reproductive cycle and segregation defects result in aneuploid eggs, sperm and the resulting zygotes. In addition, much information about CenH3 has been obtained from single cell eukaryotes or cultured cells in multi-cellular eukaryotes, but the dynamics of CenH3 during development are unknown. Recently, the cell cycle timing of CenH3 deposition at centromeres has emerged as a key aspect of centromere specification and function. In human cultured cells, newly synthesized CenH3 is deposited at centromeres at late telephase/early G1, whereas Drosophila CID loading occurs earlier, in mitosis. Thus, the regulation of CenH3 loading may differ between organisms, tissue types and developmental stages. Here we investigate the function and timing of CenH3 deposition during development and meiosis in Drosophila melanogaster. This study aims to further our understanding of the dynamics of centromeric chromatin and the maintenance of genome stability in meiotic and mitotic cells in the multi-cellular animal.

345C
Drosophila XNP/ATRX Increases Neuronal Apoptosis via the JNK and FOXO Pathway. Yoon Ki Hong, Soojin Hwang, Min Jung Lee, Sookjin Lee, Darae Kim, Seung Hwan Park, Kyoung Sang Cho. Department of Biological Sciences, Konkuk University, Seoul 143-701, Republic of Korea.
Mutation of the Drosophila XNP/ATRX locus, which encodes an SNF2 family ATPase/helicase protein, leads to ATR-X syndrome and several other X-linked mental retardation syndromes. Although XNP/ATRX is a chromatin remodeler, the molecular mechanism by which mental retardation occurs in patients with ATR-X has yet to be determined. To better understand the role of XNP/ATRX in neuronal development, we expressed Drosophila XNP (dXNP/ATRX) ectopically in Drosophila neurons. Neuronal expression of dXNP/ATRX resulted in various developmental defects and induced strong apoptosis. These defects and apoptosis were suppressed by Drosophila inhibitor of apoptosis protein 1. Expression of dXNP/ATRX also increased JNK activity and the levels of reaper and hid transcripts, which are pro-apoptotic factors that activate caspase. Furthermore, dXNP/ATRX-induced rough eye phenotype and apoptosis were suppressed by dFOXO deficiency. These results suggest that dXNP/ATRX is involved in caspase-dependent apoptosis in Drosophila neurons via regulation of the JNK and dFOXO pathway.

346A
Involvement of heterochromatin proteins in the transcriptional regulation of the Drosophila sex determination masterswitch. Janel Rodriguez1, Hui Li2, Yongdong Yoo2, RamaKrishna Badugu2, Rebecca Kellum2, Jamila Horabin1. 1) Biomedical Sciences, Florida State University, Tallahassee, FL; 2) Biology Department, University of Kentucky, Lexington, KY.
We report that the highly conserved protein, heterochromatin protein 1 (HP1) and its telomeric binding partner HP1/ORC-Associated Protein (HOAP) play an intimate role in the control of expression of the sex determination masterswitch gene, Sex-lethal (Sel). Specifically, these proteins which are normally associated with gene repression and/or packaging of telomeric DNA, influence the firing of the X chromosome sensing promoter of Stel, at its establishment promoter, Stelapo. Female-specific regulation of Stelapo is essential for proper sex development in Drosophila. In females, the SSL protein made from the Stelapo transcript is necessary to control female-specific splicing of the late transcript transcribed from the maintenance promoter, Stelpo. Males do not make this early burst of SSL protein since Stelpo does not fire, resulting in splicing of Stelpo transcripts in the default mode, which is not productive for Sel translation. Mutants for HOAP show inappropriate Stelapo firing in males and improper splicing of the Stelapo-derived transcripts, while females show premature firing of Stelapo. HP1 mutants display a significant decrease in Stelapo expression and inappropriate Stelapo splicing in both sexes. We also find that overexpression of HP1 decreases Stelapo expression. Chromatin immunoprecipitation assays show that both proteins are associated with Stelapo sequences, placing them at the promoter during Stelapo firing. Our data suggests a repressive role for HOAP and a dual role for HP1 in activating and repressing Stelapo. Our analyses of these heterochromatin proteins suggest that the interaction of HP1 with HOAP may be important in the developmental control of euchromatic genes.

347B
Characterizing of Scaffold Attachment Factor A and its Dynamic Role in Nuclear Organization. John Aldrich, Keith Maggett. Department of Biology, Texas A&M University, College Station, TX.
The nuclear matrix is thought to be a meshwork of proteins and RNA that is involved in establishing and maintaining the looped domain architecture of chromatin within the interphase nucleus. Chromatin is tethered to the nuclear matrix through Scaffold Attachment / Matrix Associated Regions (S/MARs). Scaffold Attachment Factor A (SAF-A) was identified in mammals as an abundant nuclear matrix protein that interacts directly with S/MARs through its SAF domain and with RNA through its RGG domain. Additionally, SAF-A aggregates with DNA and RNA to form filaments in vitro. Here we report the initial characterization of the Drosophila SAF-A homolog (CG30122). RNA in situ hybridization of embryos, and adult and larval tissues reveals that SAF-A is expressed throughout development in actively dividing cells. A GFP-SAF-A fusion construct was used to visualize protein distributions in neuroblasts, whole-mount salivary gland nuclei, and on polytene chromosomes. Our results indicate that SAF-A binds to chromatin in both fixed and unfixed nuclei and colocalizes with sites of active transcription indicating a possible role in gene expression. SAF-A is also observed in extrachromosomal structures in salivary gland nuclei. Evidence suggests that these structures are RNA dependent and we believe they might correspond to the nuclear matrix. If current models are accurate, the nuclear matrix should play an important role in linking chromatin organization with gene expression/regulation. SAF-A has the potential to be an important component of this system. We are therefore creating GFP-tagged protein truncations to further characterize the mechanism through which SAF-A is recruited to the nuclear matrix and to specific chromosomal loci, and we are using reporter constructs to test the effects of SAF-A binding on transcription.
348C Comprehensive analysis of the Drosophila melanogaster chromatin landscape differentiates among chromosomes, genes, and regulatory elements. Artyom A Alekseyenko. Drosophila modENCODE. Chromatin Group. Our computational analysis generated a genome-wide map of the chromatin landscape for D. melanogaster, based on the distributions of 18 histone modifications and 9 combinatorial patterns. Integrative analysis with other genome-wide mapping data (non-histone chromatin proteins, GRO-seq, DNase hypersensitive sites, short/long RNA expression) reveals distinct properties of chromosomes, genes, regulatory elements and other functional domains. In addition to highlighting the special identities of the male X and the 4th chromosomes, this analysis identifies distinct chromatin signatures among active genes that are correlated with differences in gene length, exonic structure, regulatory function, and genomic context. It also reveals a diversity of chromatin signatures among Polycrypt targets, including a subset with paused RNA polymerase. This systematic profiling and integrative analysis of chromatin signatures provides important insights into the differential packaging of functional elements.

349A Functional analysis of the CHD1 chromatin remodeling and assembly factor. Jennifer A. Armstrong, Lakshmi V. Bugga, Liana Engie, Janice Cho, Eugenie S. Hong, Kelsey Schmidt. Joint Science Department, Scripps, Pitzer and Claremont McKenna Colleges, Claremont, CA. Chromatin remodeling factors utilize the energy of ATP to slide, remodel, or assemble nucleosomes. Together with histone modifying enzymes, chromatin remodelers control the transcriptional program of a cell to direct both cellular differentiation and stem cell maintenance. Our previous studies of chd1 mutant flies revealed that the CHD1 chromatin remodeling and assembly factor is important for male and female fertility and wing development. In addition to these developmental defects, we recently observed that global chromosome structure is sensitive to the levels of CHD1, as either loss of CHD1 or over-expression of CHD1 greatly compromises chromosome structure. We are taking a two-pronged approach to understanding how CHD1 regulates chromosome structure. First, we have developed a sensitized wing-based genetic assay to uncover proteins that functionally interact with CHD1. Second, we are using immuno-fluorescence of polytene chromosomes to investigate changes in the amounts of chromatin associated proteins and histone modifications following changes in CHD1 protein levels. To allow the quantification of fluorescent images of polytene chromosomes, we have developed a Matlab program that creates a mask over polytene chromosomes to remove background signals.

350B The role of su(o)/Top2 in the maintenance of chromosome structure and integrity. Silvia Bonaccorsi, Valentina Mengoli, Roberto Piergentili, Elisabetta Bucciarelli, Ramona Lattao, Maurizio Gatti. Dept Biology and Biotechnology, Univ di Roma ‘La Sapienza’, Rome, Italy. We have isolated su(o), an EMS-induced lethal allele of su(o)/Top2 (su(o)), previously identified by 2 male-sterile mutations (su(o) and su(o)) that disrupt chromosome segregation during Drosophila male meiosis (Bucciarelli et al., J Cell Biol, 160:993, 2003). Molecular analyses revealed that the su(o) lethal allele carries a G-A transition that generates a premature stop codon in the Topoisomerase 2 (Top2) gene. We have therefore renamed su(o), su(o) and su(o) into Top2, Top2 and Top2, respectively. The Top2 allele causes lethality at the third instar larval stage in both homozygous and hemizygous condition. Top2/Top2 and Top2/Top2 individuals are poorly viable, sterile and exhibit defective neuromuscular coordination. Analysis of brain preparations revealed that mutations in Top2 affect not only male meiosis but also neuroblast mitosis. Top2/Top2 and Top2/Top2 mutant brains showed metaphase figures with frequent chromosome aberrations and complex chromosome rearrangements preferentially involving heterochromatin, and anaphases with lagging chromosomes. In larval brains of Top2 hemizygous mitotic figures were extremely rare and showed morphologically aberrant chromosomes suggesting a strong defect in condensation. We found that Top2 mutations also affect polytene chromosomes structure. Interestingly, polytene chromosomes from Top2/Top2 and Top2/Top2 larvae displayed a specific alteration of X chromosome morphology in males, suggesting an involvement of Top2 in the chromatin modifications associated with dosage compensation. These results indicate that Top2 plays multiple roles in chromatin structure and is essential for the maintenance of chromosome stability.

351C Pendolino (Peo), a terminin-interacting protein required for Drosophila telomere protection. Giovanni Cenci1, Laura Ciapponi2, Grazia D. Raffa2, Romina Burla3, Isabella Saggio3, Maurizio Gatti1. 1) Biol di Base ed Applicata, Universita dell'Aquila, L'Aquila, Italy; 2) Dept. Biology and Biotechnology, Sapienza, University of Rome, Rome, Italy. We have recently shown that Drosophila telomeres are protected by terminin, a multiprotein complex that includes HOAP, Modigliani (Moi) and Verroccchio (Ver), and is functionally analogous to human shelterin. We have identified and partially characterized a gene, dubbed pendolino (peo), which encodes a terminin-interacting protein required for telomere protection. Mutations in peo cause high frequencies of telomeric fusions (~ 6 per cell). peo encodes a polypeptide of 270 amino acids that is homologous to the human AKTIP protein. Peo and AKTIP share homology with the E2 ubiquitin-conjugating enzymes but AKTIP lacks the catalytic cysteine residue in the Ub-binding domain, which is present in Drosophila Peo. However, a peo transgene carrying a mutation in this domain fully rescues the telomere-fusion phenotype of peo mutants. peo genetically interacts with cav, moi, ver and Su(var)205 (HP1); double heterozygotes for peo and either cav, moi, ver or Su(var)205 are viable but exhibit low frequencies of telomeric fusions. Consistent with these results, GST pulldown experiments showed that Peo physically interacts with HOAP, Moi and Ver but not with HP1. However, HOAP localizes normally at telomeres of peo mutants, indicating that the wild type function of peo is not required for telomeric accumulation of HOAP. The molecular mechanism underlying the telomere capping function of Peo is currently unclear. It is conceivable that Peo and Effete/Ub2/31 (an E2 ubiquitin conjugating enzyme required to prevent telomere fusion) function in an ubiquitination pathway required for telomere protection, while the substrates of this pathway remain to be identified.

352A Functional antagonism between histone H3K4 demethylases in vivo. Nicholas Dyson1, Luisa Di Stefano1, James Walker1, Giosalba Burgio1, Davide Corona1, Anders Nääri1,2. 1) Massachusetts General Hospital Cancer Center, Harvard Medical School, Charlestown, MA, USA; 2) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA. 3) Istituto Telethon Dulbecco e/o Universita degli Studi di Palermo, Palermo, ITALY; 4) Department of Cell Biology, Harvard Medical School, Boston, MA, USA. Dynamic regulation of histone modifications is critical during development and aberrant chromatin modification enzymes has been associated with diseases such as cancer. Histone demethylase have been shown to play a key role in eukaryotic gene transcription, however, little is known about how their activities are coordinated in vivo to regulate specific biological processes. In Drosophila, two enzymes, Lsd1 and Ld demethylate histone H3 at lysine 4 (H3K4), a residue whose methylation is associated with actively transcribed genes. Our studies show that compound mutation of Ld and Lsd1 results in increased H3K4 methylation levels. However, unexpectedly, Ld mutations strongly suppress Lsd1 mutant phenotypes. Investigation of the basis for this antagonism revealed that Ld opposes the functions of Lsd1 and of the histone methyltransferase Su(var)3-9 in promoting heterochromatin spreading at heterochromatin-euchromatin boundaries. Moreover, our data reveal a novel role for Lsd1 in Notch signaling in Drosophila and a complex network of interactions between Lsd1, Ld and Notch signaling at euchromatic genes. These findings illustrate the complexity of functional interplay between histone demethylases in vivo, providing insights into the epigenetic regulation of heterochromatin-euchromatin boundaries by Ld and Lsd1 and showing their involvement in Notch pathway-specific control of gene expression in euchromatin.

353B Y chromosomal determinants and transmission of position effect variegation. Keegan J. Kelsey, Elena Kwan, Andrew G. Clark. Molecular Biology and Genetics, Cornell University, Ithaca, NY. Recent work has provided evidence for natural Y-linked variation exerting an effect on the chromatin state of X-linked and autosomal loci, which is manifested by position effect
variegation (PEV). The nature of this variation is similar to the phenotypic effects induced by a class of genes know as suppressors of variegation, or Su(var), which are known to alter the kinetics of histone modifications or are structural components of heterochromatin. Here, we ask whether a newly introduced Su(var) will cause measurable modifications to the chromatin state of the Y chromosome, and, if so, whether that modified state is subsequently inherited and transmitted across generations. We reason that PEV, which is known to be affected by varying amounts of heterochromatin, will indicate the altered Y chromatin status as induced by the introduced Su(var). To answer this question, we used the system of Drosophila melanogaster and crossed seven distinct Su(var) genes with five nuclear Y chromosome variants to test their impact on the Y chromosome. Specifically, male progeny from a Su(var) x Y; Y x cross were mated to females carrying white-mottled-4 (w4), and subsequent progeny, now lacking the Su(var), were indirectly scored for heterochromatin status as measured through a variegating eye color phenotype. Clear trans-generational effects were documented. The data from this study will be used to provide information for modeling how the Y chromosome chromatin landscape interacts and affects autosomal gene expression.

**354C**

**HP1a regulates the recognition of double strand breaks.** James M. Mason, Raghovran Dronamraju. Lab Mol Gen, D3-01, NIH/NIEHS, Res Triangle Park, NC.

Chromatin structure regulates the dynamics of the recognition repair of DNA double strand breaks; open chromatin enhances the recruitment of DNA damage response factors, while compact chromatin is refractory to the assembly of radiation induced repair foci. We show that these foci form in heterochromatin but are absent in nonheterochromatic regions with time, suggesting compartmentalized repair, while hydroxurea-induced damage remains in the heterochromatic compartment. MU2, an orthologue of human MDC1, a scaffold for DNA repair foci, interacts with the chromoshadow domain of the chromatin protein HP1a in the absence of DNA damage, although HP1a does not accumulate at radiation repair sites. HP1a depletion increases apoptosis in response to irradiation and causes G2/M arrest. Further, cells irradiated in mitosis produced more and brighter repair foci than to cells irradiated during interphase. Thus, the interplay between MU2 and HP1a is dynamic and facilitates the repair of DNA damage.

**355A**

**A paternal imprint essential for the inheritance of telomere identity in Drosophila.** Yikang S. Rong, guanjun gao, yan cheng, natalia wesołowska. NCU/NIH, Bethesda, MD.

Chromatin remodeling during sperm maturation could erase epigenetic landmarks on the paternal genome creating a challenge for its re-establishment upon fertilization. Here we demonstrate that selective retention of a chromosomal protein in mature sperms protects the identity of paternal telomeres in Drosophila. The m3(k81) gene was a duplication of hiphop that encodes a telomeric protein. While HipHop protects telomeres in somatic cells, K81 is produced exclusively in males and localizes to telomeres in post-mitotic cells, including mature sperms. In embryos fathered by k81 mutants, the maternal supplies fail to re-establish a protective cap on paternal telomeres leading to their fusions. These fusions hinder the segregation of the paternal genome, and result in haploid embryos with maternal chromosomes. The functional divergence between hiphop and k81 manifests not only in expression patterns but also in the position-effect variegation (PEV) on chromosome. The nature of this variation is similar to the phenotypic effects induced by a class of genes known as Su(var), which are known to alter the kinetics of histone modifications or are structural components of heterochromatin. Here, we ask whether a newly introduced Su(var) will cause measurable modifications to the chromatin state of the Y chromosome, and, if so, whether that modified state is subsequently inherited and transmitted across generations. We reason that PEV, which is known to be affected by varying amounts of heterochromatin, will indicate the altered Y chromatin status as induced by the introduced Su(var). To answer this question, we used the system of Drosophila melanogaster and crossed seven distinct Su(var) genes with five nuclear Y chromosome variants to test their impact on the Y chromosome. Specifically, male progeny from a Su(var) x Y; Y x cross were mated to females carrying white-mottled-4 (w4), and subsequent progeny, now lacking the Su(var), were indirectly scored for heterochromatin status as measured through a variegating eye color phenotype. Clear trans-generational effects were documented. The data from this study will be used to provide information for modeling how the Y chromosome chromatin landscape interacts and affects autosomal gene expression.

**356B**

**Global nucleosome mobility during heat shock.** Sheila Teves, Jorja Henikoff, Steven Henikoff. Fred Hutchinson Cancer Research Center, Seattle, WA.

The euchromatic genome is packaged into nucleosomes, which are dynamically mobilized to allow access to DNA during transcription and other active processes. To better understand nucleosome mobility, we are studying chromatin changes that occur during the heat shock response in Drosophila S2 cells. The heat shock response is an attractive model system for studying nucleosome dynamics because it induces a fast global transcriptional response and is highly conserved across different species. We have mapped three nucleosome properties genome-wide: (1) nucleosome density using micrococcal nuclease mapping, (2) nucleosome turnover using the CATCH-IT (Covalent Attachment of Tags to Capture Histones and Identify Turnover) method, and (3) nucleosome salt solubility. We find that nucleosome density within gene bodies is reduced within 15 minutes of heat shock, and shock persists for at least one hour. Heat shock also causes nucleosome turnover to decrease over gene bodies downstream of the +1 nucleosome relative to transcription start site. Low salt solubility gradually decreases during heat shock, suggesting an increase in nucleosome stability. Taken together, these results indicate a global decrease in nucleosome mobility that might be involved in gene repression during heat shock.

**357C**

**Regulation of Heterochromatin by the Histone Demethylase KDM4A.** Serafin U. Colmenares, Joel Swenson, Cameron Kennedy, Sasha Langley, Gary H. Karpen. Genome Dynamics, Lawrence Berkeley National Lab, Berkeley, CA.

Heterochromatin, the highly repetitive and transcriptionally repressive region of the genome, is marked by enrichment of HP1a and the di- and tri-methylated forms of histone H3 K9. These marks are essential for repeat stability and position-effect variegation, and are thought to function by recruiting a host of proteins that mediate heterochromatin formation. We have identified that the histone H3 K36 demethylase KDM4A binds HP1a, co-localizes with heterochromatin in a HP1a-dependent manner, and suppresses position-effect variegation in Drosophila melanogaster. However, we also show that transient KDM4A knockdown favors HP1a accumulation in heterochromatin with relatively little change in distribution of H3 K36 methylation, suggesting that KDM4A regulates other features of heterochromatin. We are interested in investigating additional functions of KDM4A, HP1a and trimethylated H3 K36 in the regulation of heterochromatin structure.

**358A**


Integrity of chromosome structure is fundamental for proper segregation of the genetic material into daughter cells. In Drosophila melanogaster, abnormal circularized (ring-) Y chromosomes are known to cause mitotic instability, chromosome loss, sterility and even death in male carriers. We have been analyzing the lethal effects of a ring-Y chromosome generated by Oster (1964). We found that ring-Y lethality results from failure of the Y chromatids to separate at anaphase during the late cleavage cycles. Moreover, we found that separation failure of the ring-Y chromatids occurs at a large region of 359-bp satellite DNA translocated from the X chromosome. This effect occurs only with the ring-Y chromosome and not its linear precursor chromosome. Specifically, male progeny from a Su(var)i x Yj cross were mated to females carrying white-mottled-4 (w4), and subsequent progeny, now lacking the Su(var), were indirectly scored for heterochromatin status as measured through a variegating eye color phenotype. Clear trans-generational effects were documented. The data from this study will be used to provide information for modeling how the Y chromosome chromatin landscape interacts and affects autosomal gene expression.

**359B**

**Why are certain regions of the genome more susceptible to re-replication?** Madhura Kadaba1, David M. MacAlpine2, Brian R. Calvi1. 1) Department of Biology, Indiana University, Bloomington, IN; 2) Department of Pharmacology and Biology, Duke University Medical Center, Durham, NC.

Re-replication is the aberrant duplication of genomic DNA more than once per cell cycle. Previously, we have shown that over-exression of double-parked (dup), the Drosophila ortholog of Cdt1, is sufficient to cause re-replication. Array Comparative Genomic Hybridization (CGH) comparing normal and re-replicating cells from brains and discs suggests that the largely heterochromatic fourth chromosome re-replicates more than other chromosomes. In addition, labeling fixed cells with γ-H2A V suggests that heterochromatin has the most damage during re-replication. These observations imply that origins within heterochromatic regions of the genome may be especially prone to...
reinitiating DNA replication after dgo over-expression. Indeed, knockdown of a Dup inhibitor, Geminin, in Kc cells resulted in higher copy number of pericentric heterochromatic DNA (Ding et al, 2010). These observations are surprising given that heterochromatic origins are often inefficient and initiate late during a normal S phase. We are using a combination of approaches to uncover the properties that make heterochromatic regions of the genome more susceptible to re-replication and damage. This study may lead to important insights into the relationship between chromosome structure and origin activity during normal and aberrant DNA replication.


Abnormal circular ring-Y chromosomes have been found in various organisms including flies, rodents, and humans. In Drosophila melanogaster ring-Y chromosomes can induce mitotic instability, Y chromosome loss, male sterility and in some cases lethality. This last phenotype is intriguing since the Y chromosome is known to be non-essential for male survival. We have been investigating the nature of male lethality caused by a ring-Y chromosome remaining from a collection of ring-Y chromosomes made by Oster (1964). Male embryos bearing this chromosome die as early embryos due to extensive chromosome bridges just before the transition from the maternal to zygotic development. Our group has recently shown that this ring-Y contains a large amount of 359 base pair satellite DNA derived from the X chromosome. We showed that the ring-Y is the sole cause of the chromosome bridges and that the 359-bp DNA fails to separate between daughter chromatids during mitosis. Further, we found that abnormally high levels of Topoisomerase II (TopII) localized to the 359-bp DNA in early male embryos. This observation suggests that TopII may be either directly involved in separation failure of the 359-bp DNA or is a secondary response for repair of DNA damage. We are testing the first hypothesis genetically by using a collection of TopII mutants (generated by the laboratory of Ting Wu). Specifically, we are testing if certain combinations of maternal TopII mutations can suppress or enhance ring-Y lethality. We are also using cytology to investigate TopII levels on the ring-Y linked 359-bp DNA in these mutants.

361A Control of stem cell self-renewal by heterochromatin formation. Willis X. Li1,2, Yaling Xing2, 1) Dept Biomedical Genetics, Univ Rochester Medical Ctr, Rochester, NY; 2) Dept of Medicine, Univ California, San Diego, CA.

Stem cells use differing strategies to epigenetically repress the expression of differentiation genes in order to maintain stemness; the molecular mechanisms, however, remain unclear. Here we show that in the Drosophila testis, a paradigm for studying adult stem cell behavior, heterochromatin formation plays an important role in germline stem cells (GSCs) self-renewal. GSCs contain globally highly condensed DNA characteristic of heterochromatin. Disrupting heterochromatin formation by genetic mutations or RNAi causes premature expression of differentiation genes and loss of GSCs. Conversely, increasing heterochromatin formation by over-expressing Heterochromatin Protein 1 (HP1) dramatically increases GSC number and rescues the GSC loss phenotype. These results indicate that heterochromatin formation is an essential epigenetic mechanism that not only silences differentiation genes but also promotes stem cell self-renewal.

362B CG7172 acts as a tumor suppressor by regulating heterochromatin. Su Jun Lim, Pranabananda Dutta, Willis Li. University of Rochester Medical Center, Rochester, NY.

Heterochromatin is a compact DNA structure that is crucial for gene expression regulation. Recently, increasing evidence has emerged to suggest that heterochromatin serves as a mechanism for cells to counteract tumorigenesis. Here, we demonstrate that CG7172, a previously uncharacterized but well-conserved Drosophila gene, plays a role in heterochromatin formation and acts as a tumor suppressor. Knocking down CG7172 expression by RNAi results in reduced heterochromatin protein 1 (HP1) localization at heterochromatin regions; and loss-of-function of CG7172 suppresses position-effect-variegation. Interestingly, knockdown of CG7172 also results in tissue hyper-proliferation and severely enhances tumorigenesis caused by oncogenic Ras in the adult fly eyes. Consistently, overexpressing CG7172 partially rescues the oncogenic Ras phenotype. These results show that CG7172 antagonizes cell proliferation and suppresses tumorigenesis by regulating the formation of heterochromatin.


1) Dept Genome Dynamics, Lawrence Berkeley National Lab, Berkeley, CA; 2) Drosophila modENCODE Consortium.

Eukaryotic genomes are packaged in two basic forms, euchromatin and heterochromatin. We have examined the composition and organization of Drosophila melanogaster heterochromatin in different cell types using ChIP-array analysis of histone modifications and chromosomal proteins. As anticipated, the pericentric heterochromatin and chromosome 4 are on average enriched for the ‘silencing’ marks H3K9me2, H3K9me3, HP1a, and SU(VAR)3-9 and are generally depleted for marks associated with active transcription. The locations of the euchromatin-heterochromatin borders identified by these marks (‘epigenomic’ borders) are similar in animal tissues and most cell lines. However, in S2 cells the borders are shifted distally in the chromosome arms (heterochromatic ‘extensions’), indicating that the amount of heterochromatin is variable in different cell types. Combinatorial analysis of chromatin patterns reveals distinct profiles for euchromatin, pericentric heterochromatin, and the 4th chromosome. Both silent and active protein-coding genes in heterochromatin display complex patterns of chromosomal proteins and histone modifications; a majority of the active genes exhibit both ‘activation’ marks (e.g. H3K4me3 and H3K27ac) and ‘silencing’ marks (e.g. H3K9me2 and HP1a). The hallmark of active genes in heterochromatic domains appears to be a loss of H3K9 methylation at the transcription start site. An unexpectedly large fraction of sequences in the euchromatin-rich euchromatin arms exhibits a heterochromatic chromatin signature, which differs in size, position, and impact on gene expression among cell types. We conclude that patterns of heterochromatin/euchromatin packaging show greater complexity and plasticity than anticipated.


SUUR protein is often found in heterochromatic regions of polytene chromosomes in drosophila salivary gland cells. The number of bound regions varies from chromosomeoc and numerous bands on the chromosome arms (e.g. intercalary heterochromatin) to chromocenter only depending on cell cycle stage. It was shown in Kc cells that SUUR chromosomal targets are mostly tissue specific genes that are generally silenced throughout development. This suggests that SUUR probably regulates heterochromatic state establishment or maintenance in a replication dependent manner. However, the mechanism of SUUR action has not been elucidated yet. All of the above coincides with the fact that SUUR copurifies with several proteins involved in replication, DNA double strand break repair, cell cycle control and heterochromatin maintenance (tandem affinity purification followed by mass spectrometry analysis). Here we report the results of genetic analysis of SUUR interactions. To study SUUR interactions we used RNAi silencing of SUUR potential partners to test whether malfunction of these genes affects SUUR phenotype and localization in salivary gland cells.


Mcm10 is an abundant nuclear protein that is essential for DNA replication. This replication factor, first discovered in S. cerevisiae, has been shown to interact with proteins of the pre-replication complex (Pre-RC), the pre-initiation complex (Pre-IC), and heterochromatin protein 1(HP1). As it is now becoming more apparent that the processes of DNA replication and the establishment of epigenetic chromosome states are more intimately linked than once thought; the D. melanogaster homolog Mcm10 provides an excellent subject by which the connections of these two essential processes can be investigated. Recently our lab conducted an analysis of two Drosophila Mcm10 mutants which demonstrated that
Mcm10 not only plays a role in DNA replication, but also has a role in heterochromatin silencing and chromosome condensation. Interaction studies in yeast, as well as both phenotypic and genetic analyses in Drosophila, imply that the conserved C terminus is important for the many interactions carried out by this promiscuous protein. In light of these recent results, we have continued our investigation of the conserved Drosophila Mcm10 protein using three mutants which truncate the C terminus by 85, 263, and 388 amino acids respectively. Genetic and phenotypic analysis of the Mcm10 Trp and Mcm10 Ser truncation alleles have revealed a homozygous female sterility issue, that ultimately results from apoptotic-like event in late stages of oogenesis. The apoptotic-like phenotype observed in the ovaries of these young mutant females phenocopies that of the minifly (mfli) mutant described by Giordano et al. 1999. These mutant phenotypes taken together with yeast interaction data suggesting an interaction between Mcm10 and RNA PolI associated factor PAF67 (CG5642), and both in vitro and in vivo cellular localization results depicting Mcm10 in the nucleolus; suggest that Mcm10 may function in ribosome biogenesis as well as its established functions in DNA replication and chromatin dynamics.


The distal 1.2 Mb of chromosome 4 of D. melanogaster is a unique chromatin domain. It exhibits features usually associated with heterochromatin (ie late replication, association with H3K9me2/3, high repeat content) as well as features typical of euchromatin (amplification in polytene chromosomes, high gene density). In addition, transgene reporter assays have discovered several small regions of chromosome 4 that support high reporter expression, while in the majority of chromosome 4 euchromatic reporter genes (hsp70-white) are silenced. These observations lead to the hypothesis that on chromosome 4, euchromatic domains are interspersed with heterochromatid domains. We have used the genome-wide binding profiles of histone modifications and chromosomal proteins generated by modENCODE to investigate this model. In addition to examining data from cell lines, we have generated select data sets from mutants in proteins enriched on chromosome 4, including POF, HP1a, and the histone methyltransferases EGF and Su(VAR)3-9. We find that the association of chromosome 4 with HP1a, and H3K9me2/3 is almost ubiquitous, with the exception of several Polycomb domains, which lack these marks. However, these Polycomb domains differ between cell types, and in their absence, HP1a and H3K9me2/3 can spread over these sequences. These findings argue against a simple interspersion of classical heterochromatic and euchromatic domains on chromosome 4. Further analysis of chromosome 4 sequences associated with HP1a indicates that they represent two classes, one enriched for POF, and one that lacks POF. These two classes differ in their composition with regard to H3K9me2, H3K9me3, Su(VAR)3-9, and EGG enrichment. On-going analyses aim to discover the interdependencies of these proteins and modifications and their effects on the unique chromatin structure on chromosome 4.

367A Gain of essential centromere function by the young HP1 gene Umbrea. Benjamin Ross1, Mary Alice Hiant1, Danielle Vermaak1, Harmit Malik1. 1) Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Dept. of Molecular and Cellular Biology, University of Washington, Seattle, WA.

The canonical Heterochromatin Protein 1 genes in Drosophila are present in the genomes of all fly species. HP1 genes use the same two domains to perform diverse functions. However, most HP1 genes were “born” too long ago to uncover how they acquired these differences in function. We have discovered that Umbrea (HP1b/CG15636) is a young gene, born from a duplication of HP1b ~12 million years ago. In D. melanogaster, Umbrea differs from HP1b at 51 of 106 amino acid positions. To test the idea that this divergence reflects altered function, we reduced the expression of Umbrea during development using both RNAi knockdown and genetic knockouts. Loss of function of Umbrea was lethal, indicating that HP1b cannot complement Umbrea and that Umbrea has gained a new essential function. To understand the molecular basis of this new function, we compared the localization of GFP-tagged Umbrea and HP1b. HP1b localized largely to heterochromatin in Kc cells, as expected. Strikingly, Umbrea localized to punctate dots embedded within heterochromatin that are perfectly coincident with Cid at centromeres. By creating chimeras with HP1b, we found that the chromoshadow domain and tails of Umbrea were separately sufficient for localization. Finally, we tested whether Umbrea has evolved adaptively since its birth. Several codons in the tail domains have evolved under recurrent positive selection. Thus, Umbrea may have acquired essential function so quickly because of strong diversifying selective pressure. We hypothesize that the rapid evolution of Umbrea is associated with the suppression of centromere drive, wherein species-specific satellite DNA expansions are suppressed by centromere-binding proteins. Umbrea’s localization to the centromere is surprising because most genes with centromere or kinetochore function are conserved from yeast to humans. Umbrea’s evolution suggests that the spectrum of centromere genes could be much larger than currently believed.

368B Testing interactions between the hybrid lethality genes Hmr and Lhr in D. melanogaster. Rajavasireddy P. Satyaki, Cuykendall Tawny, Kwak Hojoong, Ji Shuqing, Barbash Daniel. Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Crosses of Drosophila melanogaster females and D. simulans males produce lethal male and sterile female F1 hybrid progeny. The inviability of mutations in two fast evolving genes- Lethal hybrid rescue (Lhr) from D. simulans and Hybrid male rescue (Hmr) from D. melanogaster. The interaction between these two hybrid incompatibility genes can be explained by the Dobzhansky-Muller (DM) model. Under the DM model, negative epistasis between divergent genes causes hybrid incompatibility. However, the model does not provide a mechanistic basis for this “negative epistasis”. A key goal of our work has therefore been to understand the interactions between Lhr and Hmr. Lhr and Hmr mutants in D. melanogaster have reduced female fertility. We were therefore motivated to examine the phenotype of D. melanogaster mutant for both Lhr and Hmr. We report here that the double mutant has comparable fertility to either single mutant, suggesting that these genes may function in a common process or pathway. Lhr encodes a HIP1 interacting, BESS domain protein while Hmr encodes a MADF and BESS domain-containing protein. Interestingly, it has been established in the case of other chromatin-associated proteins that BESS and MADF domains can interact. We therefore hypothesized that LHR and HMR may physically interact. We report here new data that are consistent with this hypothesis. First, we find that HMR, like LHR, also localizes to heterochromatin. Second, we find that LHR and HMR can be co-immunoprecipitated. These results predict that LHR and HMR will localize to the same region in heterochromatin. We are currently testing this prediction by high resolution FISH experiments. We also hypothesize that the LHR-HMR physical interaction is key to the localization of either one or both of these proteins to heterochromatin. We are currently testing this hypothesis by examining Lhr mutants for HMR localization and Lhr mutants for LHR localization.

369C The Role of Heterochromatin in the DNA Damage Response. Joel Swenson1,2, Irene Chio2, Serafin Colmenareo2, Cameron Kennedy2, Gary Karpen1,2. 1) Molec & Cell Biol, Univ California-Berkeley, Berkeley, CA; 2) Genome Dynamics, Lawrence Berkeley National Laboratory, Berkeley, CA.

Improper repair of DNA double-strand breaks (DSBs) in repetitive elements may lead to expansion or contraction of sequences, translocations, or aneuploidy. Heterochromatin is characterized by the presence of Heterochromatin Protein 1a (HP1a) and is enriched for repeated sequences. Whether HP1a forms a repair complex after DNA damage is not known. Here we purify HP1a before and after damage and show a change in associated proteins as identified by mass spectrometry analysis. Several of these proteins are shown to modulate HP1a localization and/or affect the levels of the DNA damage marker γH2A.V. A candidate screen using identified HP1a-interacting proteins is currently being conducted to identify proteins involved in the repair of heterochromatin DNA damage.

370A Homolog pairing and sister chromatid cohesion in heterochromatin in Drosophila male meiosis I. Jui-He Tsai, Rihui Yan, Bruce McKee. Biochem, Cellular & Molec Biol, Univ Tennessee, Knoxville, TN.

Drosophila males lack meiotic recombination and chiasmatas yet homologous chromosomes pair and disjoin regularly. It has been suggested that homologs may remain paired at sites within the heterochromatin of each arm. The X-Y pair utilizes a specific repeated sequence within the heterochromatric ribosomal DNA (rDNA) blocks as a pairing site. No
371B Comparing the Effects of Invader4 with 1360 on Heterochromatin Targeting. Hao Yang, Monica Sentmanat, Sarah Elgin. Biology Dept, Washington University in St. Louis, St. Louis, MO.

Position Effect Variegation (PEV) in Drosophila, a phenotype characterized by stochastic silencing of a gene in cells where it is typically active, is associated with heterochromatin formation. Previous experiments have shown an enhancement of PEV at pericentric reporters in the presence of 1360, a DNA transposable element. These findings suggest that 1360 might be a target for heterochromatin formation, but only effective in certain domains, perhaps defined by repeat density or proximity to heterochromatic regions. Although the 1360 element is known to be effective in promoting heterochromatin formation in this assay, the impact of other transposable elements is still unknown. This study aims to assess the degree of heterochromatin formation in the domains of interest using an alternative transposable element, Invader4, and comparing the results with data obtained from previous 1360 analysis. In this study, we explored the effects of the retrotransposon Invader4 as a heterochromatin target at 1360-sensitive genomic loci. The phiC31-mediated cassette exchange technique was used for site-specific integration of donor constructs. Visual inspection and pigment assays, used to quantify the levels of reporter gene hsp70-white expression in these lines, show that Invader also serves as a target for heterochromatin formation. In addition, the lines were analyzed for suppression or enhancement of PEV in the presence of second-site mutations known to affect 1360-sensitive PEV. The results indicate comparable pigment levels between Invader4 lines and corresponding 1360 lines. This initial study suggests that an alternative transposable element can support heterochromatin formation at 1360-sensitive sites, and supports the idea that both DNA transposons and retrotransposons are silenced by a similar targeting mechanism.

372C Acetylation of histone H4 by Chameau is required for proper expression of Notch target genes. Hai Huang1,2, Hanqing Chen1,2, Changqing Li1, Qinghua Wu1,2, Xuehong Liang1, Renjie Jiao1. 1) State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, the Chinese Academy of Sciences, Beijing, China; 2) Graduate School of the Chinese Academy of Sciences, Beijing, China.

Chromatin organization has profound effects on the activity of signaling pathways, which exert their functions through the regulation of gene expression. The chromatin organization is dynamically modulated by epigenetic modification including histone acetylation. In a genetic screen, we identified a gene, chameau, encoding the Drosophila MYST domain acetyltransferase, whose loss-of-function results in phenotypes that resemble those caused by downregulation of the Notch activity. By manipulating the Notch pathway activity, we found Chameau genetically interacts with Notch pathway. Moreover, Chameau depletion impairs the expression of Notch target genes. Further molecular and cytological experiments indicate that the acetylation on histone H4 in promoter regions of these genes is specifically compromised as a result of Chameau depletion. We undertook in vitro and in vivo experiments to test an association between Chameau and Su(H), which may be responsible for local histone H4 acetylations at the Notch target genes. We are currently conducting investigations to explore other factors that cooperatively work with Chameau in regulating the Notch pathway at the chromatin level.

373A Functional interaction between the dSet1 and dTip60 complexes in chromatin regulation at promoters of transcribed genes. Thomas Kusch, Amanda Mei, Matthieu Caron. Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ.

Chromatin protects the fragile genomic DNA, but also impedes processes requiring its access like transcription. To overcome this barrier, cells introduce combinations of chromatin modifications that are believed to serve the coordinated passage of RNA pol II, pre-mRNA processing, and others. One prominent, yet poorly understood combination is formed at promoters of transcribed genes in all eukaryotes. The nucleosomes at promoters are distinguished by the combination of histone H3 trimethylated at lysine 4 (H3K4me3), hyperacetylated histones, and the histone variant H2A.Z (aka H2Av in Drosophila). The biogenesis and the role of these marks in transcription regulation are still unclear. Here, we report the biochemical and functional characterization of the main H3K4 trimethyltransferase complex from Drosophila, the dSet1 complex. dSet1-dependent H3K4me3 is crucial during phases of maximal transcription up-regulation. The loss of promoter-proximal H3K4me3 causes an accumulation of Pol II at transcription start sites due to reduced Pol II release into productive elongation. These findings provide a direct mechanistic role of H3K4me3 as transcription rate-promoting modification in higher eukaryotes. We further observed that H3K4me3 is required for the recruitment of the dTip60 histone acetyltransferase complex to active promoters. Biochemical assays identified the tumor suppressor homolog, dlg3, as major H3K4me3 binder within the dTip60 complex. H3K4me3 is also crucial for dTip60-dependent histone acetylation and H2A2 incorporation at active promoters. Using biochemical assays with recombinant nucleosomes and purified complexes, we confirmed that nucleosomal H3K4me3 is sufficient to strongly stimulate histone acetylation and H2A2 exchange by the dTip60 complex. Our data supports a model in which dSet1-dependent H3K4me3 functions as a docking platform and key regulator for chromatin modifiers like the dTip60 complex, which further alter the structure of promoter-proximal nucleosomes to ensure optimal release rates of Pol II throughout multiple rounds of transcription.

374B Histone modification as a way to identify DNA elements critical for the acquisition of drug tolerance. Xiaolei Li, Harish Krishnan, Ankur Abhishek, Nigel Atkinson. Sect Neurobiology, Univ Texas at Austin, Austin, TX.

In Drosophila the slo gene encodes the BK-type Ca2+-activated K+ channel that is involved in the acquisition of functional tolerance to the sedative effect of drugs. A single sedative dose of the anxiolytic benzyl alcohol induces dynamic spatiotemporal changes in histone H4 acetylation across the slo regulatory region and leads to slo induction and tolerance (1). Mutations that abolish the expression of slo also block the acquisition of tolerance, while induction of a transgenic slo expression causes resistance to drug sedation. Moreover, artificially inducing histone acetylation with histone deacetylase inhibitor results in similar H4ac changes, induction of slo, and function tolerance to the drug. Epigenetic changes occur over two highly conserved DNA regulatory elements of the slo gene - 6b and 55b. To investigate the function of these two elements, we generated individual knock-out mutants through Ends-Out gene targeting. Both of the deletions affected the epigenetic profile and animal behavior. The 6b deletion significantly changed the duration of tolerance. In wild-type flies tolerance lasts only about one week, however, the 6b deletion mutant showed a persistent tolerance for at least a month. Deletion of 55b element did not affect the ability to acquire tolerance, however the mutant showed increased mobility. To manipulate the endogenous acetylation level over 6b and 55b elements, we built another two mutants that have a UAS sequence next to each element. These UAS mutants were crossed to a transgenic fly carrying a heat shock driver driven GAL4DBD-HDAC (GAL4 DNA binding domain linked histone deacetylase) or GAL4DBD-HAT (GAL4 DNA binding domain linked histone acetylase). The mutants were heat-shocked to induce the expression of HDAC or HAT, followed by drug tolerance assays. We found that activation of HDAC abolished the ability to acquire functional tolerance. On the contrary, induction of HAT further enforced drug induced tolerance. >722; 1) Y. Wang, H. R. Krishnan, A. Ghezzi, J. C. Yin, N. S. Atkinson, PLoS Biol 5, e265 (2007).
Assembly of Drosophila centromeric chromatin proteins during mitosis. Barbara Mellone1, Kathryn Greve1, Vladimir Shteyn1, Isaac Oderberg2, Gary Karpen1. 1) Molecular and Cell Biology, University of Connecticut, Storrs, CT; 2) Brown University, Providence, RI; 3) Yale University, New Haven, CT; 4) MIT, Cambridge, MA; 5) Lawrence Berkeley National Laboratory, Berkeley, CA.

Semi-conservative segregation of nucleosomes to sister chromatids during DNA replication creates gaps that must be filled by new nucleosome assembly. We analyzed the cell cycle timing of centromere assembly in Drosophila, which contains the H3 variant Cid (CENP-A in humans), as well as CENP-C and CAL1, which are required for Cid localization. Pulse-chase experiments show that Cid and CENP-C levels decrease by 50% at each cell division, as predicted for semi-conservative segregation and inheritance, whereas CAL1 displays higher turnover. Quench-chase-pulse experiments demonstrate that there is a significant lag between replication and replenishment of centromeric chromatin. New Cid is recruited to centromeres in metaphase, by a mechanism that does not require an intact mitotic spindle, inactivation of the mitotic checkpoint, or chromosome segregation, while new CAL1 is recruited before Cid in prophase. The unusual timing of Cid recruitment and the unique dynamics of CAL1 identify a distinct centromere assembly pathway in Drosophila, and suggest that CAL1 is a key player in centromere assembly.

376A

Analysis of ATXN7 function in the SAGA chromatin modifying complex. Ryan D Mohan, Vikki M Weake, Lauren G Shelton, Laurence Florens, Michael P Washburn, Susan M Abmayr, Jerry L Workman. Stowers Institute for Medical Research, Kansas City, MO.

The Drosophila genome comprises four chromosomes and approximately 14,000 genes encoded by 180 Mb of DNA. Storage of this genetic information within the nucleus is facilitated by compaction of DNA into chromatin. A complex structure, chromatin primarily consists of a core histone octamer - two each of histones H2B, H2A, H3, and H4 - around which DNA is tightly looped. Histones possess outward-facing tails which provide a surface for interactions with DNA and nuclear proteins. These tails are subject to covalent modifications such as acetylation and ubiquitination which can act to modulate histone-DNA as well as histone-protein interactions thereby contributing to regulation of gene expression. An important mediator of chromatin modifications is the SAGA (Spt-Ada-Gcn5 acetyltransferase) chromatin modifying complex. SAGA contains two enzymatic subunits which confer histone acetyltransferase (Gcn5) and ubiquitin protease (Nonstop) activities to the complex. Accordingly, SAGA has been shown to participate in regulation of gene expression. We have undertaken a functional characterization of this highly conserved complex in Drosophila and have identified a novel component which shares some sequence similarity to the human yeast SAGA subunits ATXN7/Sgf73. Within their respective organisms, these proteins function to ensure integrity of SAGA by tethering the ubiquitin protease and acetyltransferase modules of the complex to each other and dysfunction of ATXN7/Sgf73 results in alterations to global levels of histone H2B ubiquitination (H2Bub). In Drosophila, misregulation of H2Bub levels upon loss of Nonstop expression has been shown to result in defective neuronal connectivity within the developing visual system. Here we provide evidence that Drosophila ATXN7 is an integral component of SAGA and is necessary for maintenance of histone acetylation, transcriptional regulation, and development.

377B

A genetic screen for mutations affecting the germline function of the Suppressor of Hairy-wing insulator protein. Ryan M. Baxley1, Michael W. Klein2, Ashley B. Gaeth2, Joel A. Morales-Rosado2, Amber M. Hohl3, Pamela K. Geyer1,2,3. 1) Molecular and Cellular Biology Program, University of Iowa, Iowa City, IA; 2) Biochemistry Department, University of Iowa, Iowa City, IA; 3) Genetics Program, University of Iowa, Iowa City, IA.

Suppressor of Hairy-wing [Su(Hw)] is a twelve zinc finger DNA binding protein discovered for its role in gypsy insulator function. Further, loss of Su(Hw) causes female sterility due to a block in oogenesis. While the insulator function of Su(Hw) is well characterized, the requirement for Su(Hw) during female germline development is not well understood. To gain insights into the role of Su(Hw) in oogenesis, an EMS mutagenesis screen was performed that was designed to identify mutations that alter the female fertility or insulator functions of Su(Hw). Out of ~8,000 mutagenized chromosomes screened, four mutations were identified. Three mapped to the su(Hw) gene. Among these, a novel allele, su(Hw)G460, was identified that encodes a Su(Hw) protein with a R486C substitution in zinc finger eight. Females carrying su(Hw)G460 are fertile but suppress gypsy induced mutations. Molecular analyses shows that Su(Hw)G460 has reduced in vitro and in vivo DNA binding, affecting binding of Su(Hw) to the gypsy insulator more than to other non-gypsy genomic sites. The fourth mutation identified a second site modifier locus, called sum393 [su(Hw) modifier][G460]. Females homozygous for sum393 are sterile but retain gypsy insulator function. Immunohistochemical analyses demonstrate that a second chromosome localization of Su(Hw) is altered in sum393 mutant somatic and germline cells. These data imply that sum393 preferentially affects occupancy at genomic sites required for female fertility. Our studies demonstrate that the insulator and fertility functions of Su(Hw) are genetically separable. Further, these data suggest that Su(Hw) binding has distinct requirements throughout the genome and implicate novel factors for Su(Hw) chromosome localization.

378C

Insulator elements mediate long-range interactions between Polycyprad targets and between active enhancers in Drosophila. Hua-Bing Li, Katsuhito Ohno, Vincenzo Pirrota. Molecular Biology & Biochemistry, Rutgers University, Piscataway, NJ.

The genomic binding sites of Polycyprad (PcG) complexes have been found to cluster, forming Polycyprad “bodies” in mammalian or fly nuclei. These associations result in repression of downstream gene expression. An important mediator of chromatin modifications is the SAGA (Spt-Ada-Gcn5 acetyltransferase) chromatin modifying complex. SAGA contains two enzymatic subunits which confer histone acetyltransferase (Gcn5) and ubiquitin protease (Nonstop) activities to the complex. Accordingly, SAGA has been shown to participate in regulation of gene expression. We have undertaken a functional characterization of this highly conserved complex in Drosophila and have identified a novel component which shares some sequence similarity to the human yeast SAGA subunits ATXN7/Sgf73. Within their respective organisms, these proteins function to ensure integrity of SAGA by tethering the ubiquitin protease and acetyltransferase modules of the complex to each other and dysfunction of ATXN7/Sgf73 results in alterations to global levels of histone H2B ubiquitination (H2Bub). In Drosophila, misregulation of H2Bub levels upon loss of Nonstop expression has been shown to result in defective neuronal connectivity within the developing visual system. Here we provide evidence that Drosophila ATXN7 is an integral component of SAGA and is necessary for maintenance of histone acetylation, transcriptional regulation, and development.

379A

A Novel Chromatin Barrier Element Delimits the Formation of Facultative Heterochromatin Without Blocking Enhancer Function. Nianwei Lin1, Keji Zhao2, Guangyao Li1, Lei Zhao1. 1) Molecular Genetics and Microbiology, University of Florida, Gainesville, FL; 2) Laboratory of Molecular Immunology, National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892.

Formation of facultative heterochromatin at specific genomic loci is fundamentally important in defining cellular properties such as differentiation potentials and responsiveness to environmental stimuli. By the nature of its formation, heterochromatin and repressive histone marks will propagate until the chain reaction is broken. While certain active promoters can block propagation of heterochromatin, there are also specialized DNA elements, referred to as barrier insulators, serve to demarcate the boundary of facultative heterochromatin formation. In this study we identified a chromatin barrier that specifically limits the epigenetic regulation to a distal enhancer region so that repressive histone modification cannot reach the promoter and proximal enhancer regions of reaper. Unlike all of the known insulators identified from Drosophila, the IRER (irradiation responsive enhancer region) left barrier (ILB) does not contain enhancer-blocking activity. This is in accordance with the fact that epigenetic regulation of IRER is dynamic and reversible.
following certain stresses, under which circumstances the enhancer function of IRER is required for stress-induced pro-apoptotic gene expression. Drosophila Cut protein may be required for the barrier activity by recruiting the histone acetyltransferase CBP.

Pairing between gypsy insulator facilitates chromosomal rearrangements. Mikhail Savitsky, Oksana Krvavchuk, Artem Tkachuk, Maria Kim. Institute of Gene Biology RAS, Moscow, Russian Federation. One of the best-characterized insulators is gypsy insulator which is the Su(Hw)-binding element from gypsy retrovirus. It is known to block enhancers-promoter action when placed between them and to prevent gene silencing by Polycomb group complexes and heterochromatin. Another important feature of gypsy insulators is their ability to associate with each other. The Su(Hw) protein also binds to hundreds of non-gypsy regions in the Drosophila genome. Some of these multiple sites cluster together playing role in organizing chromatin architecture. Using transgenic system we have shown previously that gypsy insulator is able to stabilize trans-interaction between enhancers and a promoter situated on homologous chromosomes. Moreover, gypsy insulator can enhance association between very remote loci even in an interchromosomal manner. We decided to investigate if chromosomal rearrangements are also facilitated between juxtaposed loci. So, we chose two transgenic insertions to interact in presence of the insulator. Here we report that recombination frequency between loci sites inserted in the transgene decreases dramatically on Su(Hw) and mod(mdg4)u1 background. Next we found that double-strand breaks occurring upon P-element excision may result in different chromosomal rearrangements. The frequency of these events is greatly elevated in presence of gypsy insulator. We propose that the spatial proximity of linearly distant genomic loci stabilized by insulators may provide conditions favoring chromosomal interactions. We also suggest that the inversions between spatially proximal loci are less likely to disturb the overall nuclear architecture and therefore would be easily fixed by natural selection.

The role in regulating TEs in the germline. In the soma, where natural selection acts on both host and TE lineages to reduce TE activity, endogenous siRNAs also play a role in TE regulation. A syndrome of hybrid dysgenesis in Drosophila virilis offers a powerful model for determining the role that these two classes of silencing RNA have in maintaining control of TEs in an epigenetic manner across generations. Unlike syndromes of hybrid dysgenesis in D. melanogaster, hybrid dysgenesis in D. virilis is characterized by the mobilization of multiple unrelated TEs. Whether this is caused by the cascading effects of one TE or multiple independent mobilizations is not known. Preliminary studies using small RNA sequencing and cytological analysis indicate that hybrid dysgenesis in D. virilis arises from the interaction of multiple TE families that exhibit differences in the level of RNA silencing between the two parental strains. This interaction across different TE families drives a cascade of events in the hybrid that lead to hybrid sterility.

DNA methylation and DNA methyltransferase in Drosophila melanogaster. Deepti D Deobagkar, Chitra Panikar. Zoology, University of Pune, Pune, India. DNA methylation is a common epigenetic modification that is a unique way of encoding information which is heritable but can still be edited. DNA methylation plays a crucial role in development of an organism and it modulates expression of phenotype by modulation of genotype. The earlier reports of presence of DNA methylation in Drosophila melanogaster were later confirmed. However no precise role has yet been assigned to DNA methylation in insects. D. melanogaster is a dipteran insect that is used as a model system for many diverse phenomena. DNA methylation could play a role in the differential regulation of several genes during development and in response to stress. A novel DNA methyltransferase assay was employed to check for the methyltransferase activity. The activity of the DNA methyl-transferase enzyme is high in eggs and adults, moderate in pupae and low in larva. The de novo methyltransferase activity was also investigated. Our work demonstrates the presence of CpC methylation in Drosophila melanogaster and specification of different regions in the Hae III and Hha I shows differences in digestion pattern during suggesting differential methylation pattern during the life cycle of Drosophila. We have investigated presence of genome wide methylation in genes in Drosophila using a novel approach using cDNA microarray combined with antibodies to modified nucleotides. This high throughput approach helped us to generate a genome wide epigenetic map in Drosophila. The results suggested that DNA methylation in genes varies between different developmental stages. This method generates methylation landscape in and around genes in a sequence independent manner. The DNA methylation could be detected in genes involved in transcription, DNA binding and epigenetic regulation. This study thus provides a link between epigenetic landscape of Drosophila genes and gene function.

Identification of Genes Involved in Somatic Homolog Pairing. Eric Joyce, C.-Ting Wu. Genetics, Harvard Medical School, Boston, MA. Homolog pairing refers to the physical alignment of homologous chromosomal segments. Although most notable for its role in chromosome segregation during meiosis, recent studies have demonstrated homologous interactions to be a powerful mechanism for controlling gene expression. We now know that these interactions play key roles in many processes, including X-inactivation, T-cell development, and silencing of the X and Y chromosomes during spermatogenesis. Most recently, extensive somatic pairing of the q arm of human Chromosome 19 has been observed in renal oncocytesomas, emphasizing the importance of understanding how chromosomal interactions might impact gene regulation in the context of disease. In Drosophila, homologous chromosomes are intimately paired in virtually all cell types throughout the cell cycle. While much is known about the downstream effects of homolog pairing on gene expression, it remains unclear how homologous chromosome segments find each other, physically align and then form stable pairing interactions within somatic cells. Therefore, I am using FISH to conduct genome-wide RNAi-driven screens for gene involved in homolog pairing in Drosophila and then will characterize the genetic pathways that are identified. An update on gene hits from primary screens will be presented as well as a protocol for high-throughput FISH, which could be applied to address a number of biological questions concerning genome organization. This work is supported by NIH GM085169-01A1 and a SPARC grant from the
Epigenetic protection of telomeres in the male germline. Benjamin Loppin1, Raphaëlle Dubruille2, Latitia Delabaure1, Gabriel Marais2, Guillermo Orsi1. 1) CGMC, CNRS UMR5534, University of Lyon, France; 2) LBBE, CNRS UMR5558, University of Lyon, France. A critical function of telomeres is to protect chromosomes from deleterious end-to-end fusions. In Drosophila, assembly of the protecting capping complex at telomeres is a sequence-independent process that is initiated by HipHop, a small, rapidly evolving protein, and its partners HP1 and HOAP. We had previously shown that hip hop was duplicated before the radiation of the melanogaster subgroup of species, giving birth to $K81$, a unique paternal effect gene required for the transmission of paternal chromosomes to the zygote. We have found that $K81$ specifically associates with telomeres in male germ cells. After fertilization, $K81$ is progressively replaced at paternal telomeres by its maternally expressed sister protein HipHop. In the absence of $K81$, capping proteins are no longer maintained at paternal telomeres, resulting in telomere fusions and chromosome bridges during the first zygotic division. We also demonstrate that $K81$ and HipHop not only acquired complementary expression domains following duplication but also functionally diverged, suggesting that $K81$ specialized in the maintenance of telomere protection in the highly peculiar chromatin environment of differentiating male gametes.

Role of histone acetylation in tissue-specific gene regulation at nuclear lamina. Beatrice Milon, Haibo Cheng, Dmitry Nurminsky. Biochemistry and Molecular Biology, University of Maryland Baltimore, Baltimore, MD. Lamina-associated gene regulation is a major mechanism that links nuclear architecture, chromatin structure, and developmental gene expression. Defects in this mechanism result in tissue-specific degenerative disorders coupled with faulty cell differentiation. Diverse chromatin modifying proteins bind to nuclear lamina, however their roles in gene regulation remain unclear. Recently, we described a male germline-specific multigenic chromatin domain which is subject to laminB1-dependent repression at nuclear periphery of somatic cells. We now exploit the advantages of this model to dissect the mechanism of tissue-specific transcriptional regulation at nuclear lamina. Using systematic RNAi approach, we demonstrate a key role for broadly expressed Class I histone deacetylases in somatic repression of male germline-specific multigenic domain. Employing bioinformatics and molecular approaches, we identify a counteracting testis-specific histone acetyltransferase that is required for full expression of the multigenic domain in male germine, and causes derepression of this domain when overexpressed in somatic cells. These findings demonstrate control of tissue specific transcription at nuclear lamina by a balanced countertplay between broadly expressed histone deacetylases and tissue-specific histone acetyltransferase, implicating this novel mechanism of gene regulation in developmental processes and their pathological disruptions.

Mapping the Telomere elongation mutation in Drosophila. Hernakumar M. Reddy, James M. Mason. Laboratory of Molecular Genetics, NIH/NIEHS, Research Triangle Park, NC. Telomeres are structures at chromosome ends necessary to prevent activation of the DNA damage response and to maintain chromosome length. Drosophila differs from mammals in the mechanism of telomere maintenance, as it lacks telomerase. Drosophila telomeres contain two distinct DNA regions: an array of non-LTR retrotransposons (HTT array) and telomere-associated sequences. The HTT array contains three elements HeT-A, TART, and TAHRE, of which HeT-A is the most abundant. Transposition of these elements and gene conversion between chromosome ends have been proposed as two major mechanisms of telomere elongation. Two dominant mutations, Telomere elongation, Tel, and Enhancer of telomeric-gene conversion, E(tc), cause cytologically visible elongation of telomere gene fragments. Both of these mutations were mapped genetically to 69C on chromosome 3, which corresponds roughly to region 92 on the cytological map. $Tel$ had also been mapped to 92A2-11, by $P$ element induced male recombination. This region contains 27 genes, of which 23 have unknown or predicted function. To further map the $Tel$ locus, we selected four $P$ elements and two $Minos$ elements that subdivide this 318 kb region. Recombination was induced in males that carried each of these elements individually using transposase, and recombinants were put into stocks and DNA collected at intervals for 12 generations. Quantitative PCR was performed using He-T-A primers to estimate telomere length in these recombinants. The results show that $Tel$ maps between the transposons $P[PK]/Df(2L)P[10]$ and $P[EPgy2]/Ino80/Df(2L)P[10]^+$, a 55 kb region that has 4 unannotated genes, the 3′ end of Ino80, and a 40 kb well conserved intergenic region. To further analyze this region, next generation sequencing with a Illumina Genome analyzer IIx was done with $Tel$ and E(tc) mutants and a y w control to identify signature differences in the sequence that could lead to identification of the Tel mutation.

Characterization of genes required for cell-death induced inhibition of RNAi. Weiwu Xie, James Birchler. Biological Sci Div, Univ Missouri-Columbia, Columbia, MO. We previously showed that cell death caused by developmental defects or ectopic expression of cell death genes inhibits RNAi in different tissues of larvae or in adult eyes. The inhibition can be non-autonomous and a signal passing from dying cells to surrounding cells was hypothesized. Using the white eye color gene and its RNAi transgene as a reporter, eye pigment can be clearly restored in the anterior portion of the eye in the Bar mutant. This phenotype was used to screen for modifiers of the death-induced inhibition of RNAi (Dir). Mutations were induced by feeding EMS to males. Dominant mutations either suppressing or enhancing the phenotype were found and named Su-Dir or E-Dir. Recombination mapping analysis indicated several genes are involved. It was found that glass mutants show an enhancer phenotype. The EMS-induced enhancer mutants (E-Dirs) are located close to glass, but sequencing of the mutations showing no changes in this gene, suggest other genes were involved. The Su-Dirs eliminating the reversal of the w RNAi (i.e., restored red color) can be divided into at least 5 groups by recombination mapping: 1 on Chr. X, 2 on Chr. 2 and 2 on Chr. 3. These mutants were crossed to an isogenic strain and then backcrossed for more than 25 generations to eliminate linked non-specific mutations. Next generation genomic sequencing was applied to identify the gene for one of the Su-Dirs from the largest group on Chr. 3, which includes 7 mutants. One mutation was found in the gene $CG1647$. A transposon-insertion (inserted in the coding sequence) allele of $CG1647$ exhibited the same phenotype as the Su-Dirs. Six out of the 7 mutants were confirmed as having a mutation in this gene. Three of the mutations involved changes to nonsense codons and the other three are located in the N-terminal conserved zinc-finger associated domain (z-f-AD). Further analysis of the modifiers will potentially provide insight into the pathway involved in the mechanism of cell death mediated reversal of RNAi.

De novo establishment of Polycomb-mediated repression. Jamuna AllHaj Abed, Siddhu Desai, Judith Benes, Richard Jones. Southern Methodist University, Dallas, TX. Polycomb group proteins (PcG) are a highly conserved group of proteins that maintain the transcriptional repression of target genes. PcG proteins do not initiate transcriptional repression of target genes but rather take over repression from gene-specific transcription factors. Previous studies have shown that during the maintenance phase of repression Pleiohomeotic (PHO), and PHO-Like (PHOL) directly bind at the Polycomb Response Elements (PRE) and recruit the PRC2 complex which trimethylates H3K27, facilitating recruitment of the PRC1 complex. Once PcG-mediated repression is established, it is stably maintained through numerous cell cycles. However, very little is known about the molecular mechanisms by which PcG proteins initially recognize the repressed state of a target gene, and take over its repression. This is due to the technical limitations imposed by heterogeneous expression of target genes within developing embryos during the time that PcG-mediated repression is established.

We are investigating the molecular mechanisms of de novo establishment of PcG-mediated repression at giant (gt). Genetic manipulation will be used to obtain synctial and cellular blastoderm stage embryos in which gt is initially ubiquitously repressed at the synctial blastoderm stage by a maternally expressed transcription factor Hunchback (HB), and in which the PcG is required to maintain its ubiquitous repression by the cellular blastoderm stage. In situ hybridizations and transgenic reporter lines are being used to map the locations of PREs within the gt regulatory region. Chromatin immunoprecipitation (ChIP) assays will be used to examine the distributions of PcG proteins as well as repressors and activators at gt, in carefully staged embryos during the developmental window at which repression transitions from HB to repression by the PcG proteins.
Polycomb group proteins connect distant genes within the nucleus. Frédéric Bantignies, Virginie Rourse, Ilyis Cymet, Benjamin Leblanc, Bernd Schuettengruber, Giacomo Cavalli. Institut de Génétique Humaine, CNRS-UPR 1142, Montpellier, France.

Polycomb group (PcG) proteins are repressive chromatin factors, which bind to specific target sites within the Drosophila genome. At the homeotic bithorax complex (BX-C) locus, these proteins have been shown to form a higher-order structure called the "repressive chromatin Hub", which corresponds to a clustering of Polycomb target sites. By using a genome-wide "Chromosomal Conformation Capture" technique (4C) together with Immuno-3D-FISH in Drosophila tissues, we tested whether clustering of PcG target sites could occur at larger distances. Our results indicate that the Fab-7 Polycomb target site in the BX-C forms an extensive and specific series of contacts over large distances with other PcG target domains. Some of these contacts involve over a few Megabases along the chromosome arm, two notable examples are the Antennapedia complex (ANT-C) and the homeobox gene complex called NK-C. Strikingly, functional analysis using specific BX-C deletions and sensitized ANT-C backgrounds reveal that some of these long-range interactions are likely important for the "fine-tuning" of gene regulation during Drosophila development.


tonalli (tna) is a trithorax group gene that was identified by its genetic interaction with Brahma, the ATPase of the BRAHMA chromatin-remodeling complexes, BAP and PBAP. It also interacts genetically with other proteins required for homoeotic gene expression. Northern analyses showed at least two developmentally regulated mRNAs encoding a long (TnaA) and a short (TnaB) isoforms. TnaA has an XSPRING domain which contains an SP-RING zinc-finger, found exclusively in proteins with SUMO E3-ligase activity.

SUMOylation is a protein reversibly covalent posttranslational modification similar to ubiquitination. The main functions attributed to SUMOylation are to change the stability, the activity and/or the subcellular location of its protein targets. tna genetic interactions and the presence of the SP-RING in TnaA, allow us to hypothesize that it might be involved in the SUMOylation of proteins required for the expression of homoeotic genes in some specific subcellular compartments. In this work we show that 1) there are at least two TnaA isoforms (TnaA130 and TnaA123) differentially expressed through development, 2) TnaA130 is located in the cytoplasm while TnaA123 is in the cytoplasm and in the nucleus of 3-21 h embryos, 3) TnaA is associated to discrete sites of euchromatin in polytene chromosomes of 3rd instar wild type larvae 4) TnaA physically interacts with the E2 SUMO-conjugating enzyme Ubc9 (encoded by lesswright, lwr), by pull-down and by two-hybrid assays and 5) at least TnaA130 interacts with Brahma by coimmunoprecipitation in embryo nuclear fractions.

Do Polycomb and trithorax mutations affect reporter gene expression on the 4th chromosome of Drosophila melanogaster? Perry Marcoce, Sarah C R Elgin, Nicole C Riddle. Biology Department, Washington University in St. Louis, St. Louis, MO.

Cells must be able to silence and activate lineage specific genes. In Drosophila, this process is controlled by Polycomb (Pc) and triethorax (trx) group proteins. Among other targets, Pc group proteins act upon homoeotic genes to maintain a silenced state, while trx group proteins act upon these same genes at different developmental times to keep them activated. When investigating the chromatin environment of Drosophila melanogaster's fourth chromosome insertion lines using data produced by the modENCODE consortium, we discovered a correlation between the insertion site of reporter lines showing a red eye (as opposed to the majority, which exhibit a variegating phenotype) and domains enriched for Polycomb protein. We hypothesized that Pc or trx group proteins might be involved in the control of gene expression for red-eyed fourth chromosome reporters. We introduced mutations in Pc and trx group genes into several reporter strains to determine how reporter expression would be affected. Only mutations of ash1, encoding a trx group protein with H3K4 methyltransferase activity, consistently decreased expression from red-eyed reporter on chromosome 4, measured indirectly by assaying eye pigment levels. We then tested if the observed trend represented a global effect on gene expression or was unique to fourth chromosome insertions. We discovered that the effect of the ash1MO mutation is highly dependent on the insertion site of the transgenic reporter, ruling out a global effect on gene expression. Our current work includes chromatin immunoprecipitation experiments and additional genetic analyses using double- mutants, which should further our understanding of the relationship between ash1 and the chromosome 4 transgene reporters.

Mutational analysis of Su(z)2 reveals both essential and regulatory residues. Son C. Nguyen1, Stephanie Yu2, Elaine Oberlick1, Chao-ting Wu1. 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Stanford University, Palo Alto, CA; 3) Emory University, Atlanta, GA.

Posterior sex combs (Psc), a core PcG protein, and its paralog Suppressor 2 of zeste (Su(z)2) are essential chromatin proteins that are believed to repress gene expression through their C-terminal regions (CTRs), as suggested by in vivo and in vitro data. Mammalian homologs bmi-1 and mel-18 are also important factors in gene regulation but lack a similar CTR, calling into question the functional homology of the mammalian and Drosophila homologs. To elucidate the function of these proteins, we conducted a genetic screen, which generated mutations in the region of Su(z)2 that is most homologous with Psc, bmi-1, and mel-18 (the homology region, or HR). Specifically, I have generated mutations in two domains within the HR that are believed to be involved in protein interactions, the RING finger and the recently characterized RAWUL domain. While mutations in the RING domain in vivo reveal that the HR can be sufficient for viability. These data suggest that the essential function of Su(z)2 lies in the HR, not the CTR, and that the RAWUL domain is a regulator of this function. The Su(z)2 HR thus provides a model for examining a mechanism that has been conserved between Drosophila and mammalian homolog and which has implications in global gene regulation, stem cell maintenance, and cancer. (This work was supported by the NSF Graduate Research Fellowship and NIH GM085169-01A1).

The Polyhomeotic protein induces hyperplastic tissue overgrowth through the activation of the JAK/STAT pathway. ROCIO SIMON1, INMA GONZALEZ2, ANA BUSTURIA1. 1) CENTRO DE BIOLOGIA MOLECULAR “SEVERO OCHOA” (CSIC-UM), MADRID, SPAIN; 2) INSTITUT DE GENETIQUE HUMAINE, CNRS, MONTPELLIER, FRANCE.

Polycomb group (PcG) and Trithorax Group (trxG) proteins are key epigenetic regulators of gene expression of most developmental genes. Altered levels of expression of these proteins are associated with the development and progression of human diseases like cancer. We have studied the effects of high levels of Polyhomeotic (PH) protein, a member of the PcG, during the proliferation of the imaginal discs in Drosophila. Over expression of PH induces high rates of proliferation. As a result, massive hyperplastic overgrowths are produced and we have observed that these are mediated by the activation of the JakStat signalling through the de-repression of unpaired (upd), the extracellular ligand of the pathway. We also observed high levels of the growth factors decapentaplegic (dpp) and d-myc, two putative targets of the Jak/Stat pathway. Moreover, inactivation of the Jak/Stat pathway in the PH-induced tumors greatly reduces the tissue overgrowth, demonstrating a functional relationship between Jak/Stat signalling and PcG gene expression, both of which are found to be perturbed in tumorigenesis.

Genetic Analysis of Drosophila and Human BAP1 in Polycomb Repression. Kim Younglan1, SHIN Na-Ra2, Lee Sang-Wang2, UM Soo-Jong2, CHUNG Yun Doo2. 1) Department of Life Science, University of Seoul, Korea; 2) Dept. of Bioscience and Biotechnology, Sejong University, Korea.
BRCA1 associated protein-1 (BAP1) is a member of the ubiquitin carboxyl-terminal hydrolase (UCH) subclass of deubiquitinating enzymes. In mammalian cells, BAP1 is thought to be a tumor suppressor that functions in the BRCA1 growth control pathway. In *Drosophila*, it has recently been reported that a novel Polycomb group gene *calypso* encodes the Drosophila homolog of BAP1 (dBAP1), and dBAP1 in combination with a Polycomb group protein ASX constitutes a Polycomb repressive deubiquitinase (PR-DUB) complex which removes mono-ubiquitin from histone H2A. To investigate the role of BAP1 further, we generated transgenic flies expressing various versions of human or Drosophila BAP1. Genetic analysis of these fly lines will be presented.

396C
The role and interactions of Drosophila SAF-B in transcriptional activity. Kudakwashe L Kupara, Keith Maggert. Biology, Texas A and M University, College Station, TX.

Scaffold Attachment factor B (SAF-B) is a nuclear protein associated with Scaffold/Matrix Attachment DNA sequences. SAF-B has recently been shown to be a component of a durable nuclear matrix, showing general nucleoplasmic distribution, distinct intense foci and web-like continua. The observation of SAF-B matrix and association of SAF-B at puffs of transcriptional activity suggests that SAF-B may be involved both in the tethering of DNA looped chromatin domains to the nuclear matrix and in transcriptional regulation. The recruitment of SAF-B to puffs of transcriptional activity is predominately by the RNA binding domain. SAF-B has predicted phosphorylation sites suggesting a possible regulatory mechanism. To investigate the role of SAF-B we embarked on loss-of-function/knock-out and gain of function approaches. We expect our data to show SAF-B knock-out to cause a misregulation of Drosophila embryonic segmentation genes by observing patterning of the pair-rule gene product *fushi-tarazu*. Genetic interactions between *sa-f-b* and a phosphorylase, *Dakerner of Apricot* (DOA) kinase have suggested a direct interaction between the two proteins. Preliminary data in our laboratory has shown that SAF-B is phosphorylated by DOA kinase. By way of an RNAi approach we expect to observe a down-regulation of the w+ reporter gene in flies lacking SAF-B. Furthermore, because flies heterozygous for *sa-f-b* knock-out in a homozygous DOA hypermorph background show an ecdysis phenotype, we expect a *lacZ* reporter driven by the edysone promoter to show aberrant tissue distribution. By means of forceable recruiting of SAF-B to a reporter locus using the *lacI* DNA binding domain-SAF-B fusion protein we expect to observe an upregulation of transcription. Our working model is that SAF-B is part of a dynamic regulable nuclear matrix in which the balance of matrix-aggregated SAF-B to free nucleoplasmic SAF-B is regulated by phosphorylation of SAF-B by DOA kinase. The phosphorylation condition of SAF-B may determine upregulation or down-regulation of genes at SAF-B-associated chromatin loops.

397A
Mining functional DNA elements in the *Drosophila* genome. Per Stenberg, Philge Philip. Molecular Biology, Computational Life Science Cluster (CLiC), Umeå University, Umea, Sweden.

The control of gene expression is highly complex in all organisms, requiring major energetic inputs and the maintenance of intricate structures and mechanisms. Consequently, approximately 10% of investigated proteins in mammalian and invertebrate genomes appear to be involved in gene regulation. Many of these proteins bind directly to the DNA or chromatin and to understand gene regulation we need to understand how the regulatory proteins are targeted to their sites of action. Using modern array technology and the new generation sequencing techniques, an increasing number of binding sites for chromatin associated proteins are being mapped in detail across whole genomes of the commonly used model organisms. The standard normalization approaches of these types of data assume that there are only small differences between the control and the treatment samples (IP-samples). We have studied how data that do not fulfill this assumption are affected in terms of sensitivity and bias and have developed a statistical test that detects skewed data sets. We show that if a data set is skewed the quality of the data can be greatly improved by applying a Hidden-Markov Model (HMM) assisted normalization procedure.

Although there is evidence for sequence components involved in the targeting of many chromatin associated factors, defining these has in many cases proven difficult. We have performed an extensive study of X-chromosome sequence variation in *D. melanogaster*. We find that this chromosome contains many unique sequence features, some of which correlate well with the distribution of the dosage compensation complex (MSCL-complex). We also find sequence elements with potential roles in X-chromosome compensation that might be unrelated to the action of the MSL-complex.
A Drosophila Model of Fetal Alcohol Syndrome. Rachael L. French, Kimberly McClure, Ulrike Heberlein. 1) Biological Sciences, San Jose State University, San Jose, CA; 2) Department of Anatomy, University of California, San Francisco, 94158.

Prenatal exposure to ethanol in mammals leads to a range of developmental problems, from growth deficiency and birth defects to mental retardation and behavioral abnormalities. In humans, these symptoms are collectively described as fetal alcohol syndrome (FAS). FAS is the leading cause of congenital mental retardation in the Western world. Despite the growing awareness of FAS, the worldwide prevalence of FAS remains steady at one to three per 1000 births. The high frequency of FAS coupled with the failure of public awareness programs highlights the need for an understanding of the molecular basis of FAS and the development of novel treatments to mitigate the complications of gestational ethanol exposure.

Human epidemiological data, twin studies, and animal models indicate that many genes likely confer risk or protection from fetal alcohol injury, yet none have been conclusively identified. We have developed a genetic model of fetal alcohol syndrome in flies. We show that developmental ethanol exposure causes reduced viability, developmental delay and reduced adult body size. We also show that, as in mammals, flies reared on ethanol have altered responses to ethanol intoxication as adults. These changes include increased locomotor activation, resistance to the sedating effects of the drug, and reduced tolerance development upon repeated ethanol exposure. Finally, we have found that these defects are largely due to ethanol’s effects on insulin signaling. Ethanol exposure during development causes a dramatic reduction in Drosophila insulin-like peptide and insulin receptor expression, and transgenic expression of Dils in the larval brain rescues both developmental and behavioral abnormalities displayed by ethanol-reared flies. Our results establish Drosophila as a model system to identify neurodevelopmental pathways that are altered by exposure to ethanol, and the genes underlying those pathways. This, in turn, may lead to the identification of targets for pharmacological intervention in cases of fetal alcohol exposure.

A Low-Dose Ethanol Treatment Impairs Learning in Drosophila Larvae. Brooks G Robinson, Sukant Khurana, Jashua Pohi, Wenke Li, Martin Hatch, Amanda Cady, Kristina Najjar, Amar Bhat, Ryan Godinez, Nigel Atkinson. 1) Institute for Neuroscience, University of Texas at Austin, Austin, TX; 2) Department of Neurobiology, University of Texas at Austin, Austin, TX.

Alcohol is a widely abused substance that has a multitude of effects on the nervous system. *Drosophila melanogaster* has proven to be a very useful model for the study of ethanol-related behaviors and their underlying genetics. Drosophila adults and larvae have a diverse array of behaviors that can be used to assay drug effects. In this study, we show that even an extremely low dose of ethanol can affect behavior in Drosophila larvae. In our study, larvae were acutely exposed to 20% ethanol, resulting in low internal concentrations (below the legal driving limit for humans). We then assayed their locomotion, olfaction, and learning abilities. While the locomotion of larvae was not affected by this low dose of ethanol, their attractive olfactory response to low, but not high concentrations of odor was impaired. In addition, olfactory learning was impaired in ethanol-treated larvae. Learning has previously been shown to be affected by ethanol in other model systems including mammals. In our learning paradigm, larvae were simultaneously exposed to an odor and an aversive temperature of heat to produce associative conditioning. Following this training, larvae will avoid the odor to which they normally would be highly attracted. The learning experiments were carried out at an odor concentration to which ethanol-treated larvae responded normally. Interestingly, learning was only impaired when a 37°C heat shock reinforcement was used and not when a more salient 43°C stimulus was used. These results indicate that low, acute doses of ethanol affect larvae’s ability to detect and respond to weaker stimuli whereas their response to strong stimuli appears to remain intact. This low-dose ethanol effect coupled with the power of Drosophila genetics may provide an important tool in the identification of genes that might be involved in human ethanol addiction.

Low-Dose Ethanol Treatment Impairs Learning in Drosophila Larvae. Brooks G Robinson, Sukant Khurana, Jashua Pohi, Wenke Li, Martin Hatch, Amanda Cady, Kristina Najjar, Amar Bhat, Ryan Godinez, Nigel Atkinson. 1) Institute for Neuroscience, University of Texas at Austin, Austin, TX; 2) Department of Neurobiology, University of Texas at Austin, Austin, TX.

Alcohol is a widely abused substance that has a multitude of effects on the nervous system. *Drosophila melanogaster* has proven to be a very useful model for the study of ethanol-related behaviors and their underlying genetics. Drosophila adults and larvae have a diverse array of behaviors that can be used to assay drug effects. In this study, we show that even an extremely low dose of ethanol can affect behavior in Drosophila larvae. In our study, larvae were acutely exposed to 20% ethanol, resulting in low internal concentrations (below the legal driving limit for humans). We then assayed their locomotion, olfaction, and learning abilities. While the locomotion of larvae was not affected by this low dose of ethanol, their attractive olfactory response to low, but not high concentrations of odor was impaired. In addition, olfactory learning was impaired in ethanol-treated larvae. Learning has previously been shown to be affected by ethanol in other model systems including mammals. In our learning paradigm, larvae were simultaneously exposed to an odor and an aversive temperature of heat to produce associative conditioning. Following this training, larvae will avoid the odor to which they normally would be highly attracted. The learning experiments were carried out at an odor concentration to which ethanol-treated larvae responded normally. Interestingly, learning was only impaired when a 37°C heat shock reinforcement was used and not when a more salient 43°C stimulus was used. These results indicate that low, acute doses of ethanol affect larvae’s ability to detect and respond to weaker stimuli whereas their response to strong stimuli appears to remain intact. This low-dose ethanol effect coupled with the power of Drosophila genetics may provide an important tool in the identification of genes that might be involved in human ethanol addiction.

Deerfield on the campus of the University of Chicago, Chicago, IL.

Deerfield on the campus of the University of Chicago, Chicago, IL.

Deerfield on the campus of the University of Chicago, Chicago, IL.

Deerfield on the campus of the University of Chicago, Chicago, IL.
Genes nAchR/g302-30D and Cam as modifiers of dystrophin gene function in Drosophila melanogaster. Natalia Holub, Yulia Shalovylo, Olena Holub, Yaroslava Chernyk. Department of Genetics and Biotechnology, Ivan Franko National University, Lviv, Ukraine.

Natalia Holub, Yulia Shalovylo, Olena Holub, Yaroslava Chernyk. Department of Genetics and Biotechnology, Ivan Franko National University, Lviv, Ukraine.

Duchenne and Becker muscular dystrophies are caused by mutations in dystrophin gene. There is currently no effective treatment for these diseases. Drosophila melanogaster is an excellent genetically tractable model for searching new approaches to treatment dystrophies such as using genes-modifiers of dystrophin gene function. The aim of our work was to check up influence of genes nAchR/g302-30D and Cam (involved in functioning of muscles and cytoskeleton) as an possible genes-modifiers of dystrophin gene function. Mutant strains tg6 and NH2-Dys constructed after the method antisense-RNA were used. They are characterized by diminished on 70% and 30% expression of dystrophin gene, defective thorax muscle structure and decreased the index of physical activity (IPA). Offspring F1 which contained supplementary copy of gene-modifier and dystrophin gene inactivation construct were analysed after these phases. In all crossing systems was observed restore of thorax muscle structure with the frequency 53% - 63,2% that is in 10 times higher comparing to the strains tg6 and NH2-Dys. In climbing-test was shown increasing of IPA in progeny NH2-Dys/nAchRb in the 3 - 5 times and for hybrids NH2-Dys/Cam - in 2 - 3 times comparing to strain NH2-Dys. In adults with genotype nAchRb/+ tg6/+ IPA exceeded the value of tg6 strain in 4-7 times and in hybrids Cam/+tg6/+ in 3-4 times. Previously was shown that genes nAchRb-30D and Cam resumed of wing vein structure with frequency 51% and 33% in strains NH2-Dys and tg6. It could be concluded that genes nAchRb and Cam manifested to be dystrophin - deficiency phenotype suppressors moreover gene nAchRb is more active suppressor than Cam gene.
POSTER: Drosophila Models of Human Diseases
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

The mesoderm gives rise to and directs the differentiation of blood, fat body, and muscle cells. Proper development of the mesoderm is required for muscular differentiation and development. Me2 (Myocyte enhancer factor 2) is a transcription factor required for embryonic mesoderm development in Drosophila melanogaster. Various factors regulate Me2 transcription at early and late stages in embryo development. The transcription factors Twist, Mad/Medea, and Dorsal are candidates for Me2 regulation at the onset of its expression, and it is possible that these factors interact to impel Me2 expression. Since the enhancer regulating early Me2 expression has been identified, we can now determine if these transcription factors act independently or as part of a heterodimeric mobility shift assay will be conducted to examine the extent to which these factors bind the enhancer region. Next, constructs containing lacZ reporters carrying mutations in the transcription binding sites will be made. P-element transformations will be conducted with these constructs to examine how the transcription factors drive activity of the enhancer region in vivo. Mad-Medea has been shown to bind to the enhancer region (Nguyen and Xu 1998) and we have found that Twist also binds to the enhancer region. Binding of the Dorsal transcription factor has not yet been thoroughly demonstrated. This study will characterize and further define how these transcription factors interact to initiate and regulate Me2 expression. Due to the high conservation of these developmental mechanisms from D. melanogaster to humans, this study will serve as a useful model by which to understand the cellular mechanisms of development and disease.

408C

Drosophila tripartite-motif containing family member implicated in the normal stabilization of myofibrils. Elisa LaBeau1, Kenneth Bauman2, Richard Cripps1, Erika Geisbrecht2. 1) Department of Biology, University of New Mexico, Albuquerque, NM; 2) School of Biological Sciences, University of Missouri, Kansas City, MO.

Tripartite-motif (TRIM) containing proteins are important for proper development and maintenance of both cardiac and skeletal muscle. This family of proteins is widely implicated in the ubiquitination of actin and other cytoskeletal elements as well as playing significant roles in cell-cycle regulation. Loss of function in these proteins results in a variety of disorders such as cardiac hypertrophy, disturbed cardiac conduction, and heart failure as well as muscular atrophy resulting from destabilized muscle tissue and other cellular components. Limited data exists concerning the roles and timing of TRIM containing proteins. Understanding of the function of TRIM proteins in normal and disease states is necessary for identifying crucial points of interaction between other elements of the cell. TRIM containing proteins have been highly conserved across taxa in both sequence and function. Investigations of a new, mutant phenotype in Drosophila show morphology consistent with disruptions in the normal function of TRIM-containing proteins. We have isolated the region of the genome which carries the mutation responsible for a dystrophic mutant phenotype. Of 11 candidate genes a TRIM protein family member, another b-box affiliate (abda), was identified. We are proceeding with investigations by confirming the identity and expression pattern of the gene(s) implicated in this phenotype; observing any other phenotypes under partial, nonfunctional conditions as well as functional recovery of mutants; and Identifying proteins interacting with the gene product(s).

409A

Isolation of Zasp-interacting proteins in Drosophila muscles. Kuo-An Liao, Frieder Schöck. Department of Biology, McGill University, Montréal, Québéc, Canada.

Zasp (Z-band alternatively spliced PDZ-motif protein) is a PDZ-LIM domain protein and belongs to the Alp/Enigma family. In Drosophila, Zasp is transcribed into two major isoforms, Zasp\(^{\text{ZAM}}\) and Zasp\(^{\text{ZH}}\). As the only known Alp/Enigma family protein in Drosophila, Zasp possesses one N-terminal PDZ domain and one (Zasp\(^{\text{ZAM}}\)) or three (Zasp\(^{\text{ZH}}\)) C-terminal LIM domains. In muscles, Zasp colocalizes with integrins at myotendinous junctions and α-actinin at Z-lines. Loss of it disrupts cells spreading in S2R+ cells and leads to muscle detachment in vivo. In order to explore the mechanism by which Zasp is recruited to integrin adhesion sites, we made tagged Zasp\(^{\text{ZAM}}\) and Zasp\(^{\text{ZH}}\)-fusion proteins with three different tags at the N-terminus, to isolate proteins that interact with Zasp in muscles. We expressed the transgenes in muscles or in all tissues through the UAS/Gal4 system. We will then identify proteins interacting with Zasp by affinity purification and mass spectrometry. We will also test how well Zasp\(^{\text{ZAM}}\) and Zasp\(^{\text{ZH}}\) can rescue the Zasp mutant.

To complement our biochemical studies, we are also currently generating domain-specific Zasp mutant flies by genomic engineering and will report on our progress. In humans, mutations in Zasp are associated with cardiomyopathies. To understand the molecular mechanism of these diseases, a simpler animal model is needed. With the similarity of defects of Zasp mutants in human, mice and flies, plus the absence of redundant genes in the fly, Drosophila provides a useful model to analyze the role of Zasp during muscle development and muscle pathogenesis.

410B


Myofibers are large, multinucleated cells compartmentalized to perform specialized muscle functions. Small, disorganized myofibers and central nuclei characterize human central nervous myopathy (CNM), a disease independently associated with mutations of three genes: MTM1 (lipid phosphatase, membrane efflux), AMPH2 (BAR-domain, membrane tubulation) and DNML2 (large GTPase, membrane scission). MTM1, AMPH2 and DNML2 are predicted to jointly function in membrane trafficking by an unknown mechanism important for myofiber organization. We found that mtn, the fly homolog of MTM1, maintains cell compartmentalization during abdominal myofiber remodeling, and that mtn mutant muscle exhibits hallmarks of human CNM. Specifically, we discovered that mtn is required for normal integrin trafficking and myofiber attachments, and that similar integrin defects arise in human CNM. Thus, the fly is a good model for insights into human muscle disease. To investigate the basis for DNML2-related CNM, we explore muscle-specific roles for shibire (shi), the single Drosophila gene for muscle animal viability during metamorphosis. Muscle-targeted depletion of shi, like mtn, resulted in missing or detached abdominal myofibers, although with a distinct effect on integrin-mediated muscle attachments. To explore how DNML2 contributes to CNM, we generated shi mutant alleles analogous to DNML2 point mutations commonly associated with CNM (E368K and A618T) and single and epistasis analysis. To determine why the myofibers in flies accumulate with integrin on abnormal endosomal-related compartments associated with disrupted PI(3)P turnover and disrupted integrin trafficking, indicating a potential site for shared Mtn and Shi functions. These studies suggest a shared role for mtn and shi in integrin trafficking important in myofibers and relevant to understanding and treating human muscle disease.

411C

Minocycline Alleviates Neural Circuit Defects in the Drosophila Fragile X Model via a Tissue Inhibitor of Matrix Metalloproteinase-Dependent Pathway. Saul S Siller, Kendal Broadie. Departments of Biological Sciences and Cell and Developmental Biology, Kennedy Center for Research on Human Development, Vanderbilt University, Nashville, TN 37232 USA.

Fragile X Syndrome (FXS), caused by loss of the fragile X mental retardation 1 (FMR1) product (FMRP), is the most common cause of inherited mental retardation and autism spectrum disorders. FXS patients usually suffer multiple behavioral symptoms, including hyperactivity, disrupted circadian cycle, and learning and memory deficits. Recently, an investigation in the mouse FXS model suggested that the tetracycline derivative minocycline may be effective at ameliorating behavioral and cellular defects of the disease state. The proposed drug mechanism is matrix metalloproteinase inhibition (MMP). Here, we use the well-characterized Drosophila FXS model to explore minocycline treatment in multiple neural circuits and to test the MMP hypothesis. We first treat Drosophila FMR1 (dfmr1) null animals with minocycline to assay effects on mutant synaptic architecture in three locations: the neuromuscular junction, a key subset of clock neurons in the circadian activity circuit, and the mushroom body learning and memory center. We find that minocycline strongly restores normal synaptic structure in all these contexts, suggesting that it is a promising treatment for FXS. We next test the MMP hypothesis by assessing the effects of endogenous Tissue Inhibitor of Matrix Metalloproteinase (TIMP) on dfmr1 null phenotypes. TIMP over-expression effectively ameliorates dfmr1 null mutant defects in synaptic architecture. Conversely, removal of Drosophila FMRP (dfMRP) completely rescues TIMP over-expression phenotypes, including tracheal defects and adult viability. However, of the two Drosophila MMPs, secreted MMP-1 expression and gelatinase activity does not appear detectably altered in the dfmr1 null condition. The effect on membrane-anchored MMP-2 is currently unknown. We conclude that minocycline and endogenous TIMP over-expression similarly rectify neural circuit defects in the Drosophila.
**POSTER: Drosophila Models of Human Diseases**

See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

412A

**Modulation of high fat diet induced heart dysfunction in Drosophila by the insulin/TOR pathway.** Ryan T. Birse, Joan Choi, Kathryn Reardon, Jessica Rodriguez, Suzanne Graham, Soo Diop, Karen Ocorr, Rolf Bodmer, Sean Oldham.

Sanford Burnham Inst, Cancer Center, La Jolla, CA.

Ryan T. Birse, Rolf Bodmer and Sean Oldham. Development and Aging Program, Sanford-Burnham Medical Research Institute, La Jolla, CA. High Fat Diet (HFD)-induced obesity is a major contributor to diabetes and cardiovascular disease, but the underlying genetic mechanisms are poorly understood. We find that HFD-fed flies exhibit increased triglycerides (TG) and alterations in insulin/glucose homeostasis, similar to mammalian responses. A HFD also causes cardiac lipid accumulation, reduced cardiac contractility, conduction blocks and severe structural pathologies, reminiscent of diabetic cardiomyopathies. Remarkably, these metabolic and cardiotoxic phenotypes elicited by HFD are blocked by inhibiting insulin-TOR signaling (Birse et al. 2010). We then tested whether inhibition of insulin-TOR activity specifically in the heart could autonomously protect the fly heart from obesity-associated cardiomyopathies. Indeed, overexpression of TSC1-2, 4EBP or FOXO, or increased lipase expression in the myocardium efficiently alleviates cardiotoxicity induced by an HFD, but not overall obesity. We conclude that deregulation of insulin-TOR signaling or lipid metabolism due to a HFD causes heart function and metabolic homeostasis defects. To further test the deleterious effects of a HFD we investigated the maternal effects of obesity on the offspring of HFD-fed mothers. We find that normal-fed progeny of HFD-fed mothers develop metabolic, developmental as well as heart function abnormalities. These maternal effect studies will allow us to gain a comprehensive insight in how insulin-TOR signaling and other metabolic regulators mediate the far-reaching effects of (maternal) HFD-induced obesity. Birse, R.T., Choi, J., Reardon, K., Rodriguez, J., Graham, S., Diop, S., Ocorr, K., Bodmer, R., Oldham, S. (2010) High fat diet-induced obesity and heart dysfunction is regulated by the TOR pathway in the Drosophila model. Cell Metabolism. 12, 533-544.

413B

**Obesity Is Linked To Insulin Signaling Through spargel, a PGC-1 homologue in Drosophila.** Claudette P. Davis, Subhas Mukherjee, Atamu Duttaroy. Biol Department, Howard Univ, Washington, DC.

 Peroxoxome Proliferator-activated Receptor Gamma Coactivator (PGC-1) plays an essential role in regulation of triglyceride metabolism in mammals. Drosophila spargel is an orthologue of PGC-1, which encodes a 121kDa protein located at the base of 3R. An interesting connection is established between spargel, fat storage, and insulin signaling. Overexpression of spargel in the fat body using the fat body-specific lsp-gal4 driver, caused increased body weight due to the accumulation of abdominal fat. Measurement of total triglyceride (TGA) content of the “obese” flies showed a significant increase in triglyceride content. Since spargel overexpression in the fat body resulted in an “obese” phenotype, we determined the relationship between spargel and insulin signaling. Our data suggests spargel is downstream to Insulin Receptor (InR) since spargel expression is reduced in the InR mutants on the other hand InR overexpression cause elevated spargel transcription. Next, we checked the expression pattern of Drosophila Insulin Like Peptides (Dilps) in the spargel hypomorph, slr1. Expression of dilp2, dilp3, and dilp5 mRNAs were reduced in slr1 flies. Subsequently, spargel overexpression in the brain, as well as in the fat body leads to an increase in dilp2, dilp3 and dilp5 mRNA levels. Taken together, our results support that spargel is involved in the insulin signaling with the possibility that spargel regulates dilp through a feedback mechanism. Target of Rapamycin (TOR) is an important regulator of body fat content. Inhibition of the TOR pathway by exposing the “obese” flies to rapamycin shows that the “obesity” phenotype can be reverted suggesting that TOR is involved in regulation of total fat content in “obese” flies. Based on these observations, a model will be proposed where spargel regulates body fat content independent of FOXP transcription factor.

414C


Adiponectin is one of the major adipokines secreted from adipose tissue in mammals. It is implicated in many metabolic syndromes such as type 2 diabetes, obesity, atherosclerosis and non-alcoholic fatty liver disease. Adiponectin receptors modulate AMP kinase activity to enhance insulin sensitivity in peripheral tissues. Recently, significant conservation between mammal and Drosophila energy homeostasis has been discovered. However, very little is known for Drosophila adiponokin signaling. In order to investigate adipokin signaling in Drosophila, we have cloned CG5315, a Drosophila homolog of the adiponectin receptor. CG5315 encodes a seven transmembrane domain protein. The amino acid sequence of CG5315 shows around 60% homology with that of the human adiponectin receptor 1. The transcript of CG5315 is expressed throughout entire developmental stages and detected in various organs. Reduced activity of CG5315 causes metabolic defects. CG5315 mutant shows small body size and increased total body triglyceride and hemolymph glucose levels. As mammalian adiponectin receptor mutants, insulin signaling is slightly inhibited in the peripheral tissue of the CG5315 mutant. However, the phosphorylation status of AMPK is not affected. These data suggest that CG5315 is involved in energy homeostasis but its signaling pathway may not be identical compared to the mammalian adiponectin signaling.

415A

**Fat Flies: Effects of a High Fat Diet in Drosophila.** Erlynn Russo Heinrichsen, Gabriel O. Haddad. 1) Department of Pediatrics, University of California, San Diego, La Jolla, CA; 2) Rady Children’s Hospital, San Diego, CA.

Over 60% of the population in the United States is estimated to be obese or overweight, and with obesity come many disease complications, including sleep apnea, hypoxia, atherosclerosis, cardiovascular diseases and stroke. Several of these complications also involve hypoxia, yet the fundamental basic mechanisms underlying the interaction of obesity and hypoxia remain unknown. Drosophila, as a model organism, offers tremendous power in uncovering and studying fundamental mechanisms, given the abundance of molecular tools available to delve into the roles of specific genes and the conservation of biochemical pathways. We have characterized the phenotype of Drosophila on a high fat diet in normoxia and hypoxia (intermittent and chronic hypoxia) using triglyceride levels, response to stress and lifespan. We have found that, when female flies are put on a high saturated fat diet, they have significantly increased triglyceride and glucose levels (normalized to body weight) (p<0.001) and a shortened lifespan. The high fat diet allows for increased survival during starvation, and significantly reduces tolerance to stress conditions such as anoxia and extreme cold. Living in hypoxia appears to alter the response to stress in flies on both diets. Candidate genes from a microarray analysis are currently being studied to explain the phenotypic changes and explore the hypothesis that hypoxia alters the metabolism of Drosophila on a high fat diet.

416B

**Nanoparticles in Drosophila: Organically Modified Silica nanoparticles are biocompatible and can be targeted to neurons in vivo.** Shermali D. Gunawardena. Biological Sciences, The State University of New York, Buffalo, Buffalo, CA.

Farda Barañeda1, Rajiv Kumar2, Phuong-Lan Nguyen1, Michelle L. Kuznicki1, Andrew Kosterman1, Earl J. Bergay2 Paras N. Prasad2 and Sherma D. Gunawardena1,2*

1,2*Department of Biological Sciences, Institute of Laser, Photonics and Biophotonics, The State University of New York at Buffalo, Buffalo, NY, 14260.

With recent advances, the application of nanotechnology in biological research is expected to have a major impact leading to the development of new types of tools for human welfare. One focus of nanobiotechnology is the development and use of various nanoparticle based formulations for efficient drug delivery. However most or all of the nanoprobes currently in use show varying levels of toxicity to cells or to whole organisms and are not suitable for long term or in vivo application. Here we test if a novel silica based nanoparticle (organically modified silica, ORMOSIL) can be used as a therapeutic delivery system to target living neurons in a whole organism. We find that feeding ORMOSIL nanoparticles to Drosophila had no effect on organismal lethality in contrast to feeding of Quantum Dots (QDs). ORMOSIL nanoparticles readily penetrate into living brains, neuronal cell bodies and into axonal projections. Within neuronal cell bodies, ORMOSIL nanoparticles were present within the cytoplasm, but not within the nuclei. Importantly,
incorporation of ORMOSIL nanoparticles into the brain did not interfere with normal neuronal processes such as axonal transport or activate aberrant neuronal cell death pathways. Our results in Drosophila indicate that ORMOSIL nanoparticles are safe and can be readily used for long-term application during an organism’s development, an important requirement for the translation of these novel particles into use in humans. Thus ORMOSIL nanoparticles have great potential in the development of therapeutics for neuronal defects in a whole organism setting.

417C


There is increasing evidence that alterations in metabolism can affect seizure susceptibility in a wide range of organisms. In order to investigate the link between metabolism and seizures, we took advantage of a group of Drosophila mutants, the BS paralytics that are 3-10 times more susceptible to seizures than wild type flies. In three of the BS strains, we introduced the *attacin* (arg) mutation, a mutation in the dystroglycan gene that is known to increase metabolism in flies. Following mechanical shock, all three BS, arg double mutants displayed a reduction in seizure activity and recovered quicker than the respective single mutant BS flies. To further study the correlation between increasing metabolism and seizure susceptibility, the three BS strains were fed a sulfonylurea drug (tolbutamide) that has been shown to increase metabolism in flies. Following mechanical shock, two of the three BS mutants fed tolbutamide displayed less seizure activity and recovered quicker than unfed flies. These data suggest that treatments that increase metabolism can have a protective effect against seizure susceptibility, a result that suggests new avenues for possible drug development.

418A


Combination therapies are a promising way to effectively target tumors. Decreasing the toxicity of a single therapy and targeting multiple pathways the tumor may be sensitive to will help increase treatment effectiveness and decrease side effects. A natural product screen in our lab has identified a number of novel inhibitors that appear to synergize with both radiation and chemotherapy drugs in cell based systems. However, the molecular targets and mechanism by which our novel inhibitors exert their effects is unknown. In order to identify the molecular targets of our novel inhibitors we will be using *in vitro* biochemical assays. We aim to biotinylate the inhibitors and pull down associated targets. Analysis of these factors will be accomplished by mass spectrometry. In order to identify the signaling pathways our inhibitors are targeting, apoptosis, mitosis, DNA repair, and nucleolus staining of imaginal wing discs will be performed in wild-type, p53, and grp (Chk1) Drosophila strains. This will indicate not only which signaling pathways are affected, but how a tumor with a similar genotype may respond to such a treatment.

419B

Characterization of anti-cancer compounds targeting polyamine import into leg imaginal discs. Minpei Wang1,2, Otto Phanstiel IV2,3, Laurence von Kalm1,2. 1) Department of Biology, University of Central Florida, Orlando, FL; 2) Biomolecular Sciences Center, University of Central Florida, Orlando, FL; 3) Department of Medical Education, College of Medicine, University of Central Florida, Orlando, FL.

The native polyamines, putrescine, spermidine and spermine, are ubiquitous organic polycations essential for diverse cellular functions including growth, proliferation, transcription, chromatin structure, mRNA stability and apoptosis. Activated polyamine biosynthesis is a hallmark feature of malignant cells. Difluoromethylornithine (DFMO) is an established inhibitor of polyamine biosynthesis, however, in response to treatment with DFMO, cancer cells compensate by up-regulating import of polyamines from the extracellular environment. Thus, there is a need to develop compounds that inhibit polyamine import for use in combination therapy with DFMO. We have previously demonstrated that the leg imaginal disc, an intact epithelium, has a polyamine transport system that is similar to mammalian cells (Tsen et al., J. Med. Chem. 2008, 51:324-330). In this study, we characterize three candidate polyamine import inhibitors: Anthracen-9-ylmethyl-4,4,4-tetraamine (Ant444), N-(4-amino-butyl)-N’-(3,5-bis-[(4-(4-amino-butylamino)-butylamino)-methyl]-benzyl)-butane-1,4-diamine (Trimer44) and Benzene-1,3,5-tricarboxylic acid tris-[(4-amino-butylamino)-butyl]-amide (Triamide44). We find that Ant444 and Trimer44 are six-fold and four-fold more effective than spermine respectively in blocking uptake of a toxic polyamine analog that utilizes the polyamine transporter to gain entry into the cell. In contrast, Triamide44 is seven-fold less effective than spermine in blocking uptake of the toxic polyamine analog. In addition, we find that Ant444 efficiently blocks uptake of native polyanines into DFMO treated imaginal discs. In summary, our data provide support for future rational design of compounds targeting polyamine import.

420C

Mutations in AP-1 gamma are neurodegenerative in nature. Vafa Bayat1,2, Ke Zhang1, Manish Jaiswal1, Adeel Jawaid1, Hector Sandoval1, Michael Wagner1, Shinya Yamamoto1, Wu-Lin Charng1, Claire Haueter5, Gabriela David1, Hugo Bellen1,4,5. 1) Developmental Biology Program, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030; 2) Medical Geosciences Program, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030; 3) Structural and Computational Biology & Molecular Biophysics Graduate Program, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030; 4) Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030; 5) Biology Department, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

We performed a large forward genetic mutagenesis screen of the *X* chromosome to identify novel neurodegenerative genes among others. We thus identified neurodegenerative mutations in components of the cytoskeleton and MAPs, especially *tau*, have been linked to a number of human neurodegenerative diseases including Alzheimer’s and Parkinson’s Diseases. We have been focusing on the role of neuronally expressed MAPs, Tau and Futsch (MAP1B). The *futsch* mutants show progressive neurodegeneration in the olfactory system. Degeneration is seen primarily in projection neurons, which connect the antennal lobes and the mushroom bodies but is also seen in the lamina of older flies. RNAi driven loss of Tau in neurons (elav-Gal4) causes a decrease in longevity as well as vacuole formation in the central brain. However, these vacuoles do not increase in number or size with age. This loss of Tau in a *futsch* heterozygote background results in developmental lethality. Conversely, over-expression of Tau in *futsch* mutants is able to partially rescue the
POSTER: Drosophila Models of Human Diseases

See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

degenerative phenotype suggesting that Futsch and Tau have a certain amount of functional overlap. RNAi mediated loss of Tau in the eye leads to vacuoles and eventual nearly complete degeneration of the adult eye. While holes are not obvious with light microscopy until 36hrs after eclosion TEM shows holes present in the eye and lamina cortex of newly eclosed flies.

Interestingly, our results show that Tau and Futsch genetically interact with Amyloid Precursor Protein (APPL). APPL is also expressed in developing and mature neurons and is required for synapse formation. APP and its cleavage products have also been projected to affect the phosphorylation state of Tau.

422B
Exportin-1 modulates polyglutamine toxicity. Ho Y E Chan1, H. Tsoi1, W.M. Chan1, C.C. Wu1, C.H. Wong2, T.C. Cheng1, H.Y. Li2, K.F. Lau1, N. Perrimon1, P.C. Shaw1. 1) School of Life Sciences, Chinese Univ HK, Shatin, Hong Kong; 2) School of Biological Sciences, Nanyang Technological University, Singapore; 3) Department of Genetics, Harvard Medical School, USA.

Polyglutamine (polyQ) diseases are a group of late-onset, progressive neurodegenerative disorders caused by CAG trinucleotide repeat expansion in the coding region of disease genes. The cellular and molecular pathology of polyQ diseases and transcriptional dysregulation is one of the pathologic hallmarks. We found that expanded polyQ domain, but not its unexpanded form, possesses nuclear export activity and interacts with the nuclear export receptor Xpo1. Genetic manipulation of Xpo1 expression level in transgenic Drosophila models of polyQ disease confirmed the nuclear export role of Xpo1 on expanded polyQ protein. Upon Xpo1 knockdown, expanded polyQ protein remained in the nucleus and caused impairment of cellular gene transcription. Our findings also establish a direct link between protein nuclear export and the progressive nature of polyQ neurodegeneration.

423C
The role of Superoxide Dismutase 2 in a Drosophila model of Machado-Joseph Disease. Natalie M Clark, John M Warrick. Department of Biology, University of Richmond, Richmond, VA.

Spinocerebellar ataxia 3 (SCA3), also known as Machado-Joseph Disease (MJD), is an autosomal dominant neurodegenerative disorder caused by an expanded polyglutamine repeat in the ataxin-3 protein. Research has suggested that MJD potentially increases the amount of reactive oxidative species within the body, accelerating the cell aging process and increasing neural death. It is hypothesized that the increase of naturally occurring antioxidant gene products such as Superoxide Dismutase 2 (SOD2) could decrease the severity of this disease and serve as a possible treatment. SOD2 is expressed in the mitochondria, a likely location for increased reactive oxygen species. Mild, moderate, and strongly expressing UAS alleles of mutant and normal MJD as well as UAS-SOD2 were expressed in the fly eye using the gmr-Gal4 driver. Flies were aged for one or seven days and their heads were fixed and embedded in epon resin blocks. Ultramicrotome thin sections of fly retinas were evaluated using light microscopy. We found flies expressing both MJD and increased levels of SOD2 had greater eye degeneration and faster progression of disease than flies with MJD and endogenous SOD2 levels. These results suggest that increased levels of SOD2 may not be an effective treatment for MJD.

424A
The neurodegenerative loe mutant interferes with the RHO pathway. Mandy Cook, Jill Wentzell, Doris Kretzschmar. CROET, Oregon Health and Science University, Portland, OR.

Isoprenylation is an important mechanism allowing intracellular proteins, like small G proteins (e.g. RHO), to associate with the membrane, which is then followed by activation of the protein. This step is critical for signal transduction of cellular hormones, growth factors, and cytokines from the membrane to the nucleus and influences proliferation, differentiation and survival of the cell. The isoprenoppy pathway is negatively regulated by AMPK (AMP-activated protein kinase), an inhibitor of HMG-CoA Reductase (hydroxymethylglutaryl-CoA Reductase). The Drosophila mutant loe, which lacks a neuronal isoform of the AMPK γ subunit, shows progressive neurodegeneration and neuronal cell death of the adult nervous system. In order to determine the correlation between the loe mutation, isoprenylation and the RHO1 pathway, we generated and analyzed loe flies with mutations in RHO and its downstream targets. We were able to show that the loe mutation interferes with the prenylation of RHO1 and the regulation of the downstream LIM-Kinase pathway, which plays an important role in actin turnover and axonal remodeling. We further demonstrate that neurons derived from loe mutants exhibit aberrant neurite outgrowth and axonal transport, suggesting that changes in the cytoskeletal network lead to disruptions in axonal transport and subsequent neuronal death in the loe mutant. These findings elucidate how alterations in AMPK may lead to neuronal degeneration and emphasize the importance of an intact cytoskeleton for the survival of the nervous system.

425B
Integration of hypoxia and inflammatory responses in a Parkinson’s disease model. Joseph G. Daigle1, Rami Ajjuri1, Arati Inamdar1,2, Janis O’Donnell1. 1) Dept of Biological Sciences, University of Alabama, Tuscaloosa, AL; 2) Dept of Plant Biology and Pathology, Rutgers University, New Brunswick, NJ.

Neuroinflammation, hypoxia, and oxidative stress are the primary causes for the pathophysiology of Parkinson’s disease (PD). In mammalian models it has been shown that microglia, the innate immune cells of the brain, become hyperactivated in response to neuronal dysfunction in PD. Hyperactivation of microglia results in a sustained inflammation that accelerates dopaminergic neuron loss. In addition, oxidative damage creates a hypoxic environment, which is a precursor to neurodegeneration. The cellular and genetic relationship between the induction of hypoxia and inflammatory responses is poorly defined. A tractable genetic model encompassing both responses in neurodegeneration has never been demonstrated. We have found that exposure to an herbicide, paraquat, results in dopaminergic neuronal loss that is accompanied by a cell specific induction of nitric oxide synthase in infiltrating hemocytes which resemble mammalian microglia. We have also found that the oxidative conditions result in a robust hypoxia response with tracheal branches migrating into the areas of damage. Remarkably, the tracheal and hemocyte migrations are integrated with these microglia-like cells tracking on the growing trachea. These cells are shown to express markers for the inflammatory and hypoxic responses, respectively. We assess candidate genes such as Punch (GTP cyclohydrolase) which is expressed in all three cell types, for their roles in facilitating these dynamic interactions. We conclude that this system serves as a holistic model for understanding the initial dysfunction of neurodegeneration in the context of a powerful genetic system. This genetic model not only recapitulates the disease pathology providing a system for investigating mechanisms. In addition, the model provides the basis for eliciting potential therapeutic targets for treatment and for identifying functionally significant genetic interaction in the responses to neuronal dysfunction.

426C

Prion diseases encompass a complex group of neurological disorders in humans and other mammals. The key molecular event is the conversion of the native Prion Protein (PrPC) into the pathogenic ‘scrapie’ conformation (PrPSc). The most common etiology for prion diseases is pathologic ‘scrapie’ conformation (PrPSc). The prion protein induces vacuolation of central synapses and mitochondrial pathology in muscle fibers. PrPSc is a misfolded isoform of the normal prion protein. PrPSc is thought to be the causative agent of prion disease. In this study, we investigated the effects of PrPSc on synaptic and mitochondrial function in the heart. We found that PrPSc induces vacuolation of central synapses and mitochondrial pathology in muscle fibers. These findings have important implications for the pathogenesis and treatment of prion diseases.
whereas the synapic terminals are swollen and lack synaptic specializations. Interestingly, we also observe dramatic degenerative changes in the Lateral Labial Abductor muscle, which runs posterior to the brain. These fibers present prominent mitochondrial defects, with ruptured or missing mitochondria. Overall, our results indicate that the spontaneous misfolding of PrP in flies induces specific neurodegenerative changes in brain neurons and muscle cells. These observations support and expand the application of the fly model of sporadic prion disease towards understanding PrP misfolding and neuropathology.

427A Role of TATA-box Binding Protein (TBP) in PolyQ Mediated Neurodegenerative Diseases: Implication of Transcription Dysfunction. Tun-Chieh Hsu, Chun-Yen Yang, Ming-Tsan Su. Department of Life Science, National Taiwan Normal University, Taipei, Taiwan.

TATA box binding protein (TBP) has been implicated in many polyglutamine (polyQ) induced neuropathies as it is sequestered and inactivated in polyQ containing inclusions. Among them, spinocerebellar ataxia type 17 (SCA17) is an autosomal dominant neurodegenerative disease caused by expansion of polyglutamine (polyQ) within the N terminal domain of TATA box binding protein (TBP-NTD). To investigate the mechanism of SCA17 pathogenesis, we have generated a Drosophila model for SCA17 by overexpression of TBP with extended polyQ tract (TBP-NTD-polyQ). We showed that native TBP affects polyQ tract and transactivation ability of TBP. Moreover, we found that mutant TBP can also affect the function of normal TBP. Our data suggested that deactivation of TBP is a causative factor of SCA17. Additionally, Drosophila TBP (dTbp) heterozygous mutant exhibits various age-dependent neurodegenerative phenotypes, and polyQ mediated toxicity was exacerbated in dTbp mutant background. By contrast, increasing the expression of TBP alleviates the abovementioned disorders. Additionally, we found that TBP-NTD interacting protein, HMGB1, also plays an important role in modulating TBP mediated neuronal disorders. Ectopic overexpression of HMGB1 caused retinal degeneration, and acerbated the pathological phenotypes of SCA17 fly model.

428B Drosophila melangaster as a model to study the mechanism of action of fungal volatile organic compounds. Arati A. Inamdar, Joan W. Bennett. Department of Plant Biology and Pathology, Rutgers, The State University of New Jersey, New Brunswick, NJ.

Many volatile organic compounds (VOCs) are found in indoor environment as products of microbial metabolism. In damp indoor environments, fungi are associated with poor indoor air quality and some epidemiological studies have suggested that microbial VOCs have a negative impact on human health. We employed Drosophila melanogaster as a model organism to study the mechanism of toxicity of known fungal VOCs. We tested fungal VOCs belonging to different classes of compounds such as alcohols, ethers, esters, aldehydes, terpenes and ketones. Of these, we found that compounds containing C-8 carbon compounds such as 1-octen-3-ol, 3-octanol, 3-octanol, octyl aldehyde were more toxic than C-4, C-5, C-6, C-7 carbon compounds as well as known industrial toxins, toluene, and formaldehyde (positive control). However, 1-octanol which is also C-8 compound was not toxic to Drosophila. 1-octanol is also a calcium channel blocker (T-type in mammalian studies). We fed calcium channel blocker, neomycin to flies along with exposure to fungal VOC, 1-octen-3-ol and found improved survival in presence of calcium channel blocker. We will further report the role of calcium mediated signaling and possible toxicity of fungal VOCs by performing genetic studies in flies. Therefore, these studies demonstrate that VOCs emitted by fungi are toxic to Drosophila melanogaster and may provide insights into certain fungal associated health related problems reported from damp indoor environment.

429C Mechanisms of Prion Protein Misfolding. Kurt Jensen1, Jonatan Sanchez-Garcia1, Yan Zhang1, Diego Rincon-Limas1, Pedro Fernandez-Funez1,2. 1) Dept. of Neurology, University of Florida, Gainesville, FL; 2) Dept. of Neurosciences, University of Florida, Gainesville, FL.

Prions are infectious particles that cause neurodegenerative disorders in humans and animals. In Drosophila, prion disease can occur when the prion protein (PrP) is co-expressed with the prion protein (PrP) from mouse (MoPrP) and rabbit (RaPrP). Unlike HaPrP, RaPrP did not induce locomotor dysfunction, conformational change, or neurodegeneration, consistent with the observation that the rabbit is a rare mammal that is resistant to prion disease. In addition, a point mutation that introduces an aspartic acid residue at the C-terminus of PrP from dog (another rare mammal that does not develop prion disease) into the MoPrP protein (N158D) was found to significantly decrease its toxicity, as assayed by conformational change and locomotor dysfunction. These results provide valuable insight into the mechanisms regulating the accumulation of neurotoxic PrP, and demonstrate the utility of Drosophila as a model for understanding prion diseases.

430A A deficiency screen to identify rescuers of the Drosophila Adar null neural degeneration. Xianghua Li1, Simona Paro1, Lecamene McGurk2, Liam Keegan1, Mary O'Connell1. 1) MRC Human Genetics Unit, Edinburgh, United Kingdom; 2) University of Pennsylvania, Philadelphia, PA.

We are using adar null mutant flies to study neural degeneration and try to rescue the phenotypes by a genetic approach. The Adenosine Deaminases that act on RNA(ADAR) family protein ADAR has fifty-five reported target transcripts and hundreds of editing sites in the Drosophila genome. Major targets of ADAR are expressed in the central nervous system of Drosophila, and the Adar mutant flies show neural degeneration and locomotion phenotypes. We carried out a deficiency screen for viability rescue on Adar null mutant flies using deficiency stocks from the Bloomington Stock Centre covering over 80% of the Drosophila euchromatic genome of Chromosomes II and III. We found that a deficiency on Chromosome II left arm covering Tor rescues low viability of Adar null flies, and reduced Tor can effectively reverse the neural degeneration in mushroom bodies and the locomotion defects of Adar null flies. In addition to Tor, we found that deleting several genes involved in proteolysis can rescue low viability of the mutant flies. Mutant alleles of JIL-1, Cryptochrome, Gem3, CG31475 also rescued the Adar null flies’ low viability.

431B Molecular study of age-related sensorineural hearing loss using Drosophila. Young-Mi Lim, Leo Tsuda. Laboratory for Drug Discovery, National Center for Geriatrics and Gerontology, Obu-City, Aichi, Japan.

Cell survival of sensory neurons is essential for the long-term maintenance of sensory functions. Its defect leads to the onset of age-related sensory defects suffered by a large number of aged human populations, such as hearing loss and retinitis pigmentosa. Research toward the development of effective treatments of those symptoms has been hampered due to the lack of efficient assay systems in model organisms. Here we show that the gene ebi, a fly homologue of TBL1, is required for the long-term survival of photoreceptor neurons in Drosophila. Furthermore, we demonstrate that Ebi forms a complex with AP-1 and represses its target genes. This co-repressor complex seems to be acting as part of the innate immune system and regulating the output of this signal. Our results strongly suggest that the regulatory system mediated by the Ebi/AP-1 co-repressor complex is involved in the cellular survival of sensory neurons. Given the functional homology of TBL1 as a co-repressor molecule in mammalian systems and the fact that TBL1 is a causative factor for age-related hearing disease, called OASD (ocular albinism with late-onset sensorineural deafness), our system has the potential to be a useful model for analysing age-related sensorineural hearing loss.
POSTER: Drosophila Models of Human Diseases
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

Mutation in E1/Uba1 dramatically reduces lifespan and results in motor impairment: a candidate Drosophila model for X-linked Infantile Spinal Muscular Atrophy. Hsiu-Yu Liu, Cathie Pfleger. Oncological Sciences, Mount Sinai School of Medicine, New York, NY.

Neurodegenerative diseases are a source of significant suffering for those afflicted and their families. Abnormalities in the ubiquitin pathway in many of these diseases have raised the question of whether impairment of the ubiquitin pathway on its own can increase mortality or if ongoing neurodegeneration alters ubiquitin pathway function as a side-effect. To address effects of the ubiquitin pathway on lifespan, we studied loss-of-function mutations in the Drosophila Ubiquitin Activating Enzyme, Ubal1 or E1, the most upstream enzyme in the Ubiquitin Pathway. Loss of only one functional copy of E1 caused a significant reduction in adult lifespan. Flies homozygous for hypomorphic E1 mutations survived to adulthood only rarely. Surviving E1 homozygotes exhibited further reduced lifespan and showed inappropriate Ras activation in their brains. Mutation in Ras dominantly restored the lifespan of heterozygous E1 mutants to that of wild-type flies and increased the lifespan of homozygous E1 mutants. These findings indicate that impairing the ubiquitin pathway is sufficient to cause early mortality and that this mortality may be mediated, at least in part, by increased Ras signaling. E1 homozygous mutants also exhibited severe motor impairment. Dramatically reduced lifespan and motor impairment are key aspects of the human disease X-linked Infantile Spinal Muscular Atrophy, which is associated with mutation in human E1. We propose that these Drosophila E1 mutants may represent an animal model for study of this devastating disease.

433A Development and Validation of a Late Stage Onset Alzheimer's disease model in Drosophila melanogaster. Siddhita Mhatre1, Sarah Michelson1, Radha Delvadia1, Daniel R. Marendra2,3. 1) Dept. of Biology, Drexel University, Philadelphia, PA; 2) Dept. of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA.

Alzheimer’s disease (AD) is a progressive neurodegenerative disease and the most common cause of dementia in the elderly. Neuropathology of AD is characterized by accumulation and deposition of β-amyloid (Aβ) peptide and neuro-fibrillary tangles in the brain. Aβ peptide is generated by sequential endoproteolysis of amyloid precursor protein (APP) by β-secretase (BACE1) and γ-secretase cleavage enzyme (BACE2). Differential cleavage of APP by γ-secretase produces Aβ40 and Aβ42 peptide, of which Aβ42 is found to be toxic and forms aggregates. The majority of animal models attempt to analyze this disease by expressing the human genes involved in disease (LOAD) in an in vivo model organism. Here, we are developing a novel LOAD model using Drosophila melanogaster which expresses human APP and BACE specifically in developing retina, wings or ubiquitously in all tissues. Thus, the major limitation in studying AD is the difficulty in modeling late onset Alzheimer’s disease (LOAD) in an in vivo model organism. Here, we are developing a novel LOAD model using Drosophila melanogaster which expresses human APP and BACE specifically in the nervous system. This model would enable us to observe disease pathology in a manner more consistent with LOAD observed in humans and may provide valuable tools for evaluating potential efficacy and toxicity of AD therapeutics.

434B Secreted APP and APPL ectodomains are neuroprotective in a Drosophila model of progressive neurodegeneration. Derek Musade1, Katia Carmine-Simmen2,3, Doris Kretzschmar1. 1) Center for Research on Occupational and Environmental Toxicology, Oregon Health & Science University, Portland, OR; 2) Department of Biochemistry, University of Alberta, Edmonton, Canada.

The processing of amyloid precursor proteins (APPS) results in the generation of several proteolytic fragments, including large soluble N-terminal ectodomains (sAPPs). sAPPs derived from α-cleavage of APP have been shown to have neurotrophic or neuroprotective effects in cell culture, while β-cleaved sAPPs can have deleterious effects. Here we describe a protective function of human APPs and fly APPL ectodomains on progressive neurodegeneration seen in the Drosophila mutant lorchig (loe), which contains a mutated isoform of the γ-secretase protein (APPKMP). Previously, we have shown that the loss of APP enhances the progressive neurodegeneration observed in loe. We now show that expression of either intact APP695, APPL, or N-terminal ectodomain fragments of these proteins protect the nervous system of loe flies from degeneration, while a secretion deficient form of APPL does not. In addition, we show that increasing KUZBANIAN (KUZ), an ADAM 10 homolog that can act as an α-secretase, can reduce neurodegeneration, whereas increasing fly β-secretase (BACE1) activity aggravates the phenotype. We also demonstrate that the loe mutation reduces the production of the α-cleaved C-terminal fragment (CTF), while overexpression of LOE results in an increase of this fragment. Finally, we provide evidence that the protective function of the secreted N-terminal APPL fragment in loe is mediated by binding to full-length APP. This suggests that α-cleaved ectodomains of APP proteins have a neuroprotective feature, possibly mediated through binding to the full-length protein, and that the reduced production of this protective fragment in loe exacerbates the observed progressive degeneration of the nervous system.


Our lab has developed a transgenic model of Alzheimer’s Disease (AD) by expressing the wild type human forms of both APP and BACE within the CNS of Drosophila melanogaster. These transgenes, combined with Drosophila’s endogenous functional homolog of the gamma-secretase complex, recapitulates the amyloidogenic proteolytic processing that occurs in AD brains. Calcineurin has been implicated in the pathology and progression of neurodegeneration in AD brain. We have used our transgenic APP/BACE Drosophila to test the role of calcineurin in AD using both RNAi knockdown and pharmacological techniques. Drosophila exhibit negative geotaxis by climbing upwards when tapped to the bottom of a vial, and disruption of this stereotyped response is indicative of nervous system dysfunction. Calcineurin inhibitors administered via feeding improve the impaired motor reflex behavior observed in the APP/BACE flies. RNAi-mediated knockdown of calcineurin in the APP/BACE model also partially suppresses the defective climbing behavior. These studies emphasize the utility of this model for dissecting the molecular mechanism of AD, as well as testing possible pharmacological interventions in an in vivo system.

436A Discovery of novel modulators of PINK1 combining cell based RNAi screens and Drosophila models of Parkinson’s disease. Joe Pogson, Alexander Whitworth. Department of Biomedical Science, University of Sheffield, UK.

Parkinson’s disease (PD) is the second most prevalent neurodegenerative disorder. It is characterised by the loss of dopaminergic neurons in the substantia nigra. Causes of PD are largely unknown although cases of familial PD do have to the identification of disease causing genetic factors such as loss of function mutations in Pten-induced Kinase 1 (PINK1). PINK1 is a putative kinase that contains a mitochondrial localisation sequence. PINK1 acts in a common pathway with another PD gene, parkin, that appears to regulate mitophagy but little else is known about the components of this pathway. This study aims to dissect the pathway and find novel modulators of PINK1 loss of function. Loss of PINK1 in Drosophila causes dopaminergic neuron loss, muscle degeneration, mitochondrial defects along with reduced climbing and flight ability. Previous studies have shown that PINK1 genetically interacts with genes that control mitochondrial fusion (OPA1 and Mfn) and fission (Drp1 and Fis1). RNAi mediated knock down of PINK1 in Drosophila S2R+ cells results in elongation of the mitochondrial network. This cellular phenotype was used to perform a cell based RNAi screen on a kinome and phosphatome library consisting of around 700 genes. Results were compared from two parallel screens; wild type and PINK1 knock down (KD) background. Two groups of hits were identified from the results; 40 rescuers of PINK1 KD and 23 phenocopiess of PINK1 KD. These were rescreened under more stringent conditions resulting in 24 confirmed rescuers and 11 confirmed phenocopiess. In vitro rescuers were tested for suppression of PINK1 mutants in vivo using VDRC transgenic RNAi lines. Several PINK1 phenotypes were assayed including thoracic indentations, climbing and flight ability. KD of 4 genes have shown rescue of PINK1 mutant phenotypes. Further analysis on these four genes will aim to dissect any direct interaction with the PINK1 pathway. These rescuers also represent potential therapeutic targets for the treatment of PD. In addition, the phenocopiess will also be investigated as potential new pathway components.

Parkinson’s disease (PD) is a neurodegenerative disorder characterized by the selective loss of dopaminergic (DA) neurons. The majority of PD cases are sporadic and their etiology is poorly understood. Exposure to environmental toxins increases risk of PD but individual susceptibility varies suggesting additional genetic effects. We are using Drosophila to investigate these potential gene-environment interactions. Since DA itself may be neurotoxic, genes that regulate cellular DA levels may be particularly important. We tested the effects of two genes that regulate levels of cellular dopamine and its metabolites: the Drosophila vesicular monoamine transporter (dVMAT), which packages and transports dopamine into synaptic vesicles, and Drosophila aldehyde dehydrogenase (dALDH), which converts the highly toxic dopamine metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL) into the less toxic species 3,4-dihydroxyphenyl acetic acid (DOPAC). Since both dVMAT and dALDH can decrease the toxicity of cytosolic dopamine and its metabolites we have tested whether they will genetically interact with pesticides, thereby mitigating their neurotoxic effects. To test this hypothesis, we exposed dVMAT and dALDH mutant flies to pesticides, including paraquat and rotenone, both of which are established models of PD. We then assayed organismal survival, locomotor behaviors related to DA signaling and the number of DA neurons that remained after pesticide exposure. We show that over-expression of dVMAT confers neuroprotection of DA cells against chronic exposure to rotenone but not paraquat. dALDH mutant flies display a drastic reduction in lifespan when exposed to paraquat and further analysis of their behavior and DA cell counts are in progress. In addition, we report that exposure of flies to rotenone during larval development also causes a loss of DA neurons. Our findings indicate that select DA pathway genes may play a key role in determining susceptibility to environmental risk factors for PD and provide a useful model to investigate the mechanisms by which environmental toxins and genes interact.

Circadian Rhythm and SODs in a Drosophila Model of MJD. Steven M Richards, John Warrick. Biology, University of Richmond, VA.

Machado Joseph Disease (MJD) is a dominantly inherited neurodegenerative disorder caused by an abnormal expansion of a normally occurring CAG repeat in the coding portion of the MJD1 gene. This expansion results in a misfolded, toxic gain-of-function in the Ataxin-3 protein. Built up mutant Ataxin-3 protein may trigger neuronal loss of function and cell death due to increases in reactive oxygen species and free radicals. Flies expression mutant Ataxin-3 in circadian rhythm neurons lose circadian activity. The anti-oxidant proteins Superoxide Dismutase (SOD) 1 and 2 are found in the cytoplasm (SOD 1) and mitochondria (SOD2) and act to reduce the number of free radicals in the cell. In a Drosophila model we expressed normal or mutant Ataxin-3 proteins with either UAS-SOD1 or UAS-SOD2 genes in circadian rhythm driving neurons using a tim-Gal4 driver to see if SOD over expression may rescue neuron dysfunction and degeneration. Additionally we used RNAi mediated knock-out of SOD1 or SOD2 in those cells in normal and mutant Ataxin-3 flies to determine if reduced levels of SOD would further influence neuron function and degeneration. To measure the disease progression we used circadian rhythm and anatomical analyses. As flies progress through the disease pathway circadian rhythm is significantly affected, giving us a way to quantify the severity of the disease. In addition we performed staining of whole brain mounts to check for survival of neurons. Our results suggest SODs play an important role in the function of circadian neurons and may be important in MJD pathology.

The PrP-N158D substitution confers conformational stability and prevents prion disease. Jonatan Sanchez-Garcia1, Kurt Jensen1, Yan Zhang1, Joaquin Castilla1, Pedro Fernandez-Funez2, Diego Rincon-Limas1. 1) Department of Neurology, UF, Gainesville, FL; 2) Department of Neuroscience, UF, Gainesville, FL; 3) CIC bioGUNE, Derio, Bizkaia.

Prion diseases are transmissible neurodegenerative diseases caused by misfolding and deposition of the normal prion protein (PrPC) into pathogenic ‘scrapie’ conformation (PrPSc). However, the molecular mechanisms that regulate this conformational conversion are mostly unknown. A clue to understanding the structure and conformational dynamics of PrP has come from the dog, a rare mammal resistant to prion diseases. A comparative study identified a charged amino acid (PrPD158) in dog PrP that is not conserved in other mammals susceptible to prion diseases (PrPN158). We hypothesized that altering the charge of the loop connecting Helix 1 and the first beta-sheet could affect the stability of the globular domain and, thus, the toxicity of PrP. To determine the stabilizing effect of Asp158, we compared transgenic flies expressing wild type (MoPrP) and mutant (MoPPrP-N158D) mouse PrP. We first observed that the MoPPrP-N158D protein is more stable than MoPrP since significantly lower levels of mRNAs lead to comparable levels of protein, suggesting that MoPrP is actively degraded in flies. We have shown before that MoPrP accumulates disease-specific PrP isoforms by immunoprecipitation with the 15B3 conformational antibody. However, flies expressing MoPrP-N158D do not accumulate 15B3-specific conformations, indicating its higher conformational stability. Finally, whereas expression of MoPrP in motor neurons induced aggressive locomotor dysfunction in climbing assays, flies expressing MoPPrP-N158D were similar to control flies, supporting the lack of toxicity. Furthermore, in vitro conversion experiments support the protective role of the MoPPrP-N158D substitution. These results demonstrate that Asp158 exerts a key stabilizing activity on PrP and prevents formation of disease-specific PrP isoforms. Thus, the prion loop can be targeted for the development of anti-prion therapies.

Genetic analysis of a Drosophila IBMPFD model reveals a strong correlation between energy consumption and disease pathogenesis. Tzu-Kang Sang, Yu-Chu Chang, Wei-Tsing Hung, and Jen-Da Chen. Institute of Biotechnology, National Tsing Hua University, Hsinchu, Taiwan.

Inclusion body myopathy with Paget disease of bone and frontotemporal dementia (IBMPFD) is caused by missense mutations in VCP (valosin-containing protein), an AAA (ATPase associated with diverse cellular activities) ATPase that forms homo/hexamer to execute a range of cellular functions through hydrolyzing ATP. While a number of cellular functions, including ER associated degradation (ERAD), ubiquitin-associated protein processing, autophagy maturation, and protein translocation, could be hampered by the pathogenic VCP mutants in cell and animal models, it is not clear whether these cellular mechanisms are direct cause or subsequent effect of this debilitating disorder. To investigate the molecular mechanisms underlying IBMPFD, we established a Drosophila model that can recapitulate the important features of IBMPFD by overexpressing Drosophila TER94 (the sole VCP homolog) mutants that correspond to VCP disease-causing alleles. Interestingly, we found that the harmful effects induced by TER94 mutants depend on the formation of hexameric configurations, implicating that the ATPase activity contributes to the pathology. Through genetic analysis, we showed that these IBMPFD-causing mutations are dominant-active alleles. Using fluorescent probes as readout, the ERAD or ubiquitin-proteasome system seemed to be unaffected under the insult of pathogenic mutants in this fly model. Instead, we found that expressing pathogenic mutants resulted in the reduction of cellular ATP level. Moreover, manipulating cellular ATP level could significantly modify the severity of the phenotype. Together, these data suggest that the disease-linked TER94 mutations may cause an imbalance of cellular ATP, which may perturb ATP-dependent activities within cells and progressively lead to the degeneration of tissues that demand higher energy expenditure.

Genetic Manipulation of CREB Binding Protein Expression and its Role in the Human Brain Degenerative Disease Spinocerebellar Ataxia-3 / Machado Joseph Disease in the Fly Eye. Andrew L Simmelink, John M Warrick. Department of Biology, University of Richmond, Richmond, VA.

This research seeks to understand the pathology of the inherited human brain degeneration disease Machado Joseph Disease (MJD). MJD is a polyglutamine expansion disease. These diseases are characterized by an expansion of a normally occurring polyglutamine repeat. This expansion causes the protein to acquire a toxic gain of function by an unknown mechanism. The expanded proteins form aggregates containing many other cellular components including transcriptional regulators in dying cells. This research seeks to determine the role of the transcriptional regulator CREB Binding Protein (CBP) in MJD. CBP is found in MJD aggregates in brains of those who had been affected by the disease. This experiment hypothesizes that the mutant protein is interfering with CBP’s ability to regulate genes. This interference compromises the brain cells and causes them to die due to their inability to regulate their own transcription. This is known as the Transcription Regulation Hypothesis. The model used to test this hypothesis was the fly, Drosophila.
CREB Binding Protein localization in a fly model of a human polyglutamine disease. Brian J Sorace, Kristen Qutub, John M Warrick. Department of Biology, University of Richmond, Richmond, VA.

Machado-Joseph Disease (MJD) is one of nine known members of the human polyglutamine disease family. Polyglutamine diseases are caused by an expansion of a normally occurring trinucleotide tract in the amino acid sequence of a gene. The expansion leads to a toxic gain of function in the protein ultimately leading to cell death. Ataxin-3, the protein expanded in MJD and normally contains fewer than 35 glutamines, is the disease protein. How the expanded protein causes degeneration is unknown. Transcriptional dysregulation due to inactivation or sequestration of transcriptional regulators has been hypothesized to be caused by the disease protein. CREB Binding Protein (CBP) is a histone acetyl transferase which may be affected by mutant polyglutamine protein. Up-regulation of CBP has been previously suggested to mitigate polyglutamine induced degeneration. Immunohistochemistry was used on larval discs and adult fly eyes to determine to what extent CBP localization is influenced by expression of normal and mutant Ataxin-3. Normal and mutant Ataxin-3 along with a tagged CBP were expressed using a gmr-Gal4 and rho1-Gal4 drivers. Our results suggest that CBP may interact with mutant Ataxin-3 protein as part of the disease pathology.

In vitro detection of neuroteratogen using Drosophila Melanogaster. Nicole R Sparks. Biology Dept, CSUSB, San Bernardino, CA.

The second half of pregnancy is one of the most active growth and developmental stages for the human brain. Chemicals introduced to the developing fetus may affect the brain development at various levels of brain damage. One way neurons are classified is by the type of neurotransmitters they use to communicate with other cells. Neurons that use acetylcholine (ACH) as a neurotransmitter are termed cholinergic neurons. Neurons that secrete gamma-aminobutyric-acid (GABA) are known as GABergic neurons. Lead, valproic acid, phenytoin and phenobarbital are classified as teratogens. In this study, Drosophila melanogaster is utilized to detect neuroteratogens. Two strains of transgenic flies, the first strain’s cholinergic neurons express green fluorescence protein (GFP) and the second strain’s GABergic neurons express red fluorescence protein (RFP), were used to observe the growth of the neonatal brain in the presence of neuroteratogenic chemicals. Drosophila melanogaster embryos were collected for 2 hours on food plates and incubated for 3 hours. The gastrula stage embryos were dechorionated and dissociated in Drosophila media and plated at 1.6 x 105 cells/mL. The neuroteratogenic agents were added to the primary cultures at time of plating. Between 12-24 hours of incubation at 25°C, the cholinergic and GABergic neurons were visualized under fluorescence microscopy. The cultures were examined for cell death and inhibited neurite outgrowth. The observation of growth is continued up to 48 hours. A decrease in neuronal growth compared to the controls indicated a neuroteratogenic effect. Each of the “classified” known neuroteratogens inhibited the neuronal growth greater than 50%. Assays are underway of discovering the teratogenic potential of unknown agents. This assay of investigating neuroteratogens provides a useful approach for studying the probability of being teratogenic by use of a fluorescence microscope.

Acknowledgments: Thank you to Dr. Paul Salvaterra at City of Hope for the transgenic flies. I would like to thank Dr. Nicole Bournias-Vardibasis for being my mentor.

Dysfunction of TBP dependent transcription may contribute to the pathogenesis of tauopathy. HSJANG-YU WANG. Life science, national taiwan normal university, Taipei, Taiwan.

Taubopaths are a group of neurodegenerative diseases which is characterized by abnormal deposition of the microtubule-associated protein tau. Previous studies found that the TATA box binding protein (TBP) is sequestered by tau-mediated neurofilibrillary tangles (NFTs) in the brain of postmortem AD patients, suggesting that down-regulation of TBP may play a role in tauopathy. Using Drosophila notum bristle as an assay system for tau induced toxicity, we found that natural bristles of the transgenic flies overexpressing tau can be modulated by the expression level of the endogenous TBP. Moreover, both transactivation and DNA binding ability of TBP were affected by tau. Our findings strongly suggested that ectopic tau can lead to dysfunction of TBP. Interestingly, loss of TBP function in flies also caused various age-dependent neurodegeneration, including formation of vacuole in brain, motor dysfunction and premature death. Since TBP controls virtually the transcription of all genes, to further demonstrate that transcription dysfunction is a causative factor of tauopathy, we treated transgenic fly model with various HDAC inhibitors, including SAHA. We found that HDACi can alleviate effectively the toxicity of tau.

Defects in Peroxisomal Biogenesis Lead to Age-Dependent Locomotor Deficits and Retinal Dysfunction in Drosophila. Michael Wangler1, Vafa Bayat2, Nikolaos Giagtzoglou1, Claire Haeter1, Hugo Bellen1. 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Developmental Biology Program, Baylor College of Medicine; 3) Medical Scientist Training Program, Baylor College of Medicine; 4) Howard Hughes Medical Institute, Baylor College of Medicine.

Peroxisomes are ubiquitous organelles with enzymatic functions in the metabolism of fatty acids and specialized lipids as well as the processing of reactive oxygen species. Peroxisomal biogenesis is achieved through protein expression by the evolutionarily conserved per genes. The crucial role for peroxisomal biogenesis in the nervous system is demonstrated by debilitating human diseases caused by PEX gene mutations, namely the Zellweger syndrome spectrum disorders (ZSS). These disorders exhibit multiple signs of neuronal dysfunction, including seizures, hypotonia, blindness, and deafness. However, the pathogenesis of neurologic dysfunction in these disorders is not clear. To study the biological mechanisms that underlie these diseases we study the Drosophila phenotype resulting from disrupted peroxisomal biogenesis. We have selected pex2, a RING finger domain protein, at the peroxisomal membrane, where it is required for localization of peroxisomal targeted proteins. P-element insertion mutations in the coding region of pex2 do not cause lethality but result in male sterility. Furthermore, the subcellular localization of a peroxisome targeted GFP is abnormal in these flies. Interestingly, aged pex2 flies exhibit locomotor deficits, suggesting that loss of peroxisomal integrity results in nervous system dysfunction. To further characterize the nervous system phenotype, we have examined the structure of retinas in aged flies by transmission electron microscopy (TEM). Retinas of two week old flies exhibit increased numbers of mitochondria in the photoreceptors with mitochondrial inclusions, and glial accumulations of electron dense material. Elucidation of the molecular mechanism of retinal and locomotor defects due to Pex2 loss of function in Drosophila may impact our understanding of the neurologic disability in ZSS.

Sicily, a mitochondrial protein, is implicated in neurodegeneration. KE ZHANG1, Manish Jaiswal1, Zhihong Li2, Vafa Bayat1, Hector Sandoval2, Bo Xiong2, Shinya Yamamoto1, Wu-lin Chang1, Claire Haeter1, Gabriela Davíd2, Adeel Jawaid2, Hugo Bellen12345. 1) SCBMB Program, BAYLOR COLLEGE OF MEDICINE, HOUSTON, TX; 2) MOLECULAR & HUMAN GENETICS, BAYLOR COLLEGE OF MEDICINE, HOUSTON, TX; 3) Program in Developmental Biology, BAYLOR COLLEGE OF MEDICINE, HOUSTON, TX; 4) HIIMI; 5) Neuroscience Department, Baylor College of Medicine, Houston, TX.

To identify novel genes that are implicated in neurodegeneration, we performed a large chemical mutagenesis screen on the X-chromosome and identified ~50 complementation groups. One of them maps to an uncharacterized gene, CG15738. We named it sicily, (for severe impairment of complex I with lengthened youth) as the mutants have mitochondrial complex I deficiency and a prolonged larval stage. Flies bearing sicily mutant clones in their visual system have defective ERGs that worsen with age, as well as the morphology of mutant retina, lamina, and lobula, as shown by TEM. One of the key features include defective mitochondria in the mutant flies. Corresponding to this phenotype, Sicily has a mitochondrial targeting peptide. Using a tagged genomic rescue construct of sicily, we confirmed that Sicily localizes to the mitochondria. Although the function of Sicily is still unknown, it maps to one of the candidate loci of neurodegeneration. Consequently, the identification of Sicily may help us understand the genetic basis of neurodegeneration.
Sicily remains unclear, its human homolog has been linked to Leigh syndrome with complex I deficiency. Using flies as a model system, we found that the enzymatic activity of complex I of sicily mutant flies are non-detectable and that the protein amount of at least one of the complex I subunits, NDUFS3, is decreased compared to the wild type. Moreover, blue native PAGE shows that sicily mutants have misassembled complex I. Furthermore, we found that sicily mutants have higher ROS level, a common consequence of complex I deficiency. In our yeast two hybrid screen, we identified that Sicily interacts with a complex I subunit, ND42. We also found that the mitochondrial level of ND42 is decreased in sicily mutants. Together, our observation indicates Sicily is a complex I assembly factor that potentially regulates complex I assembly through interactions to ND42.

**447C**

The effect of genotoxins on health of organisms depends of sex, age, health condition and others. Occasionally the exposure of organisms with the same genotype (homozygous) cause strong effects in some organisms but fail to produce some kind of disturbances to other. To known the damage induced by genotoxins in somatic and germinal cells *Drosophila melanogaster* has been widely used. However, the information concerning to the reprotoxic effect of genotoxins is rather scarce. In this study we show that colchicine (CO) affects the reproductive performance of complex I deficient organisms. A mature culture of wild type flies (wt) were transferred to fresh medium to obtain eggs for 8h. Three days after, larvae were collected as (Nöthiger, 1970) and groups of ca. 100 larvae were put into vials containing instant food enriched with test solution for a semichronic exposure. Thirty successive dilutions were obtained using distilled water as dissolvent and negative control. Survival flies in control and experimental series were counted and scored by sex. Treated males mated with untreated females were put by pairs into vials with standard medium to recover the progeny produced. For each concentration, the fertility and the average progeny was determined and compared with those of control series (n<0.05). Different effects were observed at low, medium and high concentrations of CO assayed. CO treatment reduced the survival of exposed flies since the lowest concentration assayed (only two exceptions were observed) and was lethal to expose flies since 0.0625 mM of CO. The amount of progeny scored exceed that of control series since 3.81-6 and higher, but males exposed to 0.0156 to 0.031 mM of CO did not produce descendents. Our results showed the relevance to explore the effect of genotoxins in the reproductive performance in addition to other genotoxic endpoints. *Drosophila melanogaster* is a reliable and prolific system allowing determine the induction of epigenetic damage.

**448A**
Distinct Mechanisms Underlying Tolerance to Intermittent and Constant Hypoxia in Drosophila melanogaster: Role of Hsp70 in Constant hypoxia. Priti Azad, Gabriel Haddad. Dept Pediatrics, Univ California, San Diego, La Jolla, CA.

Hypoxia occurs during normal conditions (high altitude) or during pathologic states (e.g., obstructive sleep apnea, and sickle cell anemia). Our research is focused on understanding the molecular mechanisms that lead to injury or adaptation to hypoxic stress using Drosophila as a model system. We performed genome-wide study to investigate gene expression changes in D. melanogaster after exposure to severe (1% O2) intermittent (IH) or constant hypoxia (CH). Our microarray analysis has identified distinct responses to IH and CH in terms of gene expression and multiple gene families were up- or down-regulated in response to these stresses. During IH, biological processes primarily involved in multi-drug resistance and defense responses were over-represented. In contrast, there were several gene families that were over-represented in CH treated flies, such as those involved in the response to unfolded proteins, chitin, lipid, carboxylic acid, amino acid-metabolic processes, and immunity. The heat shock protein family was the most up-regulated group in CH and this was exclusive to this treatment. Furthermore, we employed the UAS-Gal4 system to further disect the protective role of Hsp70 in specific tissues in vivo under CH. We found that over-expression of Hsp70 in certain tissues such as heart and hemocytes provided survival benefit during severe hypoxia exposure in flies. These data provide further clues about the mechanisms hypoxia lead to cell injury and the protective role of Hsp70 in adaptation hypoxia tolerance.

**449B**
A deficiency-based screen for dominant modifiers of slrandance bang sensitivity. Derek M. Dean, Brian Shepherd, Dan Nachum. Biology, Williams Col, Williamstown, MA.

*slamdance* (*sda*) encodes a homolog of aspartateaminopeptidase N (APN). Loss of function in this gene causes "bang sensitivity", a behavior in response to mechanical shock that resembles seizures on a physiological level. These findings implicate a novel function for an APN gene, but the underlying mechanism by which *sda* affects bang sensitivity remains unclear. To address this issue, we took advantage of the semidominant *sda*<sup>123</sup> allele. Although homozygotes for this allele are nearly 100% bang-sensitive, and wild type flies are not bang-sensitive at all, heterozygotes seize at an intermediate rate, making *sda*<sup>123</sup> an ideal tool for identifying genetic interactors. Here, we describe a deficiency-based screen to identify modifiers of the *sda*<sup>123</sup> phenotype. *sda*<sup>123</sup> flies were crossed to a series of stocks containing deletions with molecularly defined endpoints that had been generated using the FLP-FRT system (Park et al., 2004; Thibault et al., 2004). F1 flies were then assayed for bang-sensitivity. If a line exhibited a stronger enhancement or suppression of bang-sensitivity, we systematically screened the genes that were eliminated by the deletion to identify the interacting locus. Although identification of interacting loci is still underway, the results from our pilot screen have yielded several candidates, suggesting that this screen is an efficient and effective method for identifying genetic modifiers of a semidominant mutation.

**450C**
*Drosophila* model to study stress hormone-like role of extracellular adenosine in vivo. Tomas Dolezal, Monika Zuberova, Milena Novakova, Michaela Fenckova. Dept Molecular Biology, Faculty of Sciences, University of South Bohemia, Ceske Budejovice, Czech Republic.

Extracellular adenosine is produced from released ATP during various types of stress and it is a potent regulator of different responses to stress often associated with human diseases. Therefore adenosine is increasingly recognized as a potential target in search for novel therapeutic options. However, the diversity of adenosine roles in mammalian organisms complicates these attempts. We established a *Drosophila* model to study the effects of extracellular adenosine in vivo by knocking out ADGF-A - the *Drosophila* adenosine deaminase which degrades extracellular adenosine. Our recent work shows that increased level of extracellular adenosine leads to hyperglycemia in larvae and this effect may cause a loss of energy reserves and death (Dis. Model. Mech. 2010: 773). We also produced a GFP reporter for the ADGF-A expression by homologous recombination. This gene expression changes in *D. melanogaster* after exposure to severe (1% O2) intermittent (IH) or constant hypoxia (CH). Our microarray analysis has identified distinct responses to these stresses. During IH, biological processes primarily involved in multi-drug resistance and defense responses were over-represented. In contrast, there were several gene families that were over-represented in CH treated flies, such as those involved in the response to unfolded proteins, chitin, lipid, carboxylic acid, amino acid-metabolic processes, and immunity. The heat shock protein family was the most up-regulated group in CH and this was exclusive to this treatment. Furthermore, we employed the UAS-Gal4 system to further disect the protective role of Hsp70 in specific tissues in vivo under CH. We found that over-expression of Hsp70 in certain tissues such as heart and hemocytes provided survival benefit during severe hypoxia exposure in flies. These data provide further clues about the mechanisms hypoxia lead to cell injury and the protective role of Hsp70 in adaptation hypoxia tolerance.

**451A**

Interactions between the nuclear and the mitochondrial genomes (mito-nuclear interactions) are vital for cellular function. Both mitochondrial and nuclear genes encode subunits of the complexes required for oxidative phosphorylation (OXPHOS). We have developed a model in which mitochondrial and nuclear genomes can be jointly manipulated in *Drosophila*. mtDNA from different strains of *Drosophila simulans* (*Dsim*) and *D. melanogaster* (*Dmel*) have been introduced into controlled *Dmel* nuclear backgrounds. A specific *Dsims* mtDNA, simw501, shows a strong epistatic interaction with the nuclear genotype: in the *OreR* background, simw501 mtDNA reduces fitness, but these defects are not seen in an Austria background. This implicates a nuclear allele modifying fitness of the mtDNA. Meiotic mapping in the *OreR* background and DNA sequencing of the complete mtDNA have identified a mutation in the nuclear encoded mitochondrial tyrosyl-tRNA synthetase, and the mtDNA encoded Tyrosine-tRNA. Mutations in mitochondrial tRNAs in humans have been associated with a range of disease phenotypes. The activity of OXPHOS enzymes complexes was evaluated in four strains (mitochondrial genotype; nuclear genotype: 227
simw501; OreR, OreR; OreR, Sim501; Aut, and OreR; Aut. Enzyme complexes that are jointly encoded by mitochondrial and nuclear genes (I, III, IV) showed reduced activity in the simw501; OreK genotype, while nuclearily encoded complex II and citrate synthase activity levels were not significantly different. Mitochondrial respiration and ROS production were also measured. The simw501; OreR combination displays reduced uncoupled rates and increased ROS production. Interestingly, the defective genotype was more resistant to paraquat treatment, possibly due to reduced processing and compromised metabolism. Additionally, mitochondrial morphology and translational rates were assessed. This study provides insight into the joint genetic architecture of mitochondrial function and metabolic disease.

452B
DIOPT, the DRSC Integrative Ortholog Prediction Tool. Claire Y Hu1, Ian Flockhart1, Stephanie Mohr2, Norbert Perrimon1,2. 1) Drosophila RNAi Screening Center, Genetics Dept., Harvard Medical School, Boston, MA; 2) Howard Hughes Medical Institute.

Purpose: The identification of orthologs is commonly used for data mining and establishing models for human diseases. Researchers analyzing the results of screens performed at the Drosophila RNAi Screening Center (DRSC, www.flyrnai.org) frequently wish to identify mammalian orthologs of the fly genes that were “hits” in their screens. Many tools have emerged to attempt to identify orthologs of Drosophila genes. However, low coverage and heterogeneity of these tools present an obstacle to scientists who want to identify a single “best” ortholog for a given gene of interest or conversely, want to cast a wide net and follow up on all possible orthologs. Our goal is to provide an easy-to-use resource that facilitates summary, comparison and access to various sources of ortholog predictions. Method: DIOPT (DRSC Integrative Ortholog Prediction Tool) integrates human, mouse, fly, worm, zebrafish and yeast ortholog predictions made by Ensembl, HomoloGene, Inparanoid, IsoBase, OMA, orthoMCL, Phylome, Roundup, and TreeFam. DIOPT lets users find ortholog pairs for a specified gene or genes identified by one, many or all of these published approaches. This provides a streamlined method for integration, comparison and access to orthology predictions originating from algorithms based on sequence homology, phylogenetic trees, and functional similarity. Result: Using human and fly ortholog data, DIOPT achieved about 1.3 to 3 fold coverage of both Drosophila and human orthologs, respectively, as compared to individual tools. Global validation using GO and domain annotations suggests functional consistency among human and fly orthologs identified by DIOPT. When tested with manually assembled lists of high-confidence orthologs, DIOPT shows significantly improved sensitivity with a moderate decrease in specificity versus individual tools. Preliminary analysis linking fly-human ortholog information and literature citations shows potential for systematic, quantitative assessment of disease features conserved among organisms.

453C
Analysis of the functional relevance of the asthma susceptibility gene ormdl3 using the model organism Drosophila melanogaster. Kimberley Kalllsen1,2, Holger Heine1, Thomas Roeder2. 1) Research Center Borstel, Parkallee 1–40, 23845 Borstel, Germany; 2) Institute of Zoology, Department of Zoophysiology, Christian-Albrechts-Universität, Olshausenstraße 40, 24098 Kiel, Germany.

Asthma bronchiace is a chronic inflammatory disease of the lung that is becoming a major health issue in many industrialized countries. Yet the molecular mechanisms that contribute to asthma pathogenesis are not well understood. Analysis of the relevance and function of asthma susceptibility genes may lead to better understanding of pathogenic mechanisms that cause this disease. In this study we analyzed the asthma susceptibility gene ormdl3. Orml3 is an ER transmembrane protein whose function is not fully understood. To study the role of this protein we used the model organism Drosophila melanogaster, which has the unique advantages of a short life span and easy genetic manipulation. Additionally, the airways of the fly (traeachea) are made of epithelial cells exclusively and flies only have an innate immune system. This allows an exclusive view of the role of innate immunity in epithelial cells under different physiological conditions. Using the GAL4/UAS system the Drosophila homolog of human ormdl3, orml3, was either overexpressed or knocked down in the tracheae. Microarray analysis confirmed the previously described involvement of orml in the sphingolipid metabolism and the unfolded protein response. However, in addition we found modulated expression of genes involved in EGF and Notch signaling pathways, remodeling, stress and immune response after knockdown and overexpression of orml in the tracheae of Drosophila. Taken together, our data reveal new biological processes orml3 is involved in. Focusing on these processes may lead to new insights in Asthma bronchiace pathogenesis.

454A
Stress Accelerates Muscular Dystrophy: A Genetic Analysis in Drosophila. Mariya Kucherenko1, April Marrone1, Valentyna Rishko3,5, Helena de Fatima Magliarel1,5, Halyna Shcherbata1. 1) Gene expression and signaling, Max Planck Institute for biophysical chemistry, Gottingen, Germany; 2) Department of genetics and biotechnology, Ivan Franko National University of Lviv, Ukraine.

In Drosophila, like in humans, Dystrophin Glycoprotein Complex (DGC) deficiencies cause a life span shortening disease, associated with muscle dysfunction (Shcherbata et al. 2007). Our data show that stress induces muscle degeneration and accelerates age-dependant muscular dystrophy. Dystrophic muscles are already compromised; and as a consequence they are less adaptive and more sensitive to energetic stress and to changes in the ambient temperature. However, only Dystroglycan, but not Dystrophin deficiency causes extreme myodegeneration on sugar-free conditions suggesting that Dystroglycan might be a component of the low-energy pathway. We performed the first in vivo genetic interaction screen in ageing dystrophic muscles and identified genes that have not been shown before to have a role in development of muscular dystrophy and interact with the DGC. Mutations in many of the DGC interacting genes cause age-dependent morphological and heat induced physiological defects in muscles, suggesting their importance in the tissue. The majority of them are phylogenetically conserved and implicated in human disorders, mainly tumors and myopathies.

455B

Integrins are transmembrane receptors that link the cytoskeleton to the extracellular matrix (ECM) at focal adhesion sites. Functional integrin adhesion sites are important for tissue interactions during development, as loss of integrins leads to cell-spreading defects and muscle detachment. Upon activation, integrins assume an extended conformation where they have greater affinity for ECM ligands. Integrin activation occurs when the head domain of the cytoskeletal protein talin binds to the integrin β cytoplasmic tail. Zasp (Z-band alternatively spliced PDZ-motif protein) is the only member of the Alp/Enigma family of PDZ-LIM domain proteins in Drosophila. In muscles, Zasp colocalizes with integrins at myotendinous junctions and loss of Zasp disrupts cell spreading in S2R+ cells and leads to muscle detachment. During muscle detachment, integrins partially detach from the ECM in Zasp mutant embryos, suggesting a role for Zasp in integrin activation. In addition, overexpression of the talin head domain partially suppresses the Zasp mutant phenotype. Zasp binds to both talin and β integrin in vitro. We will report on the protein domains required for these interactions and their relative importance for integrin activation.

456C
ATP is involved in MAPK signaling pathway during Drosophila development. Oky Maeng, Dahye Lee, Young-Ha Lee, Guang-Ho Cha. Infection Biology, College of Medicine, Chumsang National University, Daejeon, Korea.

Copper (Cu)-transporting P-type ATPases are central regulators of cellular copper metabolism. Defects in the mammalian Menkes and Wilson copper-transporting P-type ATPases lead to disease phenotypes with severely impaired copper metabolism in humans. In this study, we conducted genetic analysis to ascertain the developmental processes involving DmAATP, the Drosophila homolog of the Menkes disease (ATP7A, MND) and Wilson disease genes (ATP7B, WND). Ectopic expression of DmAATP in the eye during early stages of eye development leads the emergence of an extra antenna or incomplete head formation. Additionally, ectopic expression of DmAATP in wings during wing development leads to deformities of the wing. A loss-of-function study for DmAATP showed that this gene is essential for body pigmentation and cuticle formation in Drosophila adults, and probably not required for copper intake from the diet provided for excess copper. In order to understand the biological role of DmAATP in development process, genetic interaction of DmAATP with MAPK kinase pathway components were tested in developing Drosophila eye and wing. Our findings revealed that co-expression of Hemipenerous with

228
DmATP7 enhanced, but co-expression of ERK upstream kinases suppressed the DmATP7 basic phenotype. In addition, Western blot analysis showed that the intracellular phosphorylation levels of MAPKinasers are changed by DmATP7 in accordance with the genetic interaction results. We also found that the Drosophila promyelocytic leukemia zinc finger (dPLZF) is able to suppress the DmATP7 overexpression phenotype. These results suggest that DmATP7 as a copper transporter has important function in regulating MAPK signaling pathway during organism development.

457A

Impact of disease-causing missense mutations in the extracellular domain of Crumbs on photoreceptor development in Drosophila. Milena Pellliska, Ulrich Tepass. Dept. of Cell & Systems Biology, University of Toronto, Toronto, ON, Canada.

The apical transmembrane protein Crumbs (Crb) is a critical regulator of epithelial polarity and apical membrane morphogenesis in photoreceptor cells (PRCs). Correct polarization of PRCs is critical for PRC function such as phototransduction in both vertebrates and invertebrates. In Drosophila PRCs, Crumbs was shown to localize to the stalk membrane, where it plays a structurally important role in the support and orientation of the rhodopsins. Previous work indicated that Crb is required for the maintenance of rhodomere shape, zona adherens integrity, stalk membrane length, and cell survival of PRCs in the eye. Mutations in one of the three human orthologs of Crb (CRB1) are linked to eye degenerative conditions such as Leber’s Congenital Amaurosis (LCA) and Retinitis Pigmentosa type 12 (RP12) (den Hollander et al., 1999; Ready and Tepass, 2004; Richard et al., 2006; Gosen et al., 2008; Bulgakova and Knust, 2009). Work in vertebrate models suggests that a loss of epithelial integrity precedes PRCs degeneration (Mehalow et al., 2003; van de Pravert et al., 2004; Omori and Malicki, 2006; Hsu et al., 2006). Over 40 distinct disease-causing missense mutations that map to the extracellular domain have been reported in human CRB1. To gain insight into how these missense mutations affect CRB1 function and to elucidate a role for the large extracellular region of Crb/CRB1 we have recreated several disease-causing missense mutations in conserved residues in Drosophila Crb. Our analysis of these Crb mutant isoforms in both wildtype and crb mutant PRCs identified specific residues/domains in Crb that are required for the normal localization of Crb at the stalk membrane. Moreover, each of the four missense mutations tested so far shows a unique cell biological profile including mutations that cause mislocalization of Crb to the rhodobium, which leads to a displacement of Rhodopsin. Loss of Rhodopsin function is a known cause of RP, thus suggesting a potential disease mechanism that may be independent from the role of Crumbs in supporting polarity for these particular mutant alleles.

459C


Epilepsy is a debilitating neurological disorder that affects about 1% of the global population. The ketogenic diet (KD) has been shown to be an effective treatment for refractory epilepsy. It is a high-fat, low-protein, low-carbohydrate, and caloric-restricted diet. Despite the clinical effectiveness of the KD, relatively little is known about the underlying mechanisms of action. We investigated the role of diet/nutritional modulation in ameliorating seizure behavior in bang-sensitive (bs) paralytic mutants of Drosophila melanogaster. Using two behavioral measures (mean recovery time and percent paralysis) and three bs mutants (easily shocked, technical knockout and hangensceless), we evaluated the mutant bs phenotype after being raised on various diets, including the standard cornmeal/molasses/yeast diet, which consistently yields flies with seizure behavior. The results indicate that a diet modeled after the traditional KD is effective in ameliorating or eliminating bs behavior in two different mutant strains. Proposed breakdown products of a high fat diet also reduced the bs phenotype. In addition, modulation of protein to carbohydrate ratios and calorie intake can also affect the bs phenotype. The findings of this study begin to support the hypothesis that the KD is mechanistically alleviating bs phenotype via increased mitochondrial biogenesis and altered energy reserves.

460A

Establishing Drosophila as a model to study human lamin processing. Sandra R. Schulze1, Katie Adolphsen1, Sara Kevorkian2, Emily Matson2, Brian Kennedy2. 1) Dept Biol, Western Washington Univ, Bellingham, WA; 2) Department of Biochemistry University of Washington Seattle, WA.

The nuclear envelope is supported by a mesh-like structure called a lamina that is composed of A and B type lamins. Lamins are prenylated (provided with a membrane anchor) by a series of enzymes including the protease Zmpste24. B-type lamins are stably prenylated, thereby providing essential structural support in all cells, but A-type lamins exhibit only transient prenylation. Failure to remove the prenyl anchor from Lamin A may have severe consequences, evidenced by the premature aging disorder Hutchinson Gilford Progeria, in which a mutation in the Lamin A gene causes the expression of a stably prenylated variant of Lamin A called Progerin. It is not clear how the build-up of Progerin leads to organism-wide rapid aging, nor has it been directly shown that Zmpste24 is the enzyme that removes the prenyl anchor from Lamin A to prevent accumulation. We are using Drosophila to study human lamin processing, by making transgenic flies that express normal or mutant human Lamin A and then testing the localization of these human lamins in presence or absence of human or fly Zmpste24. Wild type human Lamin A incorporates readily into the fly nuclear lamina. We have also expressed a form of Lamin A that cannot be prenylated, and show that in contrast this protein localizes to the fly nuclear interior. Finally, we have expressed Progerin, and find it localizes to the fly lamina, similar to wild type human Lamin A. These data show that human Lamin A is stably prenylated in the fly nucleus, and that no conserved function in the fly is removing the prenyl anchor. We are currently making transgenic flies that co-express human Zmpste24 in order to observe how this prenyl protease interacts with human Lamin A in an in vivo context.

461B


Inclusion body myopathy 3 (IBM3) is an autosomal dominant myopathy associated with a missense mutation (E706K) in the myosin heavy chain Ila gene (MYH2). The disease is mild in childhood but appears progressive in adulthood, with proportional muscle weakness affecting movement. Biopsies from adult patients reveal dystrophic alterations and rimmed vacuoles that correlate with an increased expression of the mutant motor with advanced age. We introduced the E706K substitution into Drosophila myosin (E699K) and expressed it in indirect flight and jump muscles using a transgene. Flight ability was absent in heterozygous and homozygous flies. Jump ability was reduced in heterozygous flies and was nearly absent in homozygotes. This significantly reduced locomotion is consistent with the proposed dose-dependent muscle weakness reported in patients. Decreases in
E706K (E699K) chemomechanical properties were also consistent with the clinical data. The mutant myosin displayed 80% lower actin sliding velocity and 74 and 83% reductions in basal and actin stimulated ATPase activities, respectively, compared to wild-type myosin. Electron microscopy revealed E706K (E699K) myosin heads bear a substantial propensity to collapse and to aggregate relative to wild-type heads. At 23°C, 77.5% of mutant myosin molecules exhibited intra- or intermolecular aggregates compared to only 22.5% of control molecules. A five minute, 37°C incubation induced 95.3% of the mutant myosin heads to aggregate versus 80.9% of the control molecules’ heads. This test directly assessed motor integrity and suggests E706K (E699K) myosin is far more labile than wild-type myosin. We are imaging myosin myofibers to determine if the ultrastructural hallmarks seen in adult patients also appear in our fly model. The depresed motor properties and instability of the mutant myosin likely contribute to the muscle weakness observed in our fly model and possibly in senescent patients.


Fragile X syndrome (FXS) is the most common form of inheritable mental impairment in humans. It is caused by the transcriptional silencing of the fragile X mental retardation 1 (fmr1) gene. FXS is characterized predominantly by cognitive impairment, which has been indicated to progress with age. Additionally, a spectrum of other behavioral deficits is common in many patients, including hyperactivity and repetitive behavior. Mouse and Drosophila models of FXS exhibit behavioral and morphological abnormalities reminiscent of the human disorder and have been valuable tools for research of the disorder. A major advancement in the understanding of FXS has been the finding that fmr1 mutations increase translation of a number of target genes and enhance metabotropic glutamate receptor (mGluR) signaling in the brain. Inhibition of mGluRs with the antagonist 6-Methyl-2-(phenylethynyl)pyridine (MPEP) can rescue learning, memory and some morphological defects in these animal models. Treatment with lithium (LiCl) has also been shown to rescue learning and memory in dfmr1 mutants. However, the neuronal mechanisms underlying repetitive behavior and anxiety remain poorly understood. Here, we show that dfmr1 mutants groom excessively in an age-dependent manner. Blockage of mGluRs with MPEP, although sufficient to partially rescue courtship behavior, failed to rescue the grooming defect and instead increased grooming in mutant flies. Treating dfmr1 mutants with lithium also failed to rescue excessive grooming. Flies that overexpress the Drosophila orthologue of vesicular monoamine transporter (VMAT) have been shown to groom excessively. Inhibition of synaptic release of biogenic monoamines with reserpine, a drug that blocks VMAT, effectively suppresses the excessive grooming in these flies. We find that the increased grooming of dfmr1 flies can also be reduced by treatment with reserpine. These results suggest that enhanced monoamine signaling may underlie the repetitive behaviors and hyperactivity associated with FXS. Supported by internal funds from OU.

463A Mechanisms of Notch Signaling That Potentiate Survival During Chronic Hypoxia. DeeAnn W. Visk1, Dan Zhou2, Gabriel H. Haddad2,3. 1) Division of Biological Sciences, University of California, San Diego, La Jolla, CA; 2) Department of Pediatrics, School of Medicine, University of California, San Diego, La Jolla, CA; 3) Rady Children's Hospital, San Diego, CA.

Damage caused by lack of oxygen is a key factor in many serious disease states ranging from stroke and heart attack to asthma and high altitude mountain sickness. Determining the pathways that can ameliorate injuries caused by hypoxia is key for developing treatment and prevention for hypoxia damage. This study examines the role of Notch signaling that permits Drosophila to develop under chronic low oxygen conditions. In a pathway-level analysis of micro-array data obtained from flies selected under lower and lower oxygen levels over 18 generations, the Notch signaling pathway was significantly up-regulated. To determine the role of tissue specific Notch activation in hypoxia, homogous GAL4 drivers were crossed to UAS-RNAi flies targeting genes whose down-regulation is known to potentiate hypoxic survival in worms. These genes include Akt, NPC1a, NPC1b, dfmr1 mutants groom excessively in an age-dependent manner. Blockage of mGluRs with MPEP, although sufficient to partially rescue courtship behavior, failed to rescue the grooming defect and instead increased grooming in mutant flies. Treating dfmr1 mutants with lithium also failed to rescue excessive grooming. Flies that overexpress the Drosophila orthologue of vesicular monoamine transporter (VMAT) have been shown to groom excessively. Inhibition of synaptic release of biogenic monoamines with reserpine, a drug that blocks VMAT, effectively suppresses the excessive grooming in these flies. We find that the increased grooming of dfmr1 flies can also be reduced by treatment with reserpine. These results suggest that enhanced monoamine signaling may underlie the repetitive behaviors and hyperactivity associated with FXS. Supported by internal funds from OU.


Using RNAi to knockdown expression of dGyk (CG18374) and dGK (CG7995), we have created a Drosophila model for glycerol kinase deficiency [GKD; MIM 307030]. In humans, GKD results in a wide range of phenotypic variability; patients can have severe metabolic and CNS abnormalities, while others possess hyperglycerolemia and glyceroluria with no other apparent phenotype. Both dGK-RNAi and dGyk-RNAi flies have reduced glycerol kinase phosphorylation activity and are hypersensitive to glycerol as food source. Using a survivorship assay on a defined glycerol and sucrose food source, we demonstrate that for dGyk-RNAi knockdown flies, the glycerol hypersensitivity phenotype was strongly enhanced by null mutations in the eye pigmentation genes encoding Brown, Garnet, Rose, and Vermillion. These mutations had a relatively weak affect on glycerol hypersensitivity in dGyk-RNAi flies. Interestingly, whereas a null mutation of the Brown gene results in enhancement of glycerol hypersensitivity, a point mutation that causes a premature stop codon within the Brown gene results in suppression of glycerol hypersensitivity. We predict that due to the hypoxic nature of glycerol, glycerol hypersensitivity results in reduced desiccation resistance, suggesting that glycerol kinase plays an important role in desiccation resistance in insects. Additionally, the effect of eye pigmentation genes on glycerol hypersensitivity indicates that they have additional non-eye functions important for desiccation resistance. Taken together, these data indicate that the interaction between glycerol kinase and proteins encoded by eye pigmentation genes could play an important role in regulating metabolite levels in response to environmental stress such as desiccation.

465C Role of antimicrobial peptide genes in hyperoxia. Huwien Zhao1, Dan Zhou2, Gabriel Haddad2,3. 1) Pediatrics Dept, UCSD, La Jolla, CA; 2) Neuroscience Dept, UCSD, La Jolla, CA; 3) The Rady Children’s Hospital, San Diego, CA.

Prolonged exposure to hyperoxia generates excessive reactive oxygen species (ROS) and potentially oxidant injury in every organ including lung, brain and retina; however, the mechanisms underlying tissue susceptibility or tolerance to hyperoxia-induced oxidant stress remain elusive. Through a long-term laboratory selection over many generations, we have previously generated Drosophila melanogaster flies that tolerate tremendous oxidant stress (90%-95%02), a lethal condition to naïve flies. Microarray studies have revealed that the family of antimicrobial peptides (AMP) is over-represented in these tolerant flies. In the current study, we demonstrated that overexpression of even one AMP at a time (e.g. Diptericin) allows wild type flies to survive much better in hyperoxia and investigated the potential mechanisms underlying hyperoxia tolerance in these flies using a number of experimental approaches. We report that flies with Diptericin overexpression resist oxidative stress by increasing antioxidant enzyme activities and preventing an increase in ROS level after hyperoxia. Depleting the GSH pool using buthionine sulfoximine limits fly survival, thus confirming that enhanced survival observed in these flies is related to improved redox homeostasis. We conclude that AMPs play an important role in tolerance to oxidant stress and overexpression of Diptericin changes the cellular redox balance between oxidant and antioxidant, which plays an important role in survival in hyperoxia.
POSTER: Evolution and Quantitative Genetics
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

466A
Changing the shape of the wing by directional selection in Drosophila melanogaster at two developmental temperatures. Blanche Bitmer-Mathe, Danielle Tesseroli, Bianca Menezes. Dept Genetica, Univ Federal do Rio de Janeiro, RJ, Brazil.

Evolution of biological structures can be constrained by interconnections among traits. By analyzing a reduced number of dimensions in a model organism, one might investigate how changes in genetic variation and developmental processes can influence biological structures to adapt its size and shape in response to selective pressures. In this study, we performed a directional artificial selection for increasing divergence in the outline shape of Drosophila melanogaster wings, at two developmental temperatures (16°C and 22°C). A wing shape index (SH) was estimated as the width-to-length ratio (Ww/Wl); note that Ww and Wl might estimate developmental variation in anterior-posterior and proximal-distal wing axes respectively. Divergent lines were established by the progressive selection of a rounder wing shape (R lines) or a more elongated wing shape (L lines). After 13 generations, significant effects on accumulated realized heritabilities were detected for the direction of selection and for the selection × temperature interaction. In fact, the divergence between R and L lines was less pronounced at 16°C, given a less effective response of R lines at this temperature. A wing size index (SI) was also estimated. Remarkably, average SI changed in a similar way in R and L lines, despite the progressive increase in SH divergence. Nevertheless, the relative importance of Ww and Wl varied; while the same phenotypic response in SH was observed in L lines, or by a reduction of Wl in R lines. Our results suggest that primary short-term changes in different developmental axis might adjust both size and shape for the well-adapted wing morphologies. When comparing the SH values of R and L lines with those from other related species, we also observed that wing shape variation between the selection lines was bigger than variation found among species from several groups of the genus Drosophila. Financial support: CNPq, FAPERJ and CAPES.

467B
NEW GENES IN DROSOPHILA QUICKLY BECOME ESSENTIAL FOR DEVELOPMENT. Sidi Chen, Yong Zhang, Manyuam Long. Dept Ecology & Evolution, Univ Chicago, Chicago, IL.

Essential genes are often portrayed as conserved and ancient, while younger lineage-specific genes have been considered to be more dispensable and to perform relatively minor organismal functions. It is unclear how essential genes arise and how new genes accumulate essential functions. To investigate the origin and evolution of essential genes, we used newly evolved genes as a model. Because new genes arise continuously through time, and, when first arose, they were expected to be non-essential since their immediate ancestral species survived without them, successive essential evolution should be identified. We identified and phenotyped 195 young protein-coding genes that originated 3-35 Mya in Drosophila. Knocking down expression with RNAi showed that 30% of them are essential for viability. The proportion of genes that are essential is similar in every age group examined. Silencing young essential genes lead to stage-specific termination of developmental processes as well as morphological defects. Lethality was highly enriched in the pupal stage, and also found in the larval stages. Lethality was attributed to diverse cellular and developmental defects, such as organ formation and patterning defects, showing that new genes frequently and rapidly evolve essential functions. How did essentiality evolve? A novel discovery is the presence of an LXXLL motif in the homeotic YPWM motif. The addition of LXXLL gave Ftz the ability to interact with a new cofactor, the orphan nuclear receptor NN2. Evolutionary analyses revealed strong Darwinian selection and structure renovation for these genes, as well as their independent essentiality from parental genes, support the neofunctionalization origin as a primary mechanism. Taken together, the developmental phenotypes and evolutionary signatures of the lineage specific genes implied that, under natural selection, different species might have evolved distinct genetic components for their own development.

468C
vestigial function is not limited to wing development in Tribolium. Courtney M. Clark, Yoshinori Tomoyaus. Zoology Department, Miami University, Oxford, OH.

The vestigial gene (vg) is often referred to as a wing “master gene” because of its ability to induce wings in various locations in Drosophila when it is overexpressed. The function of vg in Drosophila seems to be limited to wing formation. However, it is yet to be determined to what extent the function of vg is conserved among other insect species. We disrupted the vg function via RNA interference (RNAi) in the red flour beetle, Tribolium castaneum, and analyzed the phenotypes. Depletion of vg in the late larval stages led to a partial or entire deletion of the hindwings and elytra. Interestingly, we also found that vg RNAi induced novel body wall phenotypes in the first and third thoracic segments. Expression analysis of vg revealed that vg is expressed in both the thoracic and abdominal segments of the developing embryo. Recently, it has been reported that vg is expressed in the body wall of bristleless, which do not possess wings (Niwa et al, 2009). These results suggest that unlike in Drosophila, vg function in Tribolium is not limited to wing development and that vg has an important role in insect body wall development that has been lost in Drosophila.

469A
A quantitative framework provides insights to design features and evolution of enhancers. Thyago Duque1, Xin He2, Saurabh Sinha2. 1) University of Illinois at Urbana Champaign; 2) University of California at San Francisco.

Despite the central role of enhancers in gene expression, we know very little about the principles of their design and evolution, and how one shapes the other. While it is hard to address this issue experimentally, simulations may prove to be effective (e.g., Lusk & Eisen, PLoS Genetics, 2010). We combine evolutionary simulations with a model of transcriptional regulation in order to understand how mechanisms of enhancer action influence their composition and evolution. Our strategy consists of two parts: 1) The function output of an enhancer is defined as an expression pattern generated by a thermodynamics-based sequence-to-expression model. 2) Population-level evolutionary simulations of enhancers are carried out with fitness dependent on the predicted function of that sequence. Earlier studies of Drosophila enhancers, ..., revealed interesting aspects of their sequence content and evolution. A common theme is the abundance of weak binding sites, sometimes attributed to the role of multi-site in achieving non-linear readout of a morphogen gradient. Contrary to this view, we find this preference for multi-site enhancers to be explainable as a direct consequence of the genotype-phenotype landscape and the evolutionary process. We also find that various sources of non-linearity in the enhancer action (e.g., synergistic activation) shape the evolutionary search so as to favor multi-site solutions. In another experiment, we simulate the evolution of enhancers under stabilizing selection to quantify the rates of binding site loss and gain. By doing so, we are able to test whether such events are due to purifying selection acting on entire enhancers, where gain or loss of individual sites may be balanced by changes in other parts of the sequences. We are then able to compare the results with empirical observations from our previous study of the 12 Drosophila genomes (Kim et al, PLoS Genetics, 2009), obtaining a holistic understanding of enhancer evolution.

470B
Evolution of the Hox gene fushi tarazu in arthropods. Alison Heffer1, Yong Lu2, Jeff Shultz2, Leslie Pick1,2,7. 1) Prog. in Molecular and Cell Biology, Univ Maryland, College Park, MD; 2) Dept. Cell Biology and Molecular Genetics, Univ Maryland, College Park, MD; 3) Dept Entomology, Univ Maryland, College Park, MD.

Hox genes are evolutionary conserved transcription factors, best known for their roles in determining segment identity in insects. fushi tarazu (ftz) is a rapidly evolving Hox gene that has changed from an ancestral homeotic gene to a pair-rule segmentation gene in Drosophila. During arthropod evolution, ftz has undergone three changes thought to contribute to this switch in function. ftz gene expression during embryogenesis changed from Hox-like to seven stripes in Drosophila. The other two changes were in Ftz protein sequence: acquisition of an LXXLL motif and loss of the YPWM motif. The addition of LXXLL gave Ftz the ability to interact with a new cofactor, the orphan nuclear receptor Fz-F1, which in Drosophila is necessary for selection of target genes involved in segmentation. Drosophila Fz lost the YPWM motif conserved in other Hox genes required for interaction with Hox cofactor Exd. These changes switched the protein partner that Ftz interacts with and the DNA binding targets Ftz regulates. We have mapped these sequence and expression changes over 550 million years of arthropod evolution by isolating and sequencing ftz genes from non-model arthropods. We find that while the segmentation LXXLL motif was stably acquired once at the base of holometabolous insects, the homeotic YPWM motif has independently degenerated several times, and these “degen-YPWMs” retain varying degrees of homeotic potential when expressed in Drosophila. Also, we uncovered an additional change in ftz expression in the crustacean Artemia where
Building a dictionary of genetic effects. David Houle, Eladio Márquez. Dept Biological Science, Florida State Univ, Tallahassee, FL.

A central challenge in biology is the elucidation of the genotype-phenotype (G-P) map. Current efforts to do so depend on mutations of large effect or natural variation, each furnishing a narrow view of the map. We are taking investigating the G-P map of the Drosophila wing by quantitative targeted manipulations of gene expression. We couple the diet-inducible Gal4-GeneSwitch construct to UAS-based regulation of RNAi of specific targets to produce controlled knockdowns of individual genes under a common control, and measure the phenotypic effects on whole-wing shape. Our experimental protocol combines rapid generation of phenotypic variants at gradually increasing perturbation levels with high-throughput phenotyping, allowing us to attain an unprecedented resolution of the quantitative effects of single-gene downregulation, as opposed to the qualitative effects of perturbations of phenotypic shape data. We can navigate the entire range of phenotypic responses, including venation patterns, locally-defined wing traits, and net magnitudes of effects. We present initial results from this work, including genes from major pathways known to affect size and vein configuration in imaginal discs. In the majority of the cases analyzed thus far, there seems to be no obvious correspondence between gene function or expression domain and adult venation pattern at small perturbation levels, engrailed being an exception. However, most perturbations elicit a correlated characteristic phenotypic response, which can be large (e.g., rho, en, Dil, hpo), small (tkv, sog, vvl), gradual (rho, sog, vvl), discrete (en, hpo, Dil), linear (rho, hpo), asymptotic (en, Dil, sog), or canalized below a threshold (Dil).

Whole-phenotype characterization of quantitative variants in gene expression appears thus as a promising approach for dissection of the G-P map, allowing at once measuring the contributions of individual genes to phenotypic traits, and the study of commonalities of these effects in relation to gene-gene, gene-phenotype, and phenotype-phenotype interactions.

Mutations in a cholesterol oxygenase gene have turned Drosophila pachea into an obligate specialist species. Michael Lang1, Sophie Murat2, Geraldine Goupill2, Luciano M. Matzkin1, Takaji Yoshiyama3, Émilie Guillart2, Hiroshi Kataoka2, Ryusuke Niwa3, René LaFont1, Chantal Dauphin-Villemant1, Virginie Orgogozo1,2. 1) CNRS UMR7592, Institut Jacques Monod, Paris, France; 2) CNRS UMR7622, Université Pierre et Marie Curie, 75005 Paris, France; 3) University of California, Division of Biological Sciences, La Jolla, California 92039-0116, USA; 4) University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan.

Species restricted to a single food resource, so-called specialized species, usually display bizarre or exceptional features. Following long-term association with a particular host, specialist species can loose the ability to feed on alternate hosts and thus become obligate specialists. If the loss is irreversible and hosts happen to disappear, then the species is likely to go extinct. We investigated the genetic basis of Drosophila pachea metabolic dependence towards its cactus host plant, Lophocereus schottii. D. pachea flies require 7-dehydrosterol dehydrogenated steroids produced by this cactus to survive and they cannot utilize other food sources because they lost the capacity to transform cholesterol into 7-dehydrocholesterol (first enzymatic step of the edysone biosynthesis pathway). In insects, this enzymatic reaction is catalyzed by an evolutionary conserved Rieske-domain oxygenase named Neverland. Four amino acid changes in the Neverland protein are responsible for the loss of 7,8-dehydrogenation in D. pachea. Surprisingly, the neverland gene is still expressed in steroidaligenic tissues in D. pachea. A combination of biochemical assays and population genetics analyses indicate that Neverland has a different enzymatic activity in D. pachea. Since four amino acid changes are required to restore Neverland ancestral function, the loss of 7,8-dehydrogenase activity in D. pachea is potentially reversible but unlikely, suggesting that D. pachea is at an evolutionary dead end.

Morphological homology vs. Developmental homology - a case study using insect wing veins. Tingjia Lao, Yoshinori Tomoyasu. Department of Zoology, Miami University, Oxford, OH.

Two structures can be homologous when they have a common origin. Duplication and modification of homologous structures have been a driving force of animal evolution. Morphological similarity is an important character to identify homology (morphological homology). However, morphological similarity can be deceivable because evolutionary modification can cover morphological similarity of homologous structures. Also, convergent evolution can make two non-homologous structures look similar, which further confuses the identification of homology. Comparing developmental system is an alternative way to identify homology, sometimes independent of morphological similarity (developmental homology). Utilizing these two homology concepts is powerful to understand the evolution of homologous structures. Interestingly, however, the two concepts sometimes bring different, even controversial results. We utilize insect wing veins as a model to further understand the relationship between these two concepts. All extant insect wings are thought to be homologous, and it is even possible to homologize each wing vein among different species. We analyzed developmental homology of wing veins between two insects, Drosophila and a beetle Tribolium. Vein formation has been intensively studied in Drosophila, identifying a battery of genes important for vein formation. We depleted the function of these vein genes via RNAi in Tribolium, and compared the phenotypes to those in Drosophila. RNAi for most of these genes in Tribolium resulted in abnormality in vein formation, suggesting that the vein function of these genes has been conserved between the two species. Interestingly, however, the affected veins by the disruption of these genes were somewhat different between species. This is puzzling as these results suggest that a morphologically homologous structure can be produced by different developmental mechanisms, adding another complication to the relationship between two homology concepts. We will discuss a possible explanation for this apparent disagreement of two homology concepts in insect wing veins.


Sensory systems have wide-reaching effects on fitness. Shifts in the relative importance of different senses can produce strong selection and rapid evolutionary divergence. Such a shift is observed in male Drosophila prolongata, which display a 10-fold increase in the number of chemosensory bristles on the first leg, a unique phenotype in the Drosophila genus. The phenotype is produced by the evolutionary transformation of an otherwise deeply conserved set of mechanosensory bristles. This transition may involve changes in the transcription factor Pox Neuroun (Poxn), which has been shown to act as a developmental switch between the two bristle types. Here I report expression profiles of Poxn in the developing first legs of male and female D. prolongata and its sister species D. robula. These expression patterns indicate that Poxn expression has expanded into the derived chemosensory bristles, while it is absent from orthologous ancestral mechanosensory bristles. This trait provides an attractive model for investigating how sensory systems evolve, and how the central nervous system changes to accommodate this evolution.

Evolution of locomotor patterns in Drosophila species. Claudia M. Mizutani, Belu Mirela, Yunyi Yang. Dept Biol, Case Western Reserve Univ, Cleveland, OH.

Scaling in animals pose serious challenges to gene networks that need to adapt to variations in size and still produce coherent and functional tissues. So far, most examples of extreme variations in size have been shown in highly divergent species. Here we began to investigate the partition of three embryonic layers that give rise to the nervous system, muscles and epidermis of flies in three closely related species that diverged very recently (D. melanogaster, D. simulans and D. sechellia) and have sharp variations in egg size. In addition, we also analyzed two other more distantly related species, D. pseudoobscura and D. buzzeki. Our analyses show that the partition of dorso-ventral embryonic layers can vary significantly in species that diverged recently. In particular, of these three layers, the mesoderm shows extreme enlargements and reductions, while the neuroectoderm remains constant, as previously shown. We expected that such variations in the mesoderm might lead to either an abnormal arrangement of muscle fibers in the larval body wall, or
POSTER: Evolution and Quantitative Genetics
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

extremes in musculature size, which in either case might modify neuromuscular junctions and consequently movement. Here we show that the identity and arrangement of muscle fibers remains highly stereotyped in all species studied, and instead more or less myoblast cells are recruited to each muscle fiber depending on the species mesodermal size. As predicted, the variation in muscular mass and relative numbers of synaptic boutons is accompanied by species-specific locomotor patterns in crawling larva. To test whether those species-specific phenotypes are due to zygotic or maternal influence, we generated hybrid larva between D. melanogaster x D. sechellia and D. simulans x D. sechellia. Both movement pattern and number of fused myoblasts resemble the maternal species, and not the paternal species. Comparative genomics analyses are currently underway to isolate the genes involved in the phenotypic differences described above of myoblast fusion and locomotor patterns in the larva.

476B
Rescue of Bicoid patterning functions by divergent homeodomain proteins. Jacqueline Moore1, Gözde Yuce1,2, Na-Eun Christine Yoo1, Stephen Small1. 1) Department of Biology, New York University, New York, NY; 2) Stanford University School of Medicine, Stanford, CA.

The homeodomain (HD) protein Bicoid (Bcd)’s vital role as the major anterior determinant in Drosophila embryonic patterning has been widely acknowledged; however, it is understood poorly conserved throughout evolution, with no bcd homolog observed outside of Dipterans. Bcd arose from the duplication of an ancestral gene that also gave rise to zerknullt (zen), which plays a well-conserved role in specifying the amnio serosa. It is thought that bcd evolved independently, and eventually took over transcriptional and translational functions provided by other genes in other insects. Recent studies have implicated Orthodenticle (Otd), another HD protein with DNA-binding specificity very similar to Bcd’s, as a potential ancestral morphogen. In Drosophila, otd is a zygotic target of Bcd with a minor role in head development. To further investigate the potential ancestral links between bcd, otd, and zen, we examined whether they might still share functional activities using a Bcd rescue assay through qPCR transgenesis to control for expression levels. Replacing bcd with a maternally derived Otd shows remarkable rescue of both thoracic and cephalic structures. In contrast, a maternal Zen gradient shows no rescue activity, but a single amino acid substitution in the Zen HD (Zen K50) shows rescue of thoracic segments. At the molecular level, the Otd and Zen K50 gradients activate different subsets of Bcd target genes. The amount of rescue and target gene activation directed by the Otd and ZenK50 gradients is highly dependant upon dosage, with each requiring much higher expression levels for function compared to the wildtype Bcd protein. This functional convergence between these proteins supports the hypothesis that otd once played a larger role in embryogenesis in Drosophila’s progenitors, and sheds light on the evolution of the bcd gene.

477C
A possible contribution of abrupt in the evolution of beetle elytra. Padmapriyadarshini Ravisankar, Tingjia Lao, Yoshinori Tomoyasu. Zoology Department, Miami University, Oxford, OH.

Morphological innovation is a fundamental process in evolution; however, the molecular mechanism underlying the evolution of morphologically novel structures is still elusive. Coleoptera (beetles) is the most successful animal group on the planet, accounting for over 20 percent of extant animals. Innovation of elytra, which are highly sclerotized and modified forewings, is an important trait driving the successful radiation of beetles. We are using the red flour beetle, Tribolium castaneum, as a model system to understand the molecular basis of elytral evolution in beetles. Tribolium is rapidly gaining momentum as a genetic model system due to its availability of several modern genetic tools. Systemic RNAi technique is one of the important advantages in Tribolium, which has paved the way to create gene ‘knock down’ phenotypes by simple injection of double stranded RNA (dsRNA). Our initial screening for genes important for the evolution of elytra has identified abrupt (ab) as a gene involved in the formation of unique elytral features. ab encodes an evolutionarily conserved transcription factor that contains a BTB zinc finger domain. A mutation in ab in the fruit fly Drosophila melanogaster results in the loss of a particular wing vein. Depleting ab function via RNA interference (RNAi) in Tribolium also caused defects in some wing veins, suggesting that the function of ab in the wing vein formation is conserved among insects. ab functions before the mid-zygotic stage in elytra. For example, ab transcripts are expressed in a parallel vein pattern, which is atypical compared to other insect wing vein patterns. This unique parallel vein pattern was disrupted in ab RNAi beetles. The unique overall shape of elytra was also altered by ab RNAi. These results suggest that ab has gained a new function in the beetle lineage, which might have contributed to the elytral evolution. We will present detailed ab phenotypes in Tribolium, and discuss a possible involvement of ab in the evolution of elytra in beetles.

478A

Abdominal pigmentation is a phenotype that widely varies both across and within Drosophila species. The montium subgroup of Drosophila is ideal for investigating the types of genomic changes that are responsible for the rapid evolution of this morphological trait because in several species, a single distinct locus of large effect controls the female polymorphism. We employ a forward genetics approach with Drosophila serrata, introgressing the dark allele into a light pigmentation background for 35 generations. Then we perform bulked segregant analysis via RNA-seq to identify single nucleotide polymorphisms that segregate with the dark phenotype. Following this initial screen on our introgressed lines, we intend to perform a similar strategy on D. serrata from wild populations to obtain a higher resolution map.

479B

The genetic basis of adaptive evolution may vary among traits, and may be biased by the structure of the regulatory networks that control these traits. To test for such biases, it is necessary to investigate the genetic basis of parallel evolutionary changes in the same traits in closely related species. This meta-model approach may help us discern the general rules that govern the evolution of certain types of traits and gene networks. Drosophila pigmentation is a good system to develop such comparisons, as there are many cases of independent gain and loss of the same pattern elements. As part of this effort I am investigating the genetic basis of pigmentation differences in two subspecies of Drosophila malerkotliana, which differ by the presence of male-specific abdominal pigmentation. A previous analysis using RFLP markers narrowed down the responsible regions to three QTLs on Muller E, and determined that no known members of the pigmentation pathway are involved in this instance of color pattern evolution. However, this analysis did not provide enough resolution to find the causative genes. In order to map these QTLs with higher resolution I used high throughput sequencing of normalized parental cDNA libraries to develop densely spaced SNP markers. A panel of individuals for genotyping was generated using advanced hybrids of the parental strains. Males were scored for pigmentation and 480 were genotyped using the Illumina BeadXpress system. A QTL analysis was performed which localized the QTLs to relatively narrow regions of Muller E. This has allowed for the identification of candidate causative genes, which are now in the process of being tested and characterized. The characterization of these genes will contribute to the reconstruction of the structure of the pigmentation pathway, and shed light upon the dynamics of parallel evolution in this network.

480C

Gene expression analyses have noted that a substantial portion of active transcription in Drosophila melanogaster occurs outside of annotated regions, however little is known about the spatial and temporal patterns of expression of unannotated transcribed elements (UTEs). Additionally, it has been suggested that these UTEs may be non-functional by-products of transcriptional functional genomic targets. This hypothesis predicts that UTEs will degrade neutrally over evolutionary time, therefore we used a comparative evolutionary approach to determine if UTEs showed conserved expression among other Drosophila species. We assessed patterns of expression of 2,789 UTEs (1,269 found within the introns of annotated genes and 1,610 that are intergenic) identified in the modENCODE D. melanogaster ontogeny RNA-Seq transcription profile and compared these data to profiles in male and female dissected heads of D. melanogaster and heads of two distantly related species: D. pseudoobscura (~45 million years diverged [MYD]) and D. melanogaster.
mojavensis (~60 MYD). We find that the sequences containing UTEs are significantly more conserved than randomly extracted intronic or intergenic sequence, though they are less conserved than exonic sequence. UTEs are, on average, expressed at lower levels than annotated genes, yet we note a distinct spike in expression of UTEs beginning during the 3rd instar larval stage (corresponding to the onset of spermatogenesis), until reaching a maximum level of expression in adult males. Furthermore adult expression of UTEs is overwhelmingly male-biased, suggesting that a substantial portion of these transcripts are regulated, and possibly involved in male gonadal function. We find that 13 to 18% of identifiable UTE orthologs show expression at conserved levels in at least one of the two distantly related Drosophila species. These data provide evidence that UTEs may encode functional transcripts that are more than by-products of transcriptional processes.

481A
Rapid Evolutionary Rewiring of a Structurally Constrained Eye Enhancer. Scott E. Barolo1, David B. Schwimmer2, Christina I. Swanson1,2. 1) Dept. of Cell & Developmental Biology, University of Michigan Medical School, Ann Arbor, MI; 2) Current Address: Dept. of Biology, UNC Chapel Hill, Chapel Hill, NC 27599.
Enhancers integrate spatio-temporal signals to control the pattern, timing, and levels of gene expression. How does the regulatory grammar of an enhancer evolve over time? Here, we ask how evolution changes to the structure and function of a flying eye enhancer which is critical for determining proper cell type-specific expression. Our fine-scale chimeric enhancer analysis indicates that, despite these severe constraints on enhancer sequence and structure, sparkling has undergone significant structural changes in its recent evolutionary history. Recently acquired sites—including binding sites for known transcription factors, as well as newly identified regulatory motifs—make large contributions to enhancer function in different fly lineages. Our experimental data, combined with a multi-species analysis, suggest that the relative strengths of the various regulatory inputs into sparkling rapidly change over evolutionary time, such that relatively weak input by certain regulators is compensated by an increased emphasis on a different regulator. These gains and losses are at least partly responsible for the changes in enhancer structure that we observe. In addition, we find evidence for strong evolutionary pressure maintaining low binding affinity for the Notch-regulated transcription factor Su(H). Increasing the affinity of Su(H) binding sites in sparkling causes ectopic gene expression in multiple Notch-responsive cell types in the developing eye, suggesting that weak binding with Su(H) is an evolutionary adaptation that allows correct cell-type-specific activation by Notch.

482B
Evolutionary significance of gene expression divergence of the neo-sex chromosomes in Drosophila albomicans. Ching-Ho Chang1, Su Fang2, Chau-Ti Ting1,3, Hwei-yu Chang4,5. 1) Institute of Ecology & Evolutionary Biology, National Taiwan University, Taiwan; 2) Biodiversity Research Center, Academia Sinica, Taiwan; 3) Department of Life Science & Institute of Zoology, National Taiwan University, Taiwan; 4) Department of Entomology, National Taiwan University, Taiwan.
Sex chromosome evolution independently from autosomes in different fly species. In general, X chromosome remains gene-rich while Y chromosome lost most but sex determination factors. The degeneration of Y-linked genes is caused by accumulation of deleterious mutations as consequence of recombination inhibition between the sex chromosomes. To study the transition from homologous autosomes to differentiated sex chromosomes, recent fused autosomal regions of the neo-sex chromosomes provide a good model. To close view the process of divergence at the early stage of neo-sex chromosome evolution, we compared both sequence divergences and whole transcriptome profiles of the neo-sex chromosomes of Drosophila albomicans. Given the divergence time between D. albomicans and the related species, D. nasuta, is less than 0.12 million years, we estimated the age of the neo-X and neo-Y chromosome in D. albomicans is no more than 60 thousand years based on the level of synonymous substitutions. Conservation of the amino acid substitution (\(d_{AS}/d_{NS}\) < 0.021) suggests that neo-sex linked genes remain under strong negative selection due to the little divergence time. Among 2587 transcripts, near 12.8% show reduced gene expression in neo-Y linked alleles whereas only 3.0% show reduced gene expression in neo-X linked alleles. These results indicate that differential expression of neo-sex chromosomes evolved prior to sequence degeneration of the neo-Y chromosome in D. albomicans. It implies that lower expression of Y-linked alleles is the first sign of neo-Y chromosome degeneration. In conclusion, we demonstrate the degradation on expression level in D. albomicans neo-Y chromosomes while most amino acid sequences are conserved. These results also served as the evidence of Muller’s ratchet on expression levels in the early stage of the neo-Y degeneration.

483C
Using next-generation sequencing to investigate patterns of regulatory divergence in Drosophila. Joseph D. Coolen1, Kraig R. Stevenson1, C. Joel McManus, Brenton R. Graeley2, Patricia J. Wittkopp1. 1) Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI; 2) Department of Genetics and Developmental Biology, University of Connecticut Stem Cell Institute, University of Connecticut Health Center, Farmington, CT.
Proper organismal function requires fine-tuned regulation of gene expression and yet variation exists within and between species. Despite its importance, the genetic mechanisms underlying regulatory evolution are not yet well understood. Regulatory divergence is governed by changes in cis- and trans-regulatory elements that affect the level of expression of genes and cis-regulatory changes have effects on allele-specific expression, while trans-regulatory variation contributes to the expression of both alleles in a diploid cell. The effects of cis- and trans-regulatory elements can be disentangled using measures of allele-specific gene expression in pairs of species and hybrids made by crossing them. A previous investigation of 78 genes in D. melanogaster and D. simulans showed that cis-regulatory divergence explained a greater proportion of expression differences between species than within, suggesting that cis-regulatory variants may preferentially accumulate over time. To determine if this pattern is similar for the rest of the genome and other species comparisons, we used next-generation sequencing for enumeration of allele-specific expression genome-wide to determine the contribution of cis- and trans-regulatory divergence to expression differences between strains and species. D. melanogaster ranging in divergence times from 10,000-4 million years ago. Preliminary results support the hypothesis that the proportion of regulatory divergence explained by cis-regulatory differences is greater between species (D. melanogaster vs. D. sechellia) than within (intraspecific comparison of D. melanogaster strains). Additionally, D. melanogaster zhr allele expression is more often dominant over alleles from D. melanogaster z30 and D. sechellia allele expression was more often dominant over D. melanogaster alleles.

484A
Arginine kinase gene arrangement in the phorid fly Megaselia scalaris. Justin Davis, Kristina Maynard, Jacob Crowley, Glen Collier. University of Tulsa, Tulsa, OK.
Arginine kinase is encoded by a single locus (ArgK) in Drosophila melanogaster that produces six putative alternative transcripts. These all share a common C-terminal catalytic domain, but differ in amino acid sequences at the N-terminus of the protein products. The gene organization seen in D. melanogaster is strictly conserved in the other eleven Drosophila genomes sequenced. To examine the extent to which this gene organization is conserved among other diptera, the organization of the gene for arginine kinase in the phorid fly Megaselia scalaris was studied. 5’RACE performed on RNA isolated different adult tissues of M. scalaris revealed the presence of four of the six transcripts found in Drosophila. The tissue distribution of these forms is similar to that seen in Drosophila. The genomic organization of these exons was examined by genome walking using the RACE products as starting points.

485B
Functional analyses of two nuclear transport retrogenes (Dntf-2r and Ran-like) in D. melanogaster. Susana Dominguez, Esther Betrán. Biology Department, The University of Texas at Arlington, Arlington, TX.
Ntf-2 and Ran are two genes that physically interact and are involved in nuclear transport. We previously showed that these two genes have retrocopies in D. melanogaster. These are both X chromosome to autosome duplicates; a retrogene pattern of duplication that is likely the result of selection rather than mutation. They both have strong testis-biased transcription pattern and have evolved under recurrent positive selection. We also know that Ntf-2 and Ran genes convergently retroduplicated in two other Drosophila lineages. These data suggest strong selective pressures acting on the origin and evolution of these retrogenes. We are studying the functions of parentals genes and retrogenes in D. melanogaster.
male germline. We have generated D. melanogaster transformants that express EGFP- and RFP- tagged proteins of the retrogenes under their putative promoters. Their cellular localization and interactions are being explored with these lines. Additional constructs to study the parental genes in testes are being made. Knockout and knockdown lines are being examined or generated to study the role in fertility and in male germline genetic conflicts of these genes.

486C Using correlated rates of protein evolution to predict reproductive protein interactions. Geoffrey D. Findlay, Nathaniel L. Clark, Mariana F. Wolfner. Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Drosophila melanogaster is a powerful model system for studying the evolutionary dynamics and protein interactions that surround reproduction. Specific seminal fluid proteins control female post-mating behaviors and impact the fitness of both sexes by altering egg-laying rates and sperm storage patterns, reducing female remating receptivity, and affecting sperm competition outcomes. Genomic and proteomic methods have identified suites of male and female reproductive proteins, but knowledge of the specific interactions between these proteins remains limited. We are using evolutionary methods to predict protein interactions by looking for pairs of proteins that show correlated evolutionary rates across the phylogeny. In particular, we are focusing on interactions surrounding a small network of seminal proteins, all of which are necessary to elicit female post-mating responses. We find that several pairs of network proteins show significantly correlated rates of evolution across the phylogeny of the 12 sequenced Drosophila genomes, consistent with physical or functional interactions. In tests for correlated evolutionary rates between these network proteins and members of large sets of proteins identified from seminal fluid, sperm and female sperm-storage organs, we have identified candidates for new members of this network. We are using RNAi methods to test these candidates for roles in female post-mating responses.

487A RNA-seq in Drosophila hybrids reveals species specific gene regulation. Rita M. Grazè, Luis L. Noleto, Victor Amini, George Casella, Sergey Y. Nuzhdin, Lauren M. McIntyre. 1) Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL; 2) Department of Statistics, University of Florida, Gainesville, FL; 3) Section of Molecular and Computational Biology, Department of Biological Sciences, University of Southern California, Los Angeles, CA.

Isoform diversity is a fundamental component of the biology of sexual dimorphism, immune response and neurological function. Regulation of multi-transcript genes is complex, different regulatory mechanisms can impact subsets of alternative transcripts depending on promoter structure, splicing, and shared regulatory regions within transcripts. Thus, isoforms can be linked in their regulation, but can also be independently regulated with independent impacts on phenotype. The aim of this study is to understand how species differences in gene regulation result in dependent and independent expression differences among transcript isoforms. Short read sequencing was used to identify cis differences in gene expression between species by measuring allele-specific expression in D. melanogaster, D. simulans F1 hybrid female head tissues. Technical and mapping error, as well as copy number differences, can result in bias of allele-specific expression measurements toward a specific allele or overall toward a specific parental genome. To control for bias in exon level estimates, a novel Bayesian model was implemented in which allele frequencies in F1 hybrid DNA reads are used to correct systematic bias in estimation of allelic imbalance from RNA-seq data. Species differences in cis regulation were surveyed across all detected exons. Of 15,678 exons examined, 41% were found to show differences in allele-specific expression. Although map bias was corrected for, many more exons were biased toward expression from the D. melanogaster allele. This raises the possibility that this pattern is in part explained by biological differences rather than error. For gene models with alternative and constitutive exons, differences between exons in allelic imbalance were examined. Genes with putative isoform specific regulatory divergence were identified.

488B Gene movement between chromosomes in Drosophila miranda. Tatiana A Gurbich, Doris Bachtrog. Department of Integrative Biology, University of California, Berkeley, CA.

Evolutionary patterns on the X chromosome differ from those on the autosomes. Several recent studies in Drosophila have shown that genes with sex-biased expression are distributed non-randomly among chromosomes, and there is a paucity of male-specific genes on the X chromosome. Often this pattern is a result of relocation of male-biased genes from the X chromosome onto the autosomes. What exactly causes this pattern, however, is unclear. Here, we present data on gene traffic between chromosomes utilizing the genome sequence of Drosophila miranda. Drosophila miranda has recently evolved neo-sex chromosomes, which were formed about 1 million years ago by the fusion of Muller element C, an autosome, to the ancestral Y chromosome, and are still in the process of acquiring characteristics of fully differentiated sex chromosomes. The young age of Drosophila miranda’s neo-X chromosome provides an excellent system for analyzing early evolution of an X chromosome. We combine the pattern of gene movement with data on gene expression, X chromosome inactivation regions and dosage compensation to investigate which forces are driving male-specific genes off the X chromosome.


Transcription factor binding site (TFBS) gain and loss (i.e., turnover) is well documented in the evolution of cis-regulatory modules (CRM). Although noncoding sequence evolution in general has been shown to be under both negative and positive selection, TFBS turnover has long been considered a compensatory neutral process driven mainly by genetic drift. Positive selection, in contrast, has been invoked in specific instances of adaptive gene expression evolution, but has received little attention as a general alternative. In this study we evaluate the neutral and selection hypotheses by analyzing patterns of single nucleotide polymorphism in a large set of well-studied CRM and TFBS in two closely related Drosophila species, D. melanogaster and D. simulans. Mutations within TFBS were classified according to the direction of their predicted effects on binding affinity, which allows gains and losses to be evaluated independently along the two phylogenetic lineages. The observed patterns of polymorphism and divergence are not compatible with neutral evolution for either class of mutations. In addition to selective constraints, multiple lines of evidence strongly suggest contributions of positive selection to both TFBS gain and loss. This result challenges the prevailing view of a compensatory neutral evolution model, and importantly, suggests that CRM functions are not as constrained as we have thought before, but may experience frequent adaptive changes involving gain and loss of TFBS.


Suppressed recombination leads to the degeneration of evolving Y chromosomes. However, it is not known whether gene loss is a largely random process, i.e. whether it is primarily driven by the order in which mutations occur, or whether certain categories of genes are lost less quickly than others; the latter would imply that selection counteracts the degeneration of Y chromosomes to some extent. In this study, we investigate the relationship between putative ancestral expression levels of neoY-linked genes in Drosophila miranda and their rate of degeneration. We use RNA-Seq gene expression data of neoY homologs in D. pseudoobscura to show that genes that have become non-functional on the D. miranda neoY had, on average, lower ancestral expression levels than genes with intact reading frames, and that male-biased genes are retained for longer on the neoY compared to female-biased genes. Our results imply that gene loss from the neoY is not a purely random process, but that selection is, at least to some extent, preserving gene function at genes that are more costly to lose, despite the strongly reduced effective population size of the neoY.

Understanding the evolutionary forces shaping polymorphism and divergence of protein sequences has been a long-standing interest in evolutionary genetics. Here, we used Drosophila melanogaster population genomic data, which consist of 39 genomes from a North American population and 6 from an African population (dpgrp.org), to investigate the evolution of annotated D. melanogaster coding regions. Our observations generally support previous findings and theoretical predictions, such as the higher polymorphism in African than non-African populations, faster adaptive protein evolution on the X chromosome, reduced heterozygosity of X chromosomes in cosmopolitan populations, and the effects of linked selection on polymorphism and adaptive protein evolution. In both African and North American populations, adaptive divergence was found to be similar between genes, rejecting the null hypothesis of neutral evolution using the McDonald-Kreitman test. Most of the significant MK results for the African population sample were deviated in the direction of adaptive protein evolution. However, in North American samples a substantial fraction of significant MK tests deviated in the direction of excess protein polymorphism. We investigated the effects of sample size difference and demographic history of the two population samples to explain such findings. GO categories related to chromosome biology and reproduction are enriched with genes having evidence of adaptive protein evolution. Interestingly, GO categories associated with light-stimuli related response and circadian cycles are especially exceptional in the African sample.

492C
Birth and rapid evolution of a new importin pair of proteins in Drosophila. Nitin Phadnis, Harmit Malik. Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.

Importin-alpha and -beta mediate the nuclear import of proteins across the nuclear membrane in all eukaryotes. Importin-alphas are adaptor molecules that bind cargo proteins, and together with importin-beta are shuttled across nuclear pores. Because all eukaryotes must perform nuclear import using importins, these proteins evolve under purifying selection are generally well conserved across metazoa taxa. Here I show the birth of a novel importin alpha and a new importin-beta pair in the last 15 million years of Drosophila evolution leading to the D. melanogaster species group. In contrast to other importins, this importin alpha-beta pair is testis-specific. Population genetic analyses show that this importin alpha-beta pair evolves extremely rapidly under positive selection in a pattern reminiscent of intra-genomic conflict. Together with the precedent that nuclear pore complexes often evolve rapidly and the finding that Ran-GAP proteins are implicated in a form of segregation distortion, these results have implications for the evolutionary causes that drive the rapid evolution of nuclear import proteins.

493A

The Drosophila Y chromosome is a degenerate, heterochromatic chromosome with few functional genes. Nonetheless, recent evidence has demonstrated a significant effect of Y-linked variation on the expression of hundreds of autosomal and X-linked genes in D. melanogaster. In order to better understand the contribution of the Y-linked variation to differences in gene expression both within and between populations, we have created a series of Y-chromosome substitution lines, in which Y chromosomes from D. sechellia and D. simulans are introgressed into a common D. simulans genetic background. We then assayed gene expression of males from four lines carrying independent D. simulans Y chromosomes and four lines carrying independent D. sechellia Y chromosomes using microarrays. We find significant differences (at a 10% FDR) in expression of almost 200 genes, attributable to the species of origin of the Y chromosome. We also identify significant intra-species differences in gene expression attributable to Y chromosomal polymorphism in both D. simulans and D. sechellia. Further analysis identifies a number of properties shared by genes whose expression is affected by Y chromosome divergence, including a tendency to be male-biased. Taken together, these results suggest that interspecific divergence of Y chromosomes may play an under-appreciated role in gene expression divergence between species.

494B
A de novo Assembly of the Lucilia sericata (Diptera: Calliphoridae) Transcriptome with Alternative Splices: Genomic Tools for a Medically, Agriculturally, Forensically, and Ecologically Important Blow Fly. Aaron M. Tarone1,2, Sing-Hoi Sze3, Joseph P. Dunham3, William A. Cresko3, Nancy L. Lemos. Department of Entomology, Texas A&M University, College Station, TX; 3) Molecular and Computational Biology, University of Southern California, Los Angeles, CA 90089; 4) Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556; 5) Center for Ecology and Evolutionary Biology, University of Oregon, Eugene, OR 97403.

With the advance of high-throughput sequencing, it is feasible to study entire transcriptomes of non-model organisms through the application of de novo assembly algorithms. While the most popular strategy is to investigate assembled contiguous sequences from these algorithms, information about alternative splicing is ignored. We have developed an algorithm to perform transcriptome assembly while preserving alternative splicing and library-specific expression information for non-model organisms. Such approaches are valuable as they will accelerate the rate of biological discoveries in the absence of prior sequence knowledge. Simulations were initially done with the Drosophila melanogaster population genomic data, which include the entire sex chromosome, as in D. melanogaster. In order to better understand the contribution of the Y-linked variation to differences in gene expression both within and between populations, we have created a series of Y-chromosome substitution lines, in which Y chromosomes from D. sechellia and D. simulans are introgressed into a common D. simulans genetic background. We then assayed gene expression of males from four lines carrying independent D. simulans Y chromosomes and four lines carrying independent D. sechellia Y chromosomes using microarrays. We find significant differences (at a 10% FDR) in expression of almost 200 genes, attributable to the species of origin of the Y chromosome. We also identify significant intra-species differences in gene expression attributable to Y chromosomal polymorphism in both D. simulans and D. sechellia. Further analysis identifies a number of properties shared by genes whose expression is affected by Y chromosome divergence, including a tendency to be male-biased. Taken together, these results suggest that interspecific divergence of Y chromosomes may play an under-appreciated role in gene expression divergence between species.

495C
Characterizing the sex chromosomes of Aedes aegypti. Melissa A. Toups1,2, David V. Severson3, William A. Cresko4, Julian M. Catchen5, Susan Bassham3, Matthew W. Hahn1.

1) Biology, Indiana University, Bloomington, IN 47401; 2) Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556; 3) Center for Ecology and Evolutionary Biology, University of Oregon, Eugene, OR 97403.

Genetic sex determination has evolved multiple times in both plants and animals. In many species, there is a nonrecombining region surrounding the sex-determining locus where sexually antagonistic genes accumulate. The size of this nonrecombining region may contain only a small proportion of the sex chromosome, as in Aedes aegypti, or may include the entire sex chromosome, as in Drosophila melanogaster and Anopheles gambiae. However, why the extent of this nonrecombining region varies among species is unknown. We investigated this phenomenon in Aedes aegypti, which has been demonstrated to have only a small nonrecombining region for the last 100-150 million years. The sequenced Aedes aegypti genome contains over 1500 scaffolds, but only ~250 scaffolds are mapped onto chromosomes. We developed DNA (RAD) tags and used Illumina sequencing to map ~95% of the largest Ae. aegypti scaffolds to chromosome using an F6 recombinant population. In order to determine which scaffolds map to the nonrecombining region of the X chromosome, we compared RAD tag read depth for a male and a female mosquito. Those RAD tags that are in the autosomes or the recombining region were enriched with genes having evidence of adaptive protein evolution. However, RAD tags that have twice the coverage in a female or are found only in males are in the nonrecombining region of the sex chromosomes. We used the scaffolds containing these RAD tags to explore the genetic content of the non-recombining region, gene movement on and off these proto-sex chromosomes, and sex-biased expression in and around the non-recombining region.

496A

The males of male-heterogametic species, such as mammals and drosophila, carry one X and one Y chromosome, whilst females carry two copies of the X. As Y chromosomes are highly degenerated and gene-poor, this leads to imbalances of expression in males. "Dosage compensation" mechanisms counteract this by increasing the expression of the X chromosome.
single X in males, thereby readjusting expression levels. In female-heterogametic species (ZZ/ZW), females carry the degenerated sex chromosome (the W). This should in principle also be coupled with mechanisms that increase the expression of their single Z chromosome. However, the two independently evolved ZW systems that have so far been studied in detail, birds and silkworm, lack dosage compensation. Understanding whether this is a mere coincidence or a characteristic of female-heterogametic species requires the study of other independent ZW systems, of which unfortunately none are model organisms. In this study, we have addressed this question in a non-model ZW diptera, Tephritis californica, using RNA-seq data to both map genes and estimate their expression levels. We assembled the transcriptome de novo, and took advantage of the sex-specific patterns of polymorphism of sex-linked sequences to assign genes to the Z-chromosome and to the autosomes. We could then examine their patterns of expression in males and females to test for the presence of dosage compensation. Our results are consistent with incomplete dosage compensation in this species, in agreement with what has been observed in birds and silkworm.

497B

**Evolution of complex gene structures in the 12 Drosophila genomes.** Layli W13, Stephen Schaeffer12. 1) Department of Biology, Penn State U, University Park, PA; 2) Institute of Genetics, Penn State U, University Park, PA.

*Drosophila* genome has complex gene structures, including overlapping, embedded and interdigitated genes. It is important to understand the structure of genes when annotating higher order changes to the genome such as chromosomal rearrangements. The completion of 12 *Drosophila* genomes and the well supported annotation of gene models provided a great opportunity to examine complex gene structures in *Drosophila*. This study targets complex gene structures in these species to investigate the degree of conservation and the underlying evolutionary mechanisms for gene reorganization. A total of 4696 overlapping genes were discovered in the *D. melanogaster* genome, representing more than 30% of coding genes in the genome. Transcripts with overlapping ends and genes embedded in the introns of other genes were the most common cases observed while genes where the peptide coding sequence overlapped, polysegmentic messages, and interdigitated gene clusters were detected less frequently. We used gene order information from CAF1 annotation and supplemented this information with new analyses that define 1.1 orthologs. A conserved call was made where multi-gene structures are the same among all species as opposed to a non-conserved case where genes undergo rearrangements destroying the structure. Overall, the overlapping genes are not strictly conserved among 12 Drosophila species. Conservation percentage decreases with increasing divergence time (highest 90% in *D. sechellia* and lowest 70% in *D. grimshawi*). This study provides quantitative measure of multi-gene structure conservation in *Drosophila*, which may elucidate the overlapping gene evolution, and will contribute to inferences about ancestral gene order and structure of *Drosophila* species.

498C

**Mechanisms of spliceosomal intron gain and loss: an investigation and review using 12 Drosophila species.** Paul Yenerall1, Leming Zhou1,2, Bradlee Krupa1, Mou-Chun Wang1,1. 1) Department of Biological Sciences; 2) Department of Health Information Management; 3) Department of Bioengineering; 4) Department of Computer Science; 5) School of Information Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

While it is known that spliceosomal introns may be gained or lost over the course of evolution in ortholog genes and general mechanisms have been proposed to account for these structural modifications, the specific mechanisms by which these phenomena occur has received little attention. As the known role of spliceosomal introns in eukaryotic organisms is rapidly expanding, determining the specific mechanisms by which spliceosomal introns may be gained or lost provides further insight into the evolution of function and regulation within eukaryotic organisms. Using the 12 sequenced and annotated genomes of the *Drosophila* species, we investigated spliceosomal intron gain and loss events in these species. We downloaded previously annotated coding sequences and their corresponding gene region sequences of 12 species of *Drosophila* from Flybase. We then performed a pairwise comparison of all genes against all coding sequences using the ClustalW program. From this alignment, Dollo parsimony was applied along *Drosophila*’s phylogenetic tree to categorize spliceosomal intron loss/gain events; Anopheles gambiae was used as an outlier. The use of 12 *Drosophila* species allows us to identify structural modifications made both recently (1 MYA) and distantly (40 MYA); this fine timescale provides greater resolution in the identification of specific mechanisms that produce structural modifications. The results from this investigation, as well as evidence collected from other studies, was then used to investigate the specific mechanisms of spliceosomal intron gain and loss. Funded by NSF CPATH program.

499A

**Genomic evolution of *Drosophila miranda*’s neo-sex chromosomes.** Qi Zhou, Doris Bachr.og. Department of Integrative Biology, University of California, Berkeley, Berkeley, CA.

The genome of *Drosophila miranda* is of particular interest because it possesses a pair of chromosomes that have acquired sex-biased transmission within the last 2 million years. This unique system provides opportunities to study the early evolution of sex chromosomes, in particular how and why Y-chromosomes degerenate from gene-rich autosomes into chromosomal “gene graveyards”. For this purpose, we assembled the neo-X and neo-Y chromosomes from Illumina short-reads by taking advantage of the male-specificity of the neo-Y reads, as well as the divergence between the neo-X and the neo-Y. We found that at least 30% of the neo-Y sequences lack homology with the neo-X, likely a consequence of the repression of recombination on the neo-Y. The neo-Y also shows a 2-fold enrichment of repetitive sequences compared to the neo-X, most of which is caused by an accumulation of transposable elements. Comparisons between more than 2000 homologous gene pairs demonstrate that the neo-Y alleles have significantly faster evolutionary rates at both silent and replacement sites. Moreover, 40% of the neo-Y genes are probably no longer functional because they have suffered mutations which disrupt their reading frames. Among these, metabolic genes show more evidence of degeneration than those of other biological processes. Surprisingly, we find that the neo-Y has 3 times as many genes undergoing adaptive evolution as the neo-X, though we failed to detect chromosome-wide evidence of hitchhiking based on the Ka/Ks distribution. Interestingly, most neo-Y genes under positive selection in *D. miranda* were shown to benefit males at the expense of females in *D. melanogaster*, suggesting sexual antagonism may have played an important role during the early evolution of Y chromosomes.

500B

**Review of reported Drosophila species (Diptera: Drosophilidae) in montane habitats in Colombia.** Diana Alvarez. Dept Biol, Univ Javeriana, Bogota, Bogota DC, Colombia.

There are descriptions for 3800 species in the Drosophilidae family, from that 1600 species belong to *de Drosophila* genus. The review of *Drosophila* family made by Grimald, 1990, propose that the genus is made of 15 subgenera, meanwhile Marcow and O’Grady, 2006, based on recent phylogenetic studies, propose a modification of the taxonomic relations made by Trockmorton, 1975. The most relevant changes are in the species radiations, the virilis-repleta considered the most basal. The first records from Drosophilids reported for Colombia begins in 1960. On the other hand, in Colombia there have been genetic studies on Drosophila pseudobscura bogotana, being a model for the study of population divergence at infra specific level. This is an endemic subspecies recognized such as studying 25 allozymes in Colombian populations compared to North American ones. The objective of this work is to obtain a theoretical basis for diversity of Drosophila species that are found in montane habitats (>1600 m) from Colombia based on information available for this genus. Various sources of information were reviewed to establish which species groups can be found in Colombia. The species groups with Neotropical distributions were based on the work of Val et al., 1981. From the Markow and O’Grady, 2006, work, that is a taxonomic key for the species maintained in the stock centers. Another source of information was the database of Taxodros and Flybase. At the end only species of the Drosophila genus were considered and those with altitudes higher than 1600 m. There are 35 genera of Drosophilidae in the neotropic; according to Val et al., 1981, it can deduce that in Colombia can be found 4 species of Drosophila subgenus and 23 species of Sophophora subgenus. This means that the 24% of the Neotropical species can be found in Colombia for both subgenera, being this value underestimated because the repleta species are not considered. According to Markow and O’Grady, 2006 from the 29 species groups of Drosophila genus, 15 species groups are found in the neotropic or include it.
501C The fast evolving neo-Y chromosome of Drosophila albomicans. Hwei-yu Chang1, Chia-hao Cheng1, Ching-Ho Chang2, 1) Dept Entomology, National Taiwan Univ, Taipei, Taiwan, Taiwan; 2) Department of Life Science, National Taiwan University, Taipei 106, Taiwan.

Numerous theories have specified that the originally autosomal neo-Y chromosome arm is expected to conduct a fast and degenerative evolution. The neo-sex chromosomes of Drosophila albomicans was originated from two centric fusion events, one for X and the other for Y, between ancestral Drosophila sex chromosomes and a large pair of autosomes homologous to the 3rd chromosomes of D. nasuta. Since the neo-Y chromosome arm of D. albomicans is still evolutionally young, our genetic approaches not only allowed us to examine the changes of the whole chromosome behavior but also to demonstrate its fast evolution. In this study, we first confirmed no male recombination in hybrid males of 2 sibling species, D. albomicans and D. nasuta, which is a crucial premise for estimation of non-disjunction rates by genetic approaches. By the aid of molecular marker genotyping or direct karyotyping, aneuploid offspring produced through specially designed crosses and backcrosses of the fertile hybrids, we revealed that non-disjunction rate was significantly higher in hybrid males with the neo-Y chromosome than hybrids without it. Although we could acquire neo-Y;3,X,X F1 females produced by those F1 hybrid males from the cross between D. nasuta females and D. albomicans males, we could not generate neo-Y;neo-X,Y F1 male offspring if neo-Y;3,X,X females were crossed to D. albomicans males. These results support the existence of recessive lethal allele on neo-Y chromosome that leads to no survival. Taken together, our results demonstrated that the neo-Y chromosomes are differentiated judging from their increased non-disjunction rate in hybrids as well as the existence of recessive lethal allele. Therefore, we may infer that the neo-Y evolves faster than neo-X.


We know relatively little about the genetic basis of complex adaptations. Still less is known about the genetics underlying behavior. By coupling a candidate gene approach with a modified genome-wide bulk segregant analysis we link an ecological behavioral adaptation to constituent genetic loci. D. sechellia is a hyperspecialist on the tropical fruit of Morinda citrifolia, Sister species, including D. melanogaster, are repulsed by Morinda fruit and its volatile organic acids. We show that relatively few loci contribute to the aversion of this fruit, with Smi35a standing out as a strong candidate. We also show that while D. sechellia uses olfaction to find Morinda host, a complex interaction between taste and smell ultimately determines where flies will settle and oviposit.


In order to examine mRNA expression responses to single-copy deletions, males and females from three DrosDel stocks (Df(2L)ED7007/+, Df(2L)ED489/+, Df(2L)ED499/+), and smell ultimately determines where flies will settle and oviposit.

504C Gene expression responses to single-copy deletions in Drosophila. Renhua Li, John H. Malone, Nicolas Mattiuzzo, Brian Oliver. Developmental Genomics Section, LCDB, NIDDK, National Institutes of Health, Bethesda, MD 20892.

In order to examine mRNA expression responses to single-copy deletions, males and females from three DrosDel stocks (Df(2L)ED7007/+, Df(2L)ED489/+, Df(2L)ED499/+), and corresponding controls were in-depth sequenced on the Illumina GA II RNA-Seq platform. We generated from 11 to 16 million total reads for each of the mRNA libraries, with 6 to 11 million reads uniquely mapped to the genome of D. melanogaster (Berkeley Drosophila Genome Project R5 Flybase). Using the statistical methods of mutual information and entropy, we were able to define the dependence between expression changes of the gene sets both inside and outside a deletion region. We refer to this information and entropy, we were able to define the dependence between expression changes of the gene sets both inside and outside a deletion region. We refer to this phenomenon as inside-outside dependence (IOD). We identified significant IOD for males and females of these deletion lines, as determined by permutation tests. In addition, the adjustment for the IOD, the fold changes reduced to magnitudes that are less than 0.5 (0.09-0.21), indicating dosage compensation in single-copy deletions.

505A The evolution of foraging behaviour and social interaction in larvae of the genus Drosophila. Christen K. Mirth1,2, Hans C.P. Kelstrup2, Lynn Riddiford2. 1) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 2) Janelle Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA, U.S.A.

Through developmental mechanisms, the environment and evolution act to generate a vast diversity of foraging strategies in insects. We have conducted a survey of larval foraging behaviour to begin to address how behavioural suites evolve to produce differences in larval foraging strategies between species of the genus Drosophila. We have surveyed larval foraging behaviour using a collection of 59 species from 10 species groups of Drosophila on a simple yeast-sucrose-agar medium. In our analysis, we characterize the degree to which individuals associate with one another when burrowing (degree of social burrowing) and the array of behaviours observed when larvae come into contact on the surface of the food. These factors significantly vary among species groups as well as within a species group and allow us to generate behavioural profiles for each species. Behavioural profiles generated from this survey will be used to identify pairs of closely related species divergent in their foraging behaviour, with the aim of identifying genomic sites responsible for the evolution of foraging strategies.

506B Preliminary genomic analysis of a DNA virus of Drosophila. Robert Unckless. Department of Biology, University of Rochester, Rochester, NY.

During a viral metagenomic screen in Drosophila innubila I discovered a DNA virus with sequence homology to a nudivirus (Oryctes rhinoceros virus). Subsequent analysis confirmed no male recombination in hybrid males of 2 sibling species, D. albomicans and D. nasuta, which is a crucial premise for estimation of non-disjunction rates by genetic approaches. By the aid of molecular marker genotyping or direct karyotyping, aneuploid offspring produced through specially designed crosses and backcrosses of the fertile hybrids, we revealed that non-disjunction rate was significantly higher in hybrid males with the neo-Y chromosome than hybrids without it. Although we could acquire neo-Y;3,X,X F1 females produced by those F1 hybrid males from the cross between D. nasuta females and D. albomicans males, we could not generate neo-Y;neo-X,Y F1 male offspring if neo-Y;3,X,X females were crossed to D. albomicans males. These results support the existence of recessive lethal allele on neo-Y chromosome that leads to no survival. Taken together, our results demonstrated that the neo-Y chromosomes are differentiated judging from their increased non-disjunction rate in hybrids as well as the existence of recessive lethal allele. Therefore, we may infer that the neo-Y evolves faster than neo-X.

507C Experimental selection of hypoxia-tolerant Drosophila melanogaster: Major role for Notch activation. Dan Zhou1, Nitin Udpa2, Merril Gersten3, DeeAnn Visk1,2, Ali Bashir1, Jin Xue1, Kelly Frazer1,2, James Posakony1,2, Shankar Subramaniam2,3, Viness Bafna1,2, Gabriel Haddad1,6,7. 1) Dept Pediatrics, Univ California, San Diego, La Jolla, CA; 2) Bioinformatics & Systems Biology Graduate Program, Univ California, San Diego, La Jolla, CA; 3) Division of Biological Sciences, Section of Cell and Developmental Biology, Univ California, San Diego, La Jolla, CA; 4) Department of Bioengineering, Univ California, San Diego, La Jolla, CA; 5) Department of Computer Science and Engineering, Univ California, San Diego, La Jolla, CA; 6) Department of Neuroscience, Univ California, San Diego, La Jolla, CA; 7) Rady Children's Hospital, San Diego, CA.

Through long-term laboratory selection (over 200 generations), we have generated Drosophila melanogaster populations that tolerate normally lethal level of hypoxia. Because of initial experiments suspecting genetic mechanisms underlying this adaptation, we compared the genomes of the hypoxia-selected flies with controls using deep re-sequencing.
By applying novel computing and analytical methods we identified a number of DNA regions under selection, mostly on the X-chromosome. Several of the hypoxia-selected regions contained genes encoding or regulating the Notch pathway. In addition, expression profiling revealed an activation of the Notch pathway in the hypoxia-selected flies. We confirmed the contribution of Notch activation to hypoxia tolerance using a specific γ-secretase inhibitor, DAPT, which significantly reduced adult survival and lifespan in the hypoxia-selected flies. We also demonstrated that flies with loss-of-function Notch mutations or RNAi-mediated Notch knockdown had a significant reduction in hypoxia tolerance, but those with a gain-of-function had a dramatic opposite effect. Using the UAS-Gal4 system, we also showed that specific over-expression of the Notch intracellular domain in glial cells was critical for conferring hypoxia tolerance in flies. Novel analytical tools, genetic and bioinformatic strategies allowed us to discover that Notch activation plays a major and unsuspected role in this hypoxia tolerance in Drosophila melanogaster.

508A
Evolution of Nora Virus Sequence Between Swedish and United States Populations of Drosophila melanogaster. Darby Carlson1, Ethan Cordes1, Benjamin Klein1,2, Camilla Stoner1, Brad Ericson1, Dawn Simon1, Kimberly Carlson1. 1) Biology Department, University of Nebraska at Kearney, Kearney, NE; 2) Lexington High School, Lexington, NE 68850.

Nora Virus is a picorna-like virus originally found in Swedish Drosophila melanogaster strains. It is unlike most other picorna-like viruses in that it has four open reading frames (ORFs), in contrast to one long ORF found in traditional picornaviruses. Previous research in our lab demonstrated that Nora Virus is found in United States D. melanogaster strains and may affect longevity. Based on this observation, we hypothesized that there are differences in sequence between the Nora Virus in the U.S. and Sweden. To test this hypothesis, the four ORFs were amplified using sequence specific primers from RNA extracted from one Swedish population and two U.S. populations of D. melanogaster via reverse transcription polymerase chain reaction (RT-PCR). The PCR products were cloned into a TOPO vector, verified with restriction enzyme digests, and sequenced. The sequences were confirmed to be Nora Virus by BLAST searching, aligned, and phylogenetic analyses performed. The results show that the ORFs from the U.S. populations are more closely related to each other than to the Swedish population. The results of this study demonstrate strain specific differences of Nora Virus sequences, which may alter their ability to affect longevity in D. melanogaster. The project described was supported by the NIH grant number P20 RR016469 from the INBRE Program of the National Center for Research Resources.

509B

Errantiviruses are insect analogues of vertebrate retroviruses. Because retroviruses have high utility for genetic transformation, errantiviruses may have a similar potential for genetic modification of pest insects such as mosquitoes. Errantiviruses may have special utility in transforming insects for which laboratory raising is infeasible, given that the prototype errantivirus, gypsy, is capable of germline integration after simple feeding of larvae. To choose suitable viruses for investigation, a catalog of intact errantiviral sequences in dipteran genomes (those of mosquitoes and flies) was constructed and the phylogenetic relationship among these viruses was determined. These intact proviral sequences represent ‘fossil relics’; of recent infections that have not experienced inactivating mutational drift and, in many cases, might be used to re-express the virus. This search of dipteran genomic sequences has identified 61 distinct errantiviral proviruses in addition to 17, mostly from Drosophila melanogaster, that had been previously characterized. Half of these newly identified errantiviral sequences come from the Aedes aegypti genome. These 78 errantiviruses divide phylogenetically into 3 major classes, one of which is restricted to mosquitoes, one restricted to Drosophila species and one with sublineages in both flies and mosquitoes. Notably, all errantiviral sequences identified in the An. gambiae and Cs. quinquefasciatus genomes have a close relative in the Ae. aegypti genome. This suggests that these viruses may have a broad host range and may readily infect diverse mosquitoes. Given that endogenous retrotransposon expression in Drosophila is suppressed by siRNA generated from pericentromeric homologous insertions, use of heterologous cross-species errantiviruses, similar but distinct from endogenous ones, may be the most suitable for development as genetic transformation vectors.

510C
Genome-wide analysis of a long-term evolution experiment with Drosophila. Molly K. Burke1, Joseph P. Dunham2, Parvin Shahrastani1, Kevin R. Thornton1, Michael R. Rose1, Anthony D. Long1. 1) Department of Ecology and Evolution, University of California Irvine, Irvine, CA 92697-2525; 2) Molecular and Computational Biology, University of Southern California, Los Angeles, CA 90098.

Experimental evolution systems allow the genomic study of adaptation, and so far this has been done primarily in asexuual systems with small genomes, such as bacteria and yeast. Here we present whole-genome resequencing data from Drosophila melanogaster populations that have experienced over 600 generations of laboratory selection for accelerated development. Flies in these selected populations develop from egg to adult 20% faster than flies of ancestral control populations, and have evolved a number of other correlated phenotypes. On the basis of 688,520 intermediate-frequency, high-quality single nucleotide polymorphisms, we identify several dozen genomic regions that show strong allele frequency differentiation between a pooled sample of five replicate populations selected for accelerated development and pooled controls. On the basis of resequencing data from a single replicate population with accelerated development, as well as single nucleotide polymorphism data from individual flies from each replicate population, we infer little allele frequency differentiation between replicate populations within a selection treatment. Signatures of selection are qualitatively different than what has been observed in asexual species; in our sexual populations, adaptation is not associated with ‘classic’ sweeps whereby newly arising, unconditionally advantageous mutations become fixed. More parsimonious explanations include ‘incomplete’ sweep models, in which mutations have not had enough time to fix, and ‘soft’ sweep models, in which selection acts on pre-existing, common genetic variants. We conclude that, at least for life history characters such as development time, unconditionally advantageous alleles rarely arise, are associated with small net fitness gains or cannot fix because selection coefficients change over time.

511A
Genetic variation and divergence within and between two Drosophila melanogaster populations revealed by whole genome sequencing. Daniel Campo, Courtney Fjeldsted, Tade Souaiaia, Joyce Kao, Sergey Nuzhdin. University of California, Southern California, Los Angeles, CA.

With the advent of whole-genome resequencing methodologies in recent years there has been a shift in population genetic studies perspective, especially in model organisms such as the fruit fly Drosophila melanogaster. Thus, simultaneous analyses of genetic variation across the whole genome, rather than in a few gene sequences, provides further insights into the evolutionary processes shaping diversity within and between populations. In this work we have obtained whole genome sequences of 35 inbred D. melanogaster lines derived from a natural population in Winters (CA) using an Illumina Genome Analyzer IIx, and compared them with a set of 35 inbred lines from Raleigh (NC) for which whole genome consensus sequences are available (http://www.dpgp.org/). We describe patterns of genetic variation within these two populations as well as levels of polymorphism and divergence between them, looking for traces of selection and other evolutionary forces.

512B
Patterns of Directional Symmetry Between and Within Species in the Genus Drosophila. Ashley Carter. California State University Long Beach, Long Beach, CA.

Directional Symmetry (DA), a consistent directional difference in the size or shape of paired structures, is a paradoxical trait in it does not respond to artificial selection in the laboratory (indicating a lack of genetic variation for DA between individuals of the same population) yet is known to evolve in nature (indicating the presence of genetic variation for DA between species). Knowledge of the level of divergence at which genetic variation for DA arises is fundamental for understanding more about the evolvability of DA and processes responsible for it. At the species level, differences in wing size DA have been reported in insects, but few studies have examined differences in DA between populations within the same species. We examined the patterns of wing size DA in multiple populations from multiple species of Drosophila to address this question. Results indicate a general bias in the direction reported for other insects as well as differences between the populations examined, indicating genetic variation for DA at the level of populations within the
Natural variation in commensal bacteria regulation across DGRP lines. Angela Early*, Andrew Clark**. 1) Field of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY; 2) Dept. of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Drosophila are in constant contact with environmental bacteria, a small proportion of which establishes permanent populations in the gut. Recent research has shown that the gut-specific immune response recognizes the presence of these bacteria, but negative feedback mechanisms prevent a strong, constant immune response. This attenuation of the immune response prevents harm caused by the over-production of anti-microbial peptides (AMPs) and reactive oxygen species (ROS), but potentially leaves the gut more vulnerable to pathogen attack. Using 39 inbred lines from the Drosophila Genetic Reference Panel, we have manipulated the flies gut microbiota by first rendering them axenic, and then re-establishing single nonpathogenic bacterium populations of our own choosing. We then used qPCR to quantify bacterial growth in each line-bacterium combination and found significant among line variation in the steady-state bacterial population size. Using the genetic resources available for these lines, we have also identified SNPs in immune-related genes that may play a causal role in the observed differences in bacterial load. The results show that there is considerable segregating variation in immune-related genes that modulate the control of commensal bacteria, suggesting that there is no simple evolutionary optimum for this trait.

Polymorphisms in chromatin accessibility state within D.melanogaster. Aaron Hardin*, Xiao-Yong Li*, Michael B. Eisen**. 1) Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA; 2) California Institute of Quantitative Biology, University of California, Berkeley, Berkeley, CA; 3) Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, CA.

Previous work in the Eisen lab has shown, using ChiP-Seq, that there is extensive quantitative variation in the binding of the factors that control anterior-posterior patterning in the early embryo (BCD, HB, KR, GT, CAD and KNI) between D. melanogaster and D. yakuba. While collectively these data demonstrate that the gain and loss of binding sites for these factors play a significant role in their binding divergence, the extent of difference between these species precludes determining the effects of individual sequence changes. Ideally we would examine binding variation within D. melanogaster, but it is unknown how much variation exists and whether individual ChiP experiments would be cost effective. We have now examined chromatin state in two natural isolates of D. melanogaster by measuring genome wide DNAase-hypersensitivity (DNAase-Seq) the early embryo, and are piloting the use of pooled samples from many isolates as an efficient means of identifying regions harboring polymorphisms that affect transcription factor binding.


Y chromosomes of Drosophila contain multi-megabase stretches of satellite DNA repeats and a handful of protein-coding genes that are monomorphic within species. Nevertheless, polymorphic variation in heterochromatin Y chromosomes of Drosophila result in genome-wide gene expression variation. Here we show that such naturally occurring Y-linked regulatory variation (YRV) contributes to the epigenetic balance of heterochromatin chromosomes at three distinct loci showing position-effect variegation (PEV). Moreover, polymorphic Y chromosomes differentially affect global gene expression in XXX female genotypes in which Y-linked protein-coding genes are not transcribed. The affected genes include major components of chromatin and DNA-binding proteins. Furthermore, we show that epistatic interactions between Y-linked regulatory polymorphisms and genetic background affect global gene expression. Accordingly, using both natural variation and loss of function mutants in chromatin components, we show extensive background-by-Y-chromosome interactions affecting global gene expression. Taken together, our results indicate that Y chromosome heterochromatin serves as a major source of epigenetic variation in natural populations that interacts with variable genetic backgrounds to modulate global expression.

Geographic variation of cold and desiccation tolerance in the red flour beetle, Tribolium castaneum. David M Linz, Benjamin N Philip, Sindhu Samba, Yoshinori Tomoyasu, Richard E Lee Jr. Zoology Department, Miami University, Oxford, OH.

A species that is widely distributed often displays impressive variation in a certain trait, corresponding to the selective pressures unique to each specific location. This study examines the effects of different geographic locations on the adaptation to cold and desiccation. Tribolium castaneum, the red flour beetle, is a tenebrionid beetle that is distributed globally in stored grain products. These beetles have the ability to withstand subzero exposure, however, as a freeze avoiding insect, their cold hardiness is fairly limited. T. castaneum also has the ability to withstand extreme desiccation stress, a trait that likely provides protection against their dry habitat. We analyzed different strains of T. castaneum from varying geographic locations, and found that there are significant differences in the ability to tolerate cold and desiccation stresses among these strains. These results will serve as the foundation for future comparative studies highlighting the importance of different alleles and genes among intraspecific populations.

Genitalic shape variation in the Drosophila mojavensis species cluster. Maxi Polihronakis Richmond, Therese Markow. Cell and Developmental Biology, University of California, San Diego, La Jolla, CA.

Insect genitalia are considered to be one of the most rapidly evolving morphological characters among diverging populations and closely related species. In the current study we assessed levels of genitalic variation within the Drosophila mojavensis species cluster using geometric morphometrics. This system is ideal for such studies due to the opportunity to test and compare levels of variation along a divergence continuum at various taxonomic levels within the group. Shape variation was quantified using Elliptic Fourier Descriptors and compared among the four D. mojavensis host races; between D. mojavensis and its sister species D. arizonae; as well as among these two species and their sister
taxon, D. navojoa. A discriminant function analysis revealed varying levels of discrete differences for each comparison relative to intra-population and intra-specific variation. These results correspond to the degree of genetic divergence and reproductive isolation previously documented for this important model system.


The availability of ~40 sequenced genomes of Drosophila melanogaster opens new windows into its recent adaptive history. Genomic windows of reduced diversity may indicate recent selective sweeps. Examining the locations of these “diversity valleys” led to several intriguing findings. First, a disproportionate number of these valleys were centered on 5' and 3' untranslated regions. Second, gene ontology (GO) analysis of genes putatively affected by selective sweeps revealed a number of biological processes that may have been important in the recent adaptive history of an African population sample (including neuron development, chromatin silencing, and mRNA splicing), but largely a different set of biological processes were implicated in a North American sample (including terms related to growth, metabolism, and immunity). Third, selection signals shared between D. melanogaster and its sibling species D. simulans were vastly over-represented in data, potentially indicating genomic “hotspots” of recurrent positive selection.

Comparison of genomic polymorphism data against neutral demographic predictions (based on historical models estimated from this data, and from previous studies) offered a number of striking contrasts. Examining the distribution of FST (genetic differentiation between the African and North American samples), the X chromosome displayed a notable excess of high and low outliers relative to neutral expectations. The X chromosome also showed a disproportionate diversity reduction in the North American sample, beyond that predicted by demographic models. Thus, selection appears to have influenced variation more strongly on the X chromosome. The empirical data also differed from neutral predictions in terms of the spatial decay of linkage disequilibrium. Demographic models were unable to account for the high levels of linkage disequilibrium observed at both short (~100 bp) and longer (5 kb) scales. These genomewide departures from neutral evolution suggest that recent hitchhiking has influenced a large portion of the D. melanogaster genome.

520A Phenotypic variation of blastoderm embryos in natural populations of Drosophila melanogaster. Sarah S Saminadin-Peter, Angela DePace, Zeba Wunderlich, Arnaud Gelas, Meghan Bragdon, Kelly Eckenrode. System Biology, Harvard Medical School, Boston, MA.

Differential gene regulation plays a critical role in development and the generation of phenotypic variation between species. The segmentation network is the conserved developmental patterning system that patterns blastoderm embryo. We are interested in how this system may vary within Drosophila melanogaster natural populations. We also aim to understand how it compares to the amount of variation observed between Drosophila species. We have chosen spatiotemporal gene expression patterns as the phenotype. Though not yet clear how quantitative variation in gene expression at this stage of development will lead to selectable organismal phenotypes, we can measure gene expression in 3D at cellular resolution, making it possible to capture even the subtle variation we expect. Morphological differences in embryo size and shape are known to exist between Drosophila species and within populations. This variation in shape can convolute comparisons of gene expression patterns in the different morphological contexts. Importantly, our methods allow us to both characterize embryo morphology, including shape, nuclear number and density patterns, and to compare gene expression patterns either within this morphological framework, or independent of it on a cell by cell basis. We have measured embryo morphology and gene expression patterns for a small subset of five genes in natural populations of Drosophila melanogaster, isolated from North Carolina, Africa, Europe and Asia. We found significant morphological variation as well as anterior-posterior shifts in gene expression patterns between populations. Here, we present these data and compare them to similar datasets for closely related species. These results might have implications for understanding the amount of variability that can be tolerated while retaining function, and more broadly, for how gene expression variation can be exploited to generate new phenotypes.


Laboratory selection experiments provide an excellent opportunity to study the genetic architecture of adaptation, as replicate experiments distinguish between stochastic effects (drift) and directional forces (selection). Starting from more than 100 freshly collected D. melanogaster isofemale lines we exposed three replica cultures of 1000 individuals to temperature regime fluctuating between 18 and 28°C. DNA of pooled individuals was sequenced at the onset of the experiment, after 15 generations (replicate 1 & 2), 23 generations (replicate 3) and after 28 generations. We find more than 300 genomewide regions deviating from neutral expectations. Notably, only very narrow windows were affected by selection in our experiments, showing that laboratory selection experiments are an excellent tool to map selected alleles.


Drosophila mojavensis is a cactophilic fly endemic to the deserts of North America. This species is composed of four host races, each utilizing a different cactus species with distinct chemical profiles. Its sister species, D. arizonae, is a cactus generalist with a broader distribution. These species have adapted to the stress of developing and feeding in necrotic cactus tissues that include a number of toxic compounds. Prior work has illustrated the genetic, transcriptional and functional changes associated with host utilization at various detoxification genes. Although cacti are mostly prevalent in the desert environment in which these species reside, the frequency of necrotic cacti is significantly lower.

Therefore, in addition to being able to detoxify its host, cactophilic Drosophila must also be able to locate appropriate oviposition and feeding sites. In this study we focus on the genetic changes at three odorant receptor genes (Or83c1, Or83c2 and Or67c) in D. mojavensis and D. arizonae. We have observed significant amino acid changes across the four D. mojavensis host races as well as between the species. For example, Or83c2 appears to have experienced positive selection in the lineage leading to D. arizonae, while within D. mojavensis, certain regions of this receptor have accumulated changes between host races suggesting local adaptation.

523A Comparative analysis of intraspecific mtDNA polymorphism of four species of Drosophila virilis group. Svetlana Y. Sorekina1, Boris V. Andriano2, Anna I. Chekunova1, Vladimir G. Mitrofanov1, 1 Koltsov Inst Dev Biology, Moscow, Russian Federation; 2 Vavilov Inst Gen Genet, Moscow, Russian Federation.

The Drosophila virilis species group is a monophyletic clade of recently divided sibling species. The group has been extensively used as a model system in studies of mechanisms of evolution and speciation. We analysed intraspecific mtDNA Hinf1 RFLP and COI polymorphism of four species of Drosophila virilis group. Our results show that populations of palaeartic species D.littoralis are differentiated to Southern and Northern groups of populations. Southern populations are genetically more variable than Northern ones. Divergence rate between mt-haplotypes of these two groups of populations is close to interspecific level. Network of statistical parsimony constructed on RFLP and sequence data shows that Southern cluster of mt-haplotypes is closer to species ancestor. There is a zone of secondary contact of Southern and Northern populations at the Black Sea coast of Krasnodar area. The nearctic species D.americana shows slightly different pattern of mtDNA polymorphism. Genetic differentiation index (D) and Shannon index of genetic diversity (H) show that D.americana populations are not differentiated but more variable than D.littoralis ones that points on larger affective size of D.americauna founder population as well as on “botleneck” in phylogeographic history of D.littoralis followed by expansion of northern part of D.littoralis present-day area. D.virilis mtDNA shows extremely low level of intraspecific polymorphism. We find only one different Hinf1 site in mtDNA of strain from Seychelles. D.virilis is only sinantropic species of the virilis group. We suppose that modern leenage of D.virilis arises recently from small monomorphic natural population of D.virilis. Populations of D.montana according to previous data
are differentiated to American and European. We show that Baikal populations, that weren't studied earlier, are closer to European than to American and Japanese ones. The study was supported by Russian State grant "Gene pools and Genetic Diversity".

524B
P element prevalence in Central Mexico Drosophila species, Aldo A Téllez-García, Juan R. Riesgo Escovar. Depto. de Neurobiología del Desarrollo, Instituto de Neurobiologia, UNAM, Querétaro, Querétaro, México.

P elements are widespread transposable elements present in several species of the Drosophilidae family. Because the presence of P elements in Drosophilidae is not necessarily congruent with the phylogeny of the family, horizontaltransfer has been proposed as a mechanism for evolution of these genes. However, research of P elements has been limited to a few species of the family considering the great diversity that it comprises. In an attempt to conduct a survey of the Drosophila species occurring in the center of Mexico. We have found seventeen species at six localities. We have also found Zaprionus indistans, a recent invasive species in the American continent. In order to analyze for the presence of P element sequences we employ both Southern blot analysis and PCR amplification from captured flies.

525C
Climatic stress adaptations in Drosophila melanogaster along a latitudinal transect: Analysis of genetic and plastic effects. Dau Dayal Aggarwal1, Rav Parkash1, Bhawna Kalra2. 1) Department of Genetics, M.D. University, Rohtak124001, India; 2) Department of Biology, University of Haifa- Oranim, Tivon 36006,Israel.

In the Indian subcontinent, northern montane are cold and dry while southern montane are humid and hot. Our investigation of northern and southern montane populations (600-2202m) differing significantly in latitude (10.8-30.03°N) have shown variation in melanisation and several stress related traits such as desiccation, heat and cold tolerance in wild as well as laboratory grown (14 - 31°C) populations of Drosophila melanogaster across seven populations and seasons. Temperatures have significant effect on mean trait values and genetic variability for body melanisation and other ecophysiological traits. In the northern montane due to variation in temperature, seasonal changes were observed that leads to plasticity for body melanisation of three posterior abdominal segments (5h + 6h + 7h) that corresponded with higher desiccation and cold while lower heat tolerance in winter season. While, due to lack of seasonal variation there was no plasticity in southern localities for three posterior abdominal segments (5h + 6h + 7h) and non significant differences for stress tolerance were observed across seasons. Further, due to plasticity of segments (5h + 6h + 7h) at low growth temperatures (14-17°C), there was higher melanisation, cold and desiccation tolerance and reverse occurred at higher growth temperature (28-31°C). However, when all the populations were grown at single growth temperature significant positive cline for body melanisation, desiccation and cold resistance and negative for heat stress was observed along latitude. Thus, we analyzed genetic and plastic effects for these ecophysiological traits by comparing wild-caught and laboratory reared individuals of D. melanogaster across seasons and populations.

Thus, it is concluding that melanisation and stress related traits either evolve convergently through the course of evolution or directly correlated with each other.

526A
From QTL to neuron: mapping natural variation in Drosophila fecundity to an underlying mechanism. Alan O. Bergland1,2, Marc Tataren. 1) Dept. of Biology, Stanford University, Stanford, CA; 2) Dept. of Ecology and Evolutionary Biology, Brown University, Providence, RI.

A major goal of contemporary evolutionary biology is to identify the molecular and physiological basis of natural variation in fitness related traits. By integrating information about the underlying pathways influencing trait variation with the molecular evolution of such pathways, we will gain deeper insights into the evolutionary process. Our research aims identify the molecular and physiological basis of natural variation in female fecundity, a core fitness trait of many organisms such as Drosophila melanogaster. We first document natural genetic variation in female fecundity in flies derived from a wild orchard population (Winters, CA). Next, we map this variation to a single QTL and using fine scale mapping techniques narrow this QTL down to 5 candidate genes. Ubiquitous over expression of RNAi against only one of these genes, Drip, reduces fecundity. Within our mapping population Drip mRNA and protein levels in the head, but not other tissue types, are positively correlated with fecundity. Finally, over expression of Drip-RNAi using the pan-neuronal ELAV-GAL4 driver reduces fecundity. Taken together these data strongly suggest that natural variation in Drip expression in the brain affects fecundity. We then characterize Drip expression patterns in the brain. Drip is expressed in small population of neurons located in the dorsal-posterior part of the brain. We shall discuss the function of those neurons and the evolutionary history of the Drip locus.

527B

In Drosophila, male seminal proteins can drastically affect the female’s physiology and behavior, including altering the female remating rate, egg laying rates, and sperm storage dynamics. Little is known about which biological pathways mediate these important post-mating changes in the female. As a way to identify genes critical for mediating female post-mating responses, we screened for differences in female sperm use (storage) patterns in a standard sperm competition paradigm. Each female is sequentially mated with males from two standard laboratory stocks. Female sperm use is defined as the proportion of offsprings each male sires. To characterize the natural variation in this critical reproductive phenotype, we have assayed sperm use in a set of 39 lines from the Drosophila Genetic Reference Panel (DGRP). To identify female genes responsible for this variation, we perform a full genome-wide association study, using the genome sequences of the DGRP lines. Surprisingly, genome-wide significance is attained by SNPs with high allele frequencies (PARA, SK, SHAB) and other genes with roles in the nervous system (CAUP, RAB2, RIM). Our association study results indicate that synaptic transmission and neurological control of behavior and muscle contractions may play an important role in female sperm use patterns, perhaps more important than processes limited to the female reproductive tract. Results from functional experiments testing RNAi knockdown of several of the top candidate genes will be also presented. The nervous system may have a previously unappreciated role in female sperm use. More importantly, study of female post-mating responses should not be limited to female-reproductive-tract-specific genes and pathways and more focus should be placed on diverse biological pathways.

528C
A genome wide association study to characterize natural genetic variation in mated lifespan, age-specific reproduction and their plastic response to dietary restriction. Mary F. Durham, Jeff Leips. Biological Sciences Dept, UMBC, Baltimore, MD.

Dietary restriction has been shown to affect longevity in a variety of organisms ranging from yeast to mice to primates. The mechanism driving this pattern and the genes involved in the response are largely unknown. Variation in resource quality and quantity is common in natural populations, and adjustment of energy allocation to different traits in response to such variation is likely to have important effects on fitness. In this study, after rearing eggs and larvae on a standard sucrose/cornmeal/agar diet, we transferred newly emerged virgin female flies on sucrose/agar based medium with either 20% yeast (regular diet) or 5% yeast (restricted diet) concentration. We quantified lifespan and age specific fecundity on each dietary regime using 184 lines of the Drosophila Genome Reference Panel (DGRP) lines derived from the natural population in Raleigh, NC. We then calculated plasticity scores for each trait by comparing the trait value for each line in a restricted diet versus a regular diet. Our results indicate that there is extensive natural genetic variation in lifespan, age-specific reproduction and the plastic response of these traits to dietary restriction, as well as genotype by environment interaction regarding dietary regime. A genome wide association mapping test is underway to identify candidate genes influencing these traits and their plastic response to dietary restriction. This will help us shed light on the genetic mechanisms that drive the dietary restriction response of these traits and elucidate shared genetic pathways affecting them. In addition, this work will explore the extent to which genes that contribute to natural variation in these traits are the same as those that influence their plastic response to the two dietary regimes.

Group size is a highly variable social trait among animals, but little is known about the genetic basis for this emergent behavior, or how such a behavior might evolve. Here we use differences in pupation site selection as a model for the evolution of aggregation. D. simulans and D. sechellia are sister species that diverged roughly 300,000 years ago. We used field experiments to show that D. sechellia larvae do not wander from their larval host fruit. In contrast, D. simulans larvae pteroparate either on fruit or in the surrounding area. We have devised a laboratory assay to test the tendency of larvae to disperse from the larval food source. We show that while all strains of D. sechellia aggregate on the larval substrate, the behavior of D. simulans larvae varies by geographic location. D. simulans larvae from sub-Saharan Africa form pupal aggregations on the larval media like D. sechellia does. However, D. simulans larvae from the Mediterranean or the New World scatter from the larval substrate, with strains from California showing the greatest extent of dispersal. These data suggest that dispersion of wandering larvae is a trait that was acquired as D. simulans migrated from Africa, through Europe to invade North America. Crosses between D. sechellia and D. simulans produce fertile females, and we have employed a back-cross strategy and QTL mapping to identify loci that evolved to produce the scattered phenotype in D. simulans. To facilitate our genotyping, we have used 'multiplexed shotgun genotyping' to economically genotype large numbers of individuals at high resolution. First, back-crosses to either the D. sechellia or D. simulans parents indicate a 2 MB region on the X chromosome that contributes to pupation site choice. To determine if the variation in pupal aggregation within D. simulans occurs through the same QTL, we are also conducting QTL analysis on F2 progeny from crosses between Californian and sub-Saharan D. simulans lines. We are currently mapping this trait more finely.

Quantitative genetics of the metabolic regulatory network. Anthony J. Greenberg1, Sean R. Hackett1, Lawrence Harshman2, Andrew G. Clark1. 1) Dept Molec Biol & Gen, Cornell Univ, Ithaca, NY; 2) School of Biological Sciences, Univ of Nebraska, Lincoln, NE.

Progress in systems biology depends on the accurate description of the relevant networks. Discovery of regulatory networks typically proceeds by conducting measurements (e.g., of mRNA abundance) in a variety of conditions. Genes that are co-regulated are said to share connections in a regulatory network. Co-regulation is quantified using a variant of the correlation coefficient, while perturbations are usually a set of environmental conditions of growth or a number of targeted or naturally-occurring mutations. Correlations are arranged in a matrix, which summarizes the degree of dependence among parameters and is used to describe the network. If we want to use network information to predict system behavior, it is important to know if the nature of perturbations affects topologies of networks they reveal. We set out to probe this question by focusing on core metabolism of D. melanogaster as a model. Our source of perturbations is a set of 92 wild-derived inbred lines from five populations, crossed in a sparse parallel diallel design. We replicated these crosses in a manner that permits assessment of the effects of uncontrolled environmental fluctuation as well as the genetic variation contributed by the mutations segregating in our lines. We implemented a systems biology multivariate Bayesian approach to analyze the data. Using this model, we assessed variability of and relationships among metabolic parameters. We found extensive variation in enzyme activities, although adult weight seems buffered against such changes. Furthermore, although both the environmental and genetic correlation matrices remain substantially the same among the five populations we sampled, genetic and environmental perturbations reveal qualitatively different metabolic networks. Our results suggest that environmental shifts, e.g., drug treatments, will have different systemic effects than genetic changes, e.g., disease-causing mutations, even if the primary targets are the same.

Estimating complete genotype data from a collection of dense semi-codominant SNP markers in the Drosophila Synthetic Population Resource (DSPR). Elizabeth G. King1, Stuart J Macdonald2, Anthony D Long1. 1) Department of Ecology & Evolutionary Biology, UC Irvine, Irvine, CA; 2) Department of Molecular Biosciences, University of Kansas, Lawrence, KS.

Recombinant inbred lines (RILs) derived from multiple founder lines have the potential to serve as a powerful method for genetic mapping of complex traits. Recently, a new, large panel of RILs was created called the Drosophila Synthetic Population Resource (DSPR). The DSPR was created by crossing two sets of eight inbred founder lines (with one founder line in common) to create two synthetic populations. These synthetic populations underwent 50 generations of recombination after which two sets of 750 RILs were generated. This design results in a set of RILs whose genomes are a fine-scale mosaic of segments from the founder lines. Each RIL in the DSPR is currently being genotyped for ~10,000 semi-codominant SNP markers via sequencing of restriction-site associated DNA (RAD) tags, in addition the 15 founder lines have been completely resequenced to ~50X Illumina PE54. Successful QTL mapping relies on the amount of genotype data available; however, genotype data between genetic markers are missing. We aim to uncover these missing data by discerning the founder genotypes present for each genomic region in each RIL. We developed a hidden Markov model that relies on a large set of SNP markers typed in both the founders and the set of RILs to resolve the founder ancestry of each genetic marker in each RIL. This model allows us to estimate a genotype probability matrix containing the probabilities that any given position in the genome was inherited from each of the eight founders for each RIL. We demonstrate how this information is incorporated into the analytical routines for high-resolution QTL mapping in the DSPR using simulated phenotypes.


Chemotherapy treatment is one of the most toxic medical treatments administered to humans. Highly individual specific toxic side effects often force a cancer patient to change or stop this treatment. We have developed high-throughput protocols for exposing adult female Drosophila to chemotherapy drugs. We observe that the toxic side effect of drugs on female fecundity is often highly heritable. For example, for the five drugs we have most extensively studied: Methotrexate - h²=99%, Gemcitabine hydrochloride - 72%, Carboplatin - 72%, Mitomycin C - 64%, and Fluorouridine - 52%. We are currently using crosses between recombinant inbred lines (RILs) of D. melanogaster to map toxicity QTL and eventually identify the actual SNPs underlying the genetic component of inter-individual variation in toxicity. We have an "A" and a "B" set of RILs, each derived from 8 founders. At 50 generations, the RILs are genetically distinct from the 8 founders. Thus our RILs are ultimately a mosaic of fragments of derived for 1 of 8 completely re-sequenced founders (with the A & B collections only having a single founder in common). Crosses pairs of A & B RILs produce trans-heterozygote outbred offspring on which the phenotype is measured, and multiple flies are phenotyped from each cross to map QTL. In order to map to a high enough level of resolution to identify single genes and causative SNPs contributing to toxicity, we plan to score toxicity phenotype for 750 pairs of RIL crosses. Here we present data from the first 150 pairs. This coarse mapping allows us to localize QT to ~10cM windows, and potentially identify drugs for which the genetics of toxicity is dominated by a single major gene. It seems reasonable, that by identifying genes that offer protection against chemotherapy toxicity in flies, we can orthologs in humans and determine if they similarly modulate chemotherapy toxicity. Since chemotherapy drugs often attack fairly basic cellular level processes, we believe that the Drosophila genes involved in toxicity may be the same as those in humans.

Mapping Loci Contributing to ADH Enzyme Expression in Drosophila melanogaster. Stuart J. Macdonald, Chris M. Merkes. Dept. Molecular Biosciences, University of Kansas, Lawrence, KS.

Segregating variation at the Alcohol dehydrogenase (Adh) locus in Drosophila melanogaster is responsible for a large fraction of the variation in the activity and amount of ADH enzyme produced by flies. However, both ADH activity and protein level are complex, polygenic traits, and phenotypic variation is also due to polymorphic loci unlinked to Adh. In an effort to identify these sites we have initiated mapping experiments employing a pair of recently-developed genetic reference populations. First, we are carrying out high resolution QTL (Quantitative Trait Locus) mapping using the Drosophila Synthetic Population Resource (DSPR), a panel of 1,700 Recombinant Inbred Lines (RILs) derived from...
two eight-way synthetic laboratory populations. Multiple replicate sets of males from each RIL are phenotyped for ADH enzyme expression using a simple, plate-based, colorimetric kinetic enzyme assay. Second, we are conducting a genomewide association study using the Drosophila Genetic Reference Panel (DGRP), a set of 190 nearly isogenic lines isolated from a single natural population. For the DGRP lines we measure ADH expression both in males taken directly from the inbred lines, as well as in the male progeny of a set of line-to-line crosses. Each of these two mapping resources offers distinct advantages, and the combination of association and QTL mapping offers the promise of identifying and characterizing the precise nucleotide polymorphisms that contribute to phenotypic variation.

534C Identification of QTL contributing to larval to adult viability in the presence of nicotine. Tara N. Marriage, Stuart J. Macdonald. Dept Molecular Biosciences, Univ Kansas, Lawrence, KS.

Nicotine is an addictive and toxic substance in humans, and many of the effects of the drug can be recapitulated in Drosophila. Previous assays have shown genetic variation for resistance to nicotine in Drosophila, and we also show this with our assay. In this study, we investigate the genetics of larval nicotine resistance by comparing larval survival on nicotine for RILs from a 8-way synthetic recombinant lab population (DSPR, Drosophila Synthetic Population Resource) maintained for over 50 generations. Using the DSPR RILS allows not only high resolution mapping of the causative QTL (QTL are mapped to at least 1cM), but also estimation of both the effect and frequency of alleles that contribute to survival in the presence of nicotine. Based on previous simulation work and empirical research, we anticipate mapping a number of QTL contributing to nicotine resistance, implicating a relatively small number of putative candidate genes in the genetic control of this trait.

536B Quantitative Genetic Mapping of Resistance to Starvation in Drosophila melanogaster. Casey L. McNeil, Clint L. Bain, Stuart J. Macdonald. Department of Molecular Biosciences, University of Kansas, Lawrence, KS.

In nature, animals may encounter periods of nutrient deprivation, making the ability to withstand starvation stress an important fitness trait. We and others have demonstrated significant genetic variation within and between populations of Drosophila for resistance to starvation. Additionally, longevity and starvation conditions have been correlated with metabolic rate and resistance to other stressors. In order to better understand this model life history trait, we employed a novel quantitative trait loci (QTL) mapping panel, the Drosophila Synthetic Population Resource (DSPR). Derived from a pair of eight-way synthetic recombinant populations, the DSPR is a panel of more than 1500 recombinant inbred lines (RIL) currently being genotyped using restriction-digest associated DNA (RAD) markers. Flies of both sexes from the DSPR were phenotyped for starvation resistance, as measured by the time until death under starvation conditions. We found striking variation among the lines for resistance to starvation, with some lines living just a few days and some as long as 10 days. We expect to generate robust estimates as to the effect, number, and frequency of quantitative trait loci (QTL) contributing to starvation resistance in the DSPR. Future studies will concentrate on mapping loci influencing starvation phenotype in crosses among RILs, in an effort to utilize starvation resistance as a model trait to identify alleles that, when homozygous, reduce organismal fitness.

537C Mito-nuclear genetic effects on sperm performance in Drosophila. Jim Mossman, David Rand. Ecology and Evolutionary Biology, Brown University, Providence, RI.

How and why some males father more offspring than others has puzzled scientists for centuries. In many species, sperm activity and fertility is highly dependent on ATP produced by oxidative phosphorylation; a biochemical pathway jointly encoded by nuclear and mitochondrial DNA (mtDNA). Population genetics theory predicts that deleterious mtDNA mutations will be removed from populations by purifying selection. However, because mtDNA is maternally inherited, mtDNA mutations that are deleterious in males but which have negligible or zero fitness consequences for females can persist in populations. As a result, male-specific phenotypes such as sperm have no fitness consequences for mitochondria. In addition, since ATP production has both nuclear DNA and mtDNA-encoded components, it is predicted that mito-nuclear epistasis is likely to modulate energetic properties of sperm. Recently, fluorescent protamine constructs have revolutionized the way sperm heads can be visualized in vivo, allowing sperm tracking in real time and in the female milieu. Here, we generated mito-nuclear introgressed lines whose sperm were fluorescently labeled with eGFP and DsRed monomer to test the hypothesis that mtDNA, nuclear DNA, and their epistasis affect sperm energetics. Sperm motility from these discreet lines was quantified using a combination of image analysis tools and the results are presented.

536A Additive and Dominance Effects on Drosophila melanogaster wings in diallel crosses using the Drosophila Genetic Reference Panel. Jessica Nye, David Houle. Evolution and Ecology, Florida State University, Tallahassee, FL.

The complex phenotype of the Drosophila melanogaster wing is highly heritable and controlled by many genes. This quantitative trait is formed from a complex genetic network that go through several stages of development. Phenotypic differences caused by combinations of various genotypes are explored through diallel crosses between lines from the Drosophila Genetic Reference Panel. The wings of offspring and parental phenotypes are imaged and twelve landmark coordinates are recorded. A G-matrix is estimated between sexes using a mixed model by restricted maximum likelihood analysis using the program WOMBAT. Flies from the DGRP originated as wild caught individuals that have been inbred for twenty generations. The DGRP serves as a genetic library of natural diversity in Drosophila melanogaster which is explored within these crosses. This study answers questions on sexual differences in phenotypes, additive effects, dominance effects, maternal and paternal effects, as well as the significance of dimensionality in quantifying a complex phenotype.

539B Transgressive segregation under laboratory condition in Drosophila. BALLAGERE P HARINI. DROSOPHILA CULTURE LABORATORY, DEPT OF ZOOLOGY, BANGALORE UNIVERSITY, BANGALORE, KARNATAKA, India.

Drosophila nasutu nasutu (2n = 8) and Drosophila nasutu albomicans (2n = 6) are a pair of sibling allopatric chromosomal cross-fertile races of the nasutu subgroup of immigrants species group of Drosophila. Intercross hybridization between these two races has given rise to new karyotypic strains called Cytorace 1 and Cytorace 2 (first phase).
Further hybridization between Thailand strain of *D. n. albopticans* and *D. n. nasuta* of Coorg strain has resulted in the evolution of two more Cytoraces, namely Cytorace 3 and Cytorace 4 (second phase). The third phase Cytoraces (Cytorace 5 to Cytorace 16) have evolved through interracial hybridization among first, second phase Cytoraces along with parental races. Each of these Cytoraces is composed of recombined genomes of the parental races. An attempt to systematically assess the impact of hybridization on karyotypes, morphometric and life history traits in 16 Cytoraces has been made. The newly evolved Cytoraces with different chromosome constitutions, exhibit decreased body size, better fitness and live longer than their parents. Particularly, Cytorace 5, 6 and 8 have evolved with very much higher range values of quantitative traits than the parents and other Cytoraces, which suggests the role of transgressive segregation in the evolution of these Cytoraces. Thus, the rapid divergence recorded in the chromosomes, karyotypes, body size and fitness traits of Cytoraces exhibit the early event of recombinational radiation in the evolution of the Cytoraces under laboratory conditions.

540C

**The Durham sex-ratio meiotic drive in *Drosophila simulans***. Yun Tao, Linbin Zhang, Jennifer Kovacs, Hailian Xiao. Dept Biol, Emory Univ, Atlanta, GA.

Recent genetic studies of speciation in *Drosophila* have convincingly shown the rapid divergence of genes for male fertility and their disproportionately dense distribution on the X chromosome. One likely evolutionary mechanism for these two genetic patterns of speciation is sex-ratio meiotic drive. Selfish sex-ratio distorters can increase their frequencies in the population even they inflict fitness cost to most of the other genes in the genome. As a defense, suppressors can also evolve. This kind of intragenomic conflicts over sex-ratio can create a perpetual dynamic for rapid evolution of male meiosis genes. Because the conflict is between the X chromosome and the rest of the genome, hybrid male sterility genes are expected to be enriched on the X. Direct evidence for the above scenario has been accumulated during our study of the Durham meiotic drive system in *D. simulans*. We identified the suppressor, *too much yin (tmy)*, which has been initially found as the strongest hybrid male sterility gene on the third chromosome between *D. simulans* and *D. mauritiana*. When the *D. simulans* allele (*Tmy*) is replaced by the *D. mauritiana* allele (*tmy*), male has much reduced fertility and sires about 70% of daughters in his progeny. The two alleles differ by the presence or absence of a pair of 1.55 kb inverted repeat (IR). Multiple copies of the *Tmy* IR homologs are detected in *D. simulans*, *D. mauritiana* and *D. sechellia* but not in *D. melanogaster*. One such X-linked homolog in *D. simulans* appears to be distorter related to *Tmy* (*Dit*). The *sex-ratio* phenotype of *tmy* male is temperature sensitive (ts). By temperature shift experiment and TEM observation, we narrowed down the critical ts stage of spermatogenesis to pre-meiotic. The Durham drive system in many aspects is similar to the Winters *sex-ratio* drive system found in the same species, both in phenotypes and in DNA sequences. It seems evident that *sex-ratio* meiotic drives in *D. simulans* have evolved multiple rounds through arms race between the X and autosomes, and one consequence of this arms race is speciation.

541A

**The genome of *Drosophila athabasca* and its application to speciation**. Karen M. Wong1, Doris Bachtrog1, Michael B. Eisen2,3. 1) Department of Integrative Biology, UC Berkeley, Berkeley, CA; 2) Department of Molecular and Cell Biology, UC Berkeley, Berkeley, CA; 3) Howard Hughes Medical Institute, UC Berkeley, Berkeley, CA.

In evolutionary genetics, a gap remains between our understanding of the population-level processes that act within species and the forces that result in speciation. Past studies focusing on speciation in *Drosophila* have looked mainly at the divergence between distantly related species at a small number of loci, an approach that yields important, yet limited information on the evolutionary forces that act along the genome early on in speciation. Utilizing an intermediate diverged system, we are more likely to identify genomic features and genetic boundaries that are important to the actual process of speciation. We describe the newly sequenced genome of *Drosophila athabasca*, which is a North American species composed of three recently diverged, but behaviorally isolated semispecies with overlapping ranges. By examining patterns within and between semispecies, we aim to identify features in the genome that may be important for creating and maintaining new species. Previous studies on a handful of loci in *D. athabasca* have shown increased divergence between semispecies along the X-chromosome. We extend this analysis using next-generation sequencing to examine genome-wide patterns of nucleotide divergence on a preliminary dataset of 12 genomes sampled across the *D. athabasca* range.
POSTER: Gametogenesis and Organogenesis
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

542B

The evolution of heteromorphic sex chromosomes is frequently associated with two kinds of chromosome-specific regulation: dosage compensation and meiotic sex chromosome inactivation (MSCI). Global regulation of the X chromosome in the Drosophila melanogaster male germline is poorly understood. We show that there are three unusual features of gene expression from the X chromosome in the male germline. First, dosage compensation appears to be absent. Second, there is no evidence for a chromosome-wide reduction in gene expression on the X during meiosis, indicating that Drosophila lacks MSCI. Third, testis-specific promoters are transcriptionally repressed on the X chromosome, and this repression occurs in both pre-meiotic and meiotic cells. The behavior of the X chromosome in the male germline has implications for our understanding of genome evolution and speciation in Drosophila.

543C
A Repressive Activity of the MSL Complex. Lin Sun1,2, Harvey Fernandez2, Ryan Donohue2, James Birchler1,2. 1) Biological Sci Div, Univ Missouri, Columbia, Columbia, MO; 2) Genetics Area Program, Univ Missouri, Columbia, MO.

It has been proposed that X chromosome dosage compensation has a component similar to intrinsic mechanisms that inversely modulate gene expression (Bhadra et al., 1999, Genetics). This hypothesis explains the two-fold up regulation of the male X as well as the two-thirds down regulation of the three X chromosomes in metameres to account for their respective dosage regulation. The male specific lethal (MSL) complex is present on the male X chromosome and would act to sequester chromatin modifiers, including the histone acetylase MOF, to the X chromosome to mute the inverse effect on the autosomes in normal males and then nullify any overcompensation that might result from the increased histone acetylation while allowing the inverse effect to act on the X itself. To investigate the repressive property of the MSL complex, selected protein components of the complex were combined with the GAL4 binding domain. When targeted to a UAS-minimal promoter mini-white reporter, which was recovered at several X and autosomal positions, MOF causes up-regulation in females but down-regulation in males. By using immuno-staining and FISIL, it was found that the whole MSL complex is recruited to the sites of the reporter genes in males and conditions an increased level of histone acetylation, but in females only the high acetylation is colocalized with the reporter, which is further confirmed by Chromatin IP. Using a GAL4-MSL2 construct does not cause dosage compensation on X and autosomal reporters in females, although its expression causes the organization of the MSL complex on the X and autosomal reporters with an increased histone acetylation. When also ectopically expressing MSL2 in GAL4-MOF targeting genotypes, we found that the increased expression in, caused by MOF fusion protein, was reversed. All these data indicate that the MSL complex does not condition the dosage compensation directly, but rather its repressive activity overrides the high level of histone acetylation and counteracts the over-expression of X-linked genes to achieve the proper two-fold up-regulation in males.

544A
Lumen formation in tracheal terminal cells - novel role of Microsomal triacylglycerol transfer protein. Magdalena M Baser1, Maria Leptin2, Markus Affolter1. 1) Department of Cell Biology, Biozentrum, University of Basel, Klingelbergstrasse 50/70, 4056 Basel, Switzerland; 2) Institute for Genetics, University of Cologne, Zülpicher Str 47, 50674 Cologne, Germany.

Tubular structures can be found in many organs, like lung, kidney or cardiovascular system. Although they have different shapes and sizes their architecture on cellular level shows some similarities and three major types of tubes can be distinguished: multicellular, unicellular with external lumen and unicellular with luminal space within the cell. How the lumen forms within a single cell is of our main interest. An excellent model to study assembly, expansion and maintenance of an intracellular lumen is Drosophila tracheal (respiratory) system with its terminal cells, forming long, luminized, branched cytoplasmic extensions. What is the mechanism of the terminal cells development and which molecules are involved is poorly understood. In attempt to answer these questions we conducted a genetic screen and employed MARCM (Mosaic Analysis with Repressible Cell Marker) system to analyse EMS-induced mutations for defects in lumen of terminal cells of third instar larvae. Here we present one of the identified mutants, carrying the mutation in gene encoding Microsomal triacylglycerol transfer protein (Mtp). Mtp is an ER protein, known to be involved in the assembly and secretion of lipoproteins - molecules required for lipid transport in animal body. The absence of Mtp in terminal cells leads to the disturbed lumen structure and lack of gas filling. Additionally we observed similar defects in clonal cells on secondary branches, where the lumen, although extracellular, is made by a single cell. These defects are cell autonomous and can be rescued by expressing Mtp cDNA in the mutant cells. Our data show that Mtp in tracheal cells acts in lipoprotein independent manner and thus indicate a possible novel function of this protein.

545B
Mipps are involved in multiple processes of Drosophila tracheal development. Yi Ling Cheng, Deborah Andrew. Cell Biology, Johns Hopkins School of Medicine, Baltimore, MD.

Tube formation is an important process in the development of many organs, including the lung, kidney, intestine, and heart, so it is critical to understand this process at both the cellular and molecular level. The Drosophila trachea is one of the best model systems for studying tubular organogenesis due to its relative simplicity and genetic tractability. Our lab and others have shown that the Tracheal (Trh) transcription factor is a major regulator of trachea formation. To learn how Trh carries out these functions, we have screened for downstream target genes using a number of approaches. Among the genes we identified is mipp1, an early expressed gene whose tracheal expression absolutely depends on Trh. mipp1 encodes a dual substrate specificity multiple isoinositol polyphosphate phosphatase that can dephosphorylate higher inositol polyphosphates to the Ca++ second messenger IP3 and dephosphorylate 2,3-bisphosphoglycerate to 2-phosphoglycerate (2,3-BPG to 2-PG). The biological function of Mipps is very poorly understood. Drosophila has two mipp genes: mipp1 and mipp2. To learn the role of mipp1 and mipp2 in tracheal development, we generated a knockout of mipp1 by homologous recombination and obtain available mipp2 mutant lines. Double mipp1+mipp2 mutant has more severe defects in dorsal trunk elongation, dorsal branch fusion and ganglionic branch migration than either single mipp mutant. We created UAS-mipp1 lines to test for rescue of the tracheal defects and to learn where this unusual enzyme localizes in the cell. We also examined if ipk (inositol phosphate kinase) mutants have related tracheal defects. Future experiment will focus on dissecting how Mipps regulate tracheal development.

546C
Tracheless (Trh) regulates all tracheal genes. Se-Yeon Chung, Cy Chaves, Deborah Andrew. Dept Cell Biol, Johns Hopkins Univ, Baltimore, MD.

Tubular organ formation is a ubiquitous process required to sustain life in multicellular organisms. The Drosophila trachea is a branching network of tubular epithelia that transports oxygen and other gases, tracheless (trh), which encodes a bHLH-PAS transcription factor, is one of the first genes to be specifically expressed in the cells that will develop as tracheal cells. Trh is essential for trachea formation; in the absence of trh function, the precursor cells fail to undergo any of the morphogenetic events of tube formation and remain at their site of origin. Expression of many trachea-specific genes is dependent on trh, but all of the known targets for Trh have relatively minor phenotypes, suggesting that there are other targets. To identify characterized transcriptional targets of Trh and to further understand the role of Trh in embryonic tracheal formation, we performed an in situ hybridization screen using a library of >100 tracheal-expressing genes identified by BDGP. Surprisingly, expression of every tracheal gene we tested was dependent on Trh, suggesting that Trh plays a major role in activating tracheal-specific gene expression. Moreover, some tracheal target genes were upregulated (or de-repressed) in the early salivary glands in trh null embryos, revealing an unexpected role for Trh as a transcriptional repressor in the secretory cells of the salivary gland. A pilot screen for the targets of Ventral veinless (Vvl) and Knirps/Knipps-related (Kni/Knrl), the other two known early-expressing transcription factors in the trachea, revealed that Vvl and Kni have only minor roles compared to Trh. Microarray experiments are currently underway to identify additional Trh targets.
**Poster:** Gametogenesis and Organogenesis

See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

---

**Domains and amino acids of Crumbs required for regulating Drosophila tracheal tube-size.** Renée M. Robins1, Milena Pellikka2, Ulrich Tepass1, Greg Beitel1. 1) Molecular Biosciences, Northwestern University, Evanston, IL, USA; 2) Department of Cell and Systems Biology, University of Toronto, Toronto, ON, Canada.

Epithelial tube-size regulation is critical for organ function, yet the molecular mechanisms controlling tube-size remain poorly understood. In the Drosophila trachea, work by multiple groups has demonstrated that the luminal/apical extracellular matrix (aECM) regulates tube morphogenesis. We have also shown that apical polarity proteins can regulate tracheal tube size. In particular, overexpression of the apical determinant Crumbs (Crb) elongates the trachea without affecting aECM. To understand how Crb functions in tracheal tube-size control, we are determining which domains and amino acids are necessary for Crb activity. Crb is unique because it is the only known transmembrane polarity protein, consisting of a highly conserved 37 amino acid intracellular domain, a single transmembrane domain (TMD), and a large 2802 amino acid extracellular domain (ECD). Overexpression of a TMD-ECD construct in tracheal cells shortens tracheal tube length in larvae. This result suggests that the Crb-ECD plays a role in regulating tube size, possibly by interacting with components of the aECM. When specific amino acids in the Crb-ECD are mutated, overexpressed Crb loses its ability to over-elongate the trachea, and in some cases, causes a complex phenotype that shortens and coils larval trachea. These results support the hypothesis that the Crb-ECD has an important role in Crb function during larval development. However, it is unclear if the ECD has a significant role in embryonic development, as overexpression of WT or mutant ECD in embryonic trachea does not appear to change tube length. In contrast, embryonic and larval overexpression of the short intracellular domain phenocopies overexpression of full length Crb, suggesting that the Crb intracellular domain can function independently of the extracellular domain.

548B

**ZPR1 mediated EGF signaling is required for subcellular lumen formation.** Oscar E. Ruiz, Mark M. Metzstein. Dept Human Genetics, Univ Utah, Salt Lake City, UT.

Branched tubular networks, such as the vascular and respiratory systems, employ common structural designs that permit the transport of liquids and gases throughout the body. The cellular and molecular mechanisms required for generating these complex structures are not well understood. Terminal cells are specialized tracheal cells that undergo subcellular branching and tubulogenesis and are responsible for transporting and exchanging gases in target tissue. In a forward genetic screen of the X chromosome for mutations disrupting different aspects of branching and lumen formation, mutations in the gene Zpr1 (zinc-finger protein 1) were identified; zpr1 mutants have a previously uncharacterized role in general EGF signal transduction, or whether ZPR1 is specifically required in tracheal development, and what the downstream targets of this pathway are.

549C

**Multiplexin promotes heart-specific lumen formation in Drosophila.** Nofar Harpaz, Talila Volk. Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel.

Hemolymph circulation in Drosophila is performed by contractions of the dorsal vessel, a linear tube comprised of two domains: the heart and the aorta. The heart lumen is significantly wider than that of the aorta, allowing the entry of a large volume of hemolymph following heart valve closure, thereby ensuring the supply of nutrients and enabling hemocytes circulation within the body of the organism. In this study we investigated the role of Multiplexin (Mpx), the ortholog of human Collagen XVIII, in the establishment of the heart lumen. In Drosophila embryos, Mpx is highly expressed in the heart cardioblasts and the protein is secreted into the lumen of the heart. Embryos homozygous for mpx mutation exhibit narrower heart lumen, whereas over-expression of Mpx is sufficient to induce wide lumen in the aorta of wild type embryos and its over-expression in the heart resulted in an abnormally wider heart lumen. Similar results were achieved by ectopic expression of Endostatin, the cleaved form of Mpx, suggesting that it is sufficient for tube widening. To get further insight to the mechanism by which Mpx promotes widening of the heart lumen, we analyzed the distribution of F-actin in the heart cardioblasts. We show that F-actin is highly detected at the basolateral surfaces of the heart cardioblasts, and is eliminated at the luminal domain. The possible effect of Mpx on inhibition of F-actin distribution at the luminal faces of the heart tube is currently examined. Genetic interaction assays showed that Mpx may function together with Slit, Robo and Laminin in promoting heart lumen formation. The precise involvement of Mpx in modulating the function of these proteins is being investigated. Taken together, our results suggest that Endostatin, the cleavage product of Mpx, is necessary and sufficient for the establishment of heart specific wide lumen. The human ortholog of Mpx, Collagen XVIII, is highly expressed in the heart and may function similarly to promote proper heart morphogenesis.

550A

**Functions of a helix-loop-helix transcription factor, Extramacrochaetae, in development of left-right asymmetry in the Drosophila embryonic hindgut.** Ryo Hatori, Kiichiro Taniguchi, Naotaka Nakazawa, Mitsotoshi Nakamura, Reo Maeda, Kenji Matsuno. Tokyo University of Science, Yamazaki, Noda, Chiba, Japan.

Left-right asymmetrical morphogenesis is a critical aspect of organogenesis in many animals. To understand the mechanisms involved in left-right asymmetric morphogenesis, we are using the embryonic hindgut of Drosophila. During development, the hindgut rotates left-handedly 90 degrees, and this rotating direction is genetically determined. In our genome wide screen, we previously found that DE-Cadherin (DE-Cad) and MyosinIII D (MyoIII D) mutants show randomized and reverse laterality of the hindgut, respectively. More recently, we identified extramacrochaetae (emc), as a gene involved in regulating the laterality of this organ. emc encodes a negative helix-loop-helix transcription factor involved in a diverse range of biological processes such as cell-fate determination. Our epistatic analysis implies that Emc functions upstream of MyoIII D and DE-Cadherin. In wild-type embryos, DE-Cadherin tended to be localized in a planar left-right asymmetrical manner at cell-boundaries of hindgut epithelial cells. However, in emc mutants, there was no left-right bias in the planar localization of DE-Cadherin. Next, we hypothesized that this planar left-right bias of DE-Cadherin localization might regulate left-right asymmetrical cell shape through differential cell adhesion, and by using differential immuno-fluorescence of the hindgut, we observed a new type of cell polarity. We propose that this new cell polarity is regulated by emc in the establishment of the left-right asymmetrical DE-Cad distribution and adhesion.

551B

**Building a complex protein network linking the membrane to the cytoskeleton: impact of gene dosage on protein stoichiometry within the integrin adhesion complex in vivo.** Yoshihiko Inoue, Nicholas H. Brown. The Gurdon Institute and Dept of PDN, University of Cambridge, U.K.

Leptothoracic morphology is a critical aspect of organogenesis in many animals. To understand the mechanisms involved in left-right asymmetric morphogenesis, we are using quantitative confocal microscopy of GFP-tagged integrin-associated proteins in the living animal to understand how the integrin adhesion complex is assembled and what mechanisms control the amount of each component in the complex. By comparing two developmentally important integrin adhesive sites in Drosophila, the larval muscle attachments and adult wings, we observed a consistent stoichiometry of some components while others vary. During this analysis, we were surprised to discover that single copies of the genes encoding integrin-associated proteins were not able to produce wild-type levels of these proteins at the muscle attachment sites, with reductions ranging from 25% to the full 50%. This was unexpected because the mutations are all recessive, and so this reduction does not cause a strong phenotype. This gave us an opportunity to...
examine the dependence of each component on the levels of the others, revealing how each component contributes to setting the overall stoichiometry. We will present our measurement of 8 GFP-tagged integrin-associated proteins in the 8 different heterozygous mutant backgrounds. This has revealed that reduction of some components not only results in the reduction of some others but an apparently compensatory increase in other components. Thus, this quantitative data analysis is revealing the molecular interactions in the integrin adhesion complex that underly the complexity and robustness of the protein network.

552C

Cell migration is a dynamic process required for tissue remodeling during early development and for organismal homeostasis throughout life. Migration often involves remodeling of the extracellular matrix (ECM), a major substrate upon which cells migrate. The ECM comprises a variety of fibrillar proteins, adhesion molecules, growth factors and their receptors, and the integrins. Remodeling of the ECM is facilitated through the action of matrix metalloproteases (MMPs) that cleave ECM constituents. The ADAMTSs are a special class of secreted MMPs, which contain Tumbthromspadin-like (TS) repeats. The Drosophila genome encodes three ADAMTS genes, including gon1 (CG14869), which is expressed in several migratory tissues during Drosophila embryogenesis. Expression of gon1 is observed in hemocytes, the caudal visceral mesoderm (CVM), the visceral branch (VB) of the trachea and the salivary gland (SG). We generated a complete loss-of-function (LOF) mutation of gon1, which results in migration defects in several different tissues, including the germ cells and CVM, as well as mis-migration of a subset of tracheal VBs. Tissue-specific rescue experiments reveal that gon1 functions both cell autonomously and non-cell autonomously. The SGs of gon1 mutants display an unexpected phenotype wherein the apical membrane of the SG is severely irregular. Consistent with the apically localized defects in the SGs, a GFP-tagged version of Gon1 localizes near the apical membrane of the SG. Through our studies, we expect to gain insight into the role of gon1 and other ADAMTS proteins in distinct cell migration events during normal embryonic development.

553A
The blade runner gene of Drosophila is required for wing structure maturation. Balaji G. Iyer2,1, Laura Fung1, Fong Cho1, Amber Ablack1, John Lewis1. 1) Department of Oncology, University of Western Ontario, London Regional Cancer Centre. London, Ontario, Canada; 2) Hamilton Health Sciences, Hamilton, Ontario, Canada.

We are interested in using reverse-genetic methods to interrogate the reciprocal interactions between differentiating cells and the extracellular matrix (ECM) in forming mature, specialized organ structures. Towards this goal, we investigated the role of Drosophila ortholog of Epidermal Growth Factor Like domain 7 (EGFL7, which we call blade runner or br), in wing development. Mammalian EGFL7 is a secreted protein that plays a critical role in vascular development, in part by remodeling the local ECM. We report that ectopic expression of br can result in abnormal wing blade shape. The first bona-fide allele of br also displayed defects on the wing-blade surface and incomplete wing-unfolding. A wing-directed knock-down of br transcript resulted in a milder wing shape defect. Using a br-regulatory-Gal4 construct and a monoclonal antibody against Br we show that the br gene codes for a secreted protein that is expressed in wing epithelial cells. This demonstrates that the wing is in a state of intracellular signaling and immunohistochemistry, that br plays a role in the stabilization of integrin-ECM contacts between the epithelial cells and the wing blade structure during maturation. Disruption of this process through misexpression or loss of function of br results in a compromised wing blade structure. Based on this study, we will discuss how cellular interactions with the ECM gives rise to the final shape and mechanical properties of the wing.

554B

The Iroquois complex (Iro-C) of Drosophila melanogaster harbours three genes araucan, caupolican (caup) and mirror, which encode transcription factors of the TALE family. Expression of these genes in the proximal-most region of the wing disc specifies development of the notum versus that of the wing hinge. EGFR/MAKP signalling pathway plays a key role in notum development, at least in part through regulation of Iro-C genes expression (reviewed in Cavodeassi, Modello and Gómez-Skarmeta, 2001, Development 128, 2847-2855). Since Iro proteins contain putative MAPK phosphorylation sites, we have analyzed the putative posttranscriptional regulation of Iro proteins activity by MAPK. This hypothesis is supported by the synergistic genetic interaction, in over expression experiments performed using the GAL4/UAS system, between Caup and the activated form of MAPK rolled Sem. We have determined that Caup is indeed phosphorylated at Ser/Thr residues in S2 cells, with constitutive activation of the EGFR pathway. Interestingly, both ERK and p38 MAPK appear to contribute to such phosphorylation. Furthermore, it is apparent that the ability of Caup in the wing disc to regulate cell proliferation and cell determination.

555C

Drosophila blisters (bs) gene is the homolog of the vertebrate Serum Response Factor (SRF) gene. bs is required for several processes throughout the life cycle of the fruit fly: formation of terminal branches in the embryonic tracheal system (Guillemin et al., 1996), specification of intervil regions in the developing wing blade in the pupa (Montagne et al., 1996), and experience-dependent regulation of sleep in adult flies (Donlea et al., 2009). Yet bs expression is observed in many other tissues and at different times during development, suggesting involvement in other processes. In order to investigate whether bs participates in other as yet uncharacterized functions, we used a well-established compound eye phenotype modifier system and tested three different bs-lack-of-function alleles for modification. We found bs mutant alleles capable of modification, implying a role in eye development. We are currently characterizing bs’ involvement in this phenotype. Donlea et al. (2009) Science 324, 105 Guillemin et al. (1996). Development 122, 1353 Montagne et al. (1996). Development 122, 2589.

556A
Brg-P9: A putative Kunitz domain inhibitor of serine proteases involved in imaginal disc morphogenesis. Sienna M Sartori1, John Emery2, Rachel Morgan1, Cynthia Bayer1, Gregory Guild3, Laurence von Kalm1. 1) Biology and Biomedical Sciences Center, University of Central Florida, Orlando, FL; 2) Department of Biology, University of Pennsylvania, Philadelphia, PA.

The Stubble-stubbloid (Sb-sbd) gene is the homolog of the vertebrate Serum Response Factor (SRF) gene. bs is required for several processes throughout the life cycle of the fruit fly: formation of terminal branches in the embryonic tracheal system (Guillemin et al., 1996), specification of intervil regions in the developing wing blade in the pupa (Montagne et al., 1996), and experience-dependent regulation of sleep in adult flies (Donlea et al., 2009). Yet bs expression is observed in many other tissues and at different times during development, suggesting involvement in other processes. In order to investigate whether bs participates in other as yet uncharacterized functions, we used a well-established compound eye phenotype modifier system and tested three different bs-lack-of-function alleles for modification. We found bs mutant alleles capable of modification, implying a role in eye development. We are currently characterizing bs’ involvement in this phenotype. Donlea et al. (2009) Science 324, 105 Guillemin et al. (1996). Development 122, 1353 Montagne et al. (1996). Development 122, 2589.

Skeletal muscles come in a variety of shapes and sizes important for functions like running or eye blinking; however, we do not yet understand the mechanisms that generate muscle fibers with discrete morphologies. In the *Drosophila* embryo, muscles arise from the fusion of a founder cell (FC) with surrounding fusion competent myoblasts (FCMs), and are distinguishable by properties such as size, shape, orientation, nuclear number, attachment and innervation. Subsets of FCS and nascent muscle fibers express transcription factors, known as identity genes, in incompletely overlapping patterns. There are at least 12 known identity genes, including *apterous* (ap) and *slouch* (slou), that have been shown to be important for muscle morphology. To determine how morphological information is translated from identity genes to cellular processes controlling muscle size and shape, we focused on the ap-expressing lateral transverse muscles (LTs). LTs have simple, elongated shapes, dorsal-ventral orientations and direct attachments. Five identity genes are known to be expressed within the LTs: Krüppel, *ap*, twist, muscle-segment homeobox and caupolican. To test the contributions of these genes to LT morphology, we have examined embryos with gain or loss of these factors. Furthermore, to analyze the genes expressed in LTs, we labeled LT FCS with an *ap*-GFP transgene, and purified them by FACS. Using microarray analysis, we compared the LT FC transcriptional profile to that of a purified population of dorsal and ventral FCs expressing a *slouch*::RFP transgene. We have identified roughly 300 genes specifically up- or down-regulated in LT FCS, reporting and interpreting diverse functions as gene expression, cytoskeletal organization and protein localization. By analyzing these genes, we aim to identify identity gene targets, other transcription factors and downstream effectors that contribute to specifying the distinct LT muscle properties. Taken together, our genetic and array data will give us a clearer picture of the hierarchical network of gene activity required to form a muscle of a specific size and shape.

The JAK/Stat pathway promotes mesoderm subdivision and maintains cardiac integrity during embryogenesis. Aaron N. Johnson, Mayssa H. Mokalled, Thomas N. Haden, Eric N. Olson. Department of Molecular Biology, Univ. of Texas Southwestern Medical Center at Dallas, Dallas, TX.

Using strong Star92E loss of function alleles, we have identified two novel functions for the JAK/Stat pathway during mesoderm development. Early in development JAK/Stat signals regulate mesoderm subdivision into cardiac, visceral, and somatic muscle domains by directly activating the expression of *E(spl)-C* genes. Notch also regulates *E(spl)-C* gene expression, and therefore JAK/Stat and Notch signals converge to regulate *E(spl)-C* loci to pattern the mesoderm. In development JAK/Stat signals are required for the proper expression and localization of the integrin β-subunit Mys in cardiomyocytes. Mys and two additional components of the integrin complex, Mew and Lanα, are essential mediators of cardiomyocyte cell-cell adhesion and in turn of cardiac integrity. Our analysis of JAK/Stat signaling was initiated by the identification of *mute* in a genetic screen for regulators of cardiogenesis. We show that *Star92E* embryos phenocopy the cardiac defects of *mute* embryos and mutations in *mute* suppress eye overgrowth induced by overexpressing the ligand *Upd*. Our in vitro studies identify Mute as a transcriptional regulator that activates a Star92E Response Element in response to Upd. These findings highlight a central role for JAK/Stat signaling during mesoderm development and identify Mute as a novel regulator of the pathway.

Two reporters are better than one: a genetic screen for novel regulators of mesoderm development. Aaron N. Johnson, Eric N. Olson. Department of Molecular Biology, UT Southwestern Medical Center at Dallas, Dallas, TX.

The molecular mechanisms regulating mesoderm development have been intensely studied for over twenty years. While this work identified a conserved gene regulatory network that specifies mesoderm progenitors and induces terminal muscle differentiation, the mechanisms directing final organ morphology remain largely unknown. To identify novel regulators of organogenesis in the mesoderm we have initiated an EMS screen that uses two distinct mesoderm reporters. One reporter drives a nuclear-localized GFP in the heart and a second reporter drives an actin-localized GFP in body wall (somatic) muscles. Since both reporters are expressed in every genetic background, we can quickly distinguish between mutations that globally disrupt mesoderm development and those that solely affect heart or somatic muscle morphogenesis. As a validation of our approach we have identified previously known yet highly specific regulators of mesoderm organogenesis including *mel2, blow*, and *Rac2*. We have chosen to focus on two phenotypic classes: mutations that affect myotube outgrowth or attachment and mutations that affect cell adhesion in the heart. One mutation with myotube outgrowth defects mapped to *boip*, a ribosomal protein implicated in Hh signaling. We subsequently found that blocking Hh signaling in somatic muscle founder cells inhibits myotube outgrowth. Our screen identified another interesting mutation that affects cell-cell adhesion between the most posterior myocardial cells of the heart. This finding suggests a unique genetic program regulates cell adhesion in the posterior cells of the dorsal vessel. Our approach has thus uncovered previously uncharacterized events directing mesoderm morphogenesis and we will present our most current insights into these processes.


Among the phenomenal coordination of events vital to the success of embryonic development are the defined proliferation, directed migration and maintenance of blood cells. Evolutionarily conserved constituents of the PDGFR and VEGFR receptor tyrosine kinase (RTK) families exhibit defined roles in these early hematopoietic processes. In Drosophila, signal transduction through the PDGFR/VEGFR-related receptor (Pvr) drives the establishment and dispersion of hemocytes during embryogenesis. Drosophila hemocytes are macrophage-like cells that arise and proliferate in the head mesoderm and subsequently populate the entire embryo in an anterior to posterior stereotypical migration across the ventral midline. This primary wave of hematopoiesis is essential for neural development, as hematocyte deposition of extracellular matrix is required for ventral nerve chord (VNC) condensation. In *Pvr* mutants, a reduced hemocyte population and a breakdown in the defined hemocyte migration results in a late embryonic lethality that stems from failures in neural development. The Pvr ligands, Pvf2 and Pvf3, also have implicated roles in these developmental processes. However, distinction between the roles of Pvf2 and Pvf3 in hematopoiesis, migration and survival remains unclear. To dissect the functions of these two ligands we generated a mutant fly line that lacks both Pvf2 and Pvf3 (*pvf2-3*). Examination of *pvf2-3* mutant embryos revealed a marked reduction in hematopoietic proliferation, a disorganized migration and failures in VNC condensation. This work establishes a signaling paradigm of Pvr/Pvf in early hematopoiesis. As a model of vertebrate RTK signaling, this work extends our understanding of the conserved contributions of RTKs in developmental proliferation, migration and even various proliferative diseases.
Transcription factor erect wing (EWG) is involved in indirect flight muscle patterning, development and maintenance in Drosophila. Mamta Rai, Upendra Nongthomba. Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore, Karnataka, India.

Muscle development is a multistep process which includes myoblast diversification, proliferation, migration, fusion, differentiation and growth. A hierarchical exhibition of myogenic factors is important for the execution of progress in external events in muscle formation. EWG ectopic expression factor known to have a role in indirect flight muscle development (IFM) in Drosophila. We marked out the precise spatio-temporal expression profile of EWG in the myoblasts, and in the developing muscles. Mutant adult flies null for EWG in myoblasts show variable number of IFM, suggesting that EWG is required for patterning of the IFM. The remnant muscle found in the EWG null flies show proper assembly of the structural proteins, which implies that some myoblasts manage to fuse, develop and differentiate normally indicating that EWG is not required for differentiation program per se. However, when EWG expression is extended beyond its expression window in a wild type background, muscle thinning is observed implying EWG function in protein synthesis inhibition. Miss-expression studies in wing disc myoblasts inhibited at its role in myoblast proliferation. We thus conclude that EWG is important for regulating fusion events which in turn decides the IFM pattern. Also IFM in EWG null mutants show clumps containing broken fibres and an altered mitochondrial morphology. The vertebrate homolog of EWG is nuclear respiratory factor1 (NRF1) which is known to have a function in mitochondrial biogenesis and protection against oxidative stress. Gene expression for inner mitochondrial membrane protein, Opal1-like was found to be absent in these mutants. Also, these flies were more sensitive to oxidative stress, indicating a compromised mitochondrial functioning. Our results therefore demonstrate that EWG functions in maintaining muscles' structural integrity by ensuring proper mitochondrial activity.

563B

Deficiency screening to identify novel genes that function during Drosophila myogenesis. Kate M. Rochlin1,2, Karen Beckert1, Mary Baylies1. 1) Dept Dev Biol, Sloan-Kettering Inst, New York, NY; 2) Weill Cornell Graduate School of Biomedical Sciences, New York, NY; 3) National Institute for Medical Research, Mill Hill, London.

During Drosophila embryonic development, syncytial somatic muscles form from the iterative fusion of two distinct types of myoblasts. Founder Cells (FCs) provide the information that dictates the size, shape, and orientation of the eventual muscle fiber whereas Fusion Competent Myoblasts (FCMs) provide the molecular machinery required for this process. Previous work has used EMS mutagenesis and forward genetic screens to identify a number of genes that are essential for fusion. Many of these identified genes regulate processes such as cell recognition and adhesion, cytoskeletal remodeling, and membrane breakdown. However, several genes that have been identified as essential for FCFM fusion have not yet been ascribed a distinct function. Moreover, many more genes involved in fusion have yet to be uncovered. These limitations have prevented the development of a comprehensive model describing the mechanism of myoblast fusion. Therefore, we have undertaken an enhancer trap screen to identify novel components that genetically interact with known regulators of myoblast fusion. We expected this approach to identify pathway components that have been missed during traditional recessive screens and lead to a greater understanding of protein function during fusion. We tested for genetic interactions by crossing flies that were heterozygous for known fusion mutants with heterozygous deficiency lines for known regulators of myoblast fusion. We have now identified various components of two signaling pathways that show a genetic interaction with fusion proteins in the context of muscle development. We speculate that these pathways regulate the activity of essential fusion genes during myogenesis. Currently we are testing this model we provide data will give greater insights to the tightly controlled process of cell-cell fusion.

564C

Identification of Genes Involved in Myoneural Positioning during Muscle Development. Victoria K. Schulman1, Thomas J. Metzger1,2, Mu Xu1,2, Mary K. Baylies1. 1) Weill Cornell Graduate School of Medical Sciences, New York, NY; 2) Sloan-Kettering Institute, Developmental Biology Program, New York, NY.

Muscles are multicellular systems formed by the fusion of mononucleated myoblasts. In Drosophila, the larval muscles are formed in the embryo from two different populations of myoblasts, Founder Cells (FCs) and Fusion Competent Myoblasts (FCMs). Each muscle is specified by a single FC, which fuses iteratively to FCMs to achieve a myofiber of a particular size, shape, and orientation within the hemisegment. We conducted a forward genetic screen to identify novel genes involved in determining these morphological traits. We used a transgenic fly line expressing the dsRed protein fused to a nuclear localization sequence in the larval transverse (LT) muscles. In wild-type embryos, the nuclei in these muscles are evenly distributed along the length of the myofiber, allowing us to assess nuclear number and organization. In our screen, we identified a mutant where the correct numbers of nuclei are present, but they fail to separate, clumping at the ventral end of each LT muscle. Based on the organization of these muscles, we named this mutant swoosh. Positional mapping and sequencing revealed that swoosh mutants contain a nonsense mutation in ensconsin (ens), a microtubule associated protein involved in RNA localization in the ovary. This led us to hypothesize that ensconsin is involved in the process of nuclear positioning. Positional complementation with another gene involved in nuclear positioning validated this hypothesis. Embryos transheterozygous for these two genes displayed a nuclear positioning phenotype in the LT muscles, while embryos homozygous for either gene showed no defect. Additionally, we discovered that Eip2 physically interacts with Ens in a yeast two-hybrid screen. We are currently working to determine the nature of this interaction, and we are examining how these two proteins contribute to the process of myoneural positioning.

565A

Spalt mediates an evolutionary conserved switch to fibrillar muscle fate. Cornelia Schönbaumer1, Jutta Distler2, Nina Jährling2, Martin Radolf3, Hans-Ulrich Dohl1, Manfred Frasch2, Fang Chen3. 1) Max-Planck-Institute of Biochemistry, Martinsried, Germany; 2) Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany; 3) Technical University of Vienna, Vienna, Austria; 4) Research Institute of Molecular Pathology, Vienna, Austria.

Human body muscles are composed of distinct muscle types that differ in their contractile properties according to their functions. These include fast and slow muscle fibres as well as a rhythmically beating heart. Several functional differences correlate with a characteristic composition of muscle contractile filaments. How these different muscle types are specified and molecularly constructed remains elusive. Many insect species possess asynchronous, stretch-activated flight muscles that enable fast and powerful wing oscillations at frequencies up to 1000 Hz, together with power outputs as high as 100 W per kg. To achieve these specific parameters, indirect flight muscles contain stretch-activated myofibrils that display a unique fibrillar morphology. This is in stark contrast to all other synchronous insect body muscles that display a tubular morphology and are more similar to vertebrate skeletal muscles. In a genome-wide screen using the RNA interference method we have identified the transcription factor spalt major (salm) as a master regulator of fibrillar flight muscle development in Drosophila. salm expression is induced specifically in the myoblasts that will form the indirect flight muscles by the transcription factor vestigial (vg). However, in contrast to vg, salm is not only required but also sufficient to induce the fibrillar muscle fate when expressed ectopically. Hence, salm can overrule other muscle identity programs. salm is responsible for all features characteristic of fibrillar flight muscles; in particular, it regulates the expression and splicing of various sarcomeric proteins that execute the fibrillar muscle fate. We find that this function of salm is conserved in insects separated by 120 million years of evolution and therefore appears to constitute an ancient developmental principle.

566B

Cdc42 is required during morphogenesis of the Drosophila heart and genetically interacts with the tyrosine kinase Ablion. Georg Vogler1, Timothy Iafe1, Jiandong Liu2, Rolf Bodmer1. 1) Development and Aging, Sanford-Burnham Medical Research Institute, La Jolla, CA; 2) Dept. Biochem. & Biophys., UCSF, San Francisco, CA.

Cardioblasts and pericardial cells, which are the cardiac precursor cells, are specified by a cascade of cell signaling events and transcriptional regulation during mid-embryogenesis. They are formed in the cardiogenic region on both sides of the lateral mesoderm and then migrate towards the dorsal midline where the two bilateral rows meet to undergo a complex morphogenetic process to form the heart. Although much has been learned about the early steps of cardiac tissue specification, understanding the genetic control of heart organ morphogenesis is still in its infancy. Recent studies have shown that cardiac alignment requires Slt/Robo signaling but how these and other signals are integrated to control heart morphogenesis is still unknown. Small GTPases are essential for the transduction of signaling events into cell behavior and may thus also be required
during heart morphogenesis. We have investigated the role of the small GTPases during embryonic heart development and found Cdc42 is required for cardiac alignment and lumen formation. Failure of the bilateral rows to close, improper alignment of cardioblasts and formation of multiple lumina in Cdc42 zygotic mutants indicates that Cdc42 is required during discrete steps of heart morphogenesis. These phenotypes seem to be independent of its role in cell polarity since known cardiac polarity markers like Discs-large or Dystroglycan still localize correctly. Since we are interested in how Cdc42 activity is regulated we are performing genetic interaction screens. We identified the tyrosine kinase Abelson (Abl) to strongly interact with Cdc42 hemizygotes in coordinating the alignment and assembly of the embryonic heart tube. Interestingly, Abl mutants also display a cardiac phenotype similar to Cdc42 mutants. Together, these data point to a novel mechanism of cardiac morphogenesis that involves the interaction between Cdc42 and Abl, possibly acting together in a common pathway in this process.

567C

**Spire, an actin nucleation factor, regulates cell division during Drosophila heart development.** Peng Xu, Tamara Johnson, Jessica Stoller-Conrad, Robert A. Schulz. Department of Biological Sciences, University of Notre Dame, Notre Dame, IN.

Heart is a beneficial model system for studying cell-cell signaling and the regulation of early heart development. Spire (Spri) is an important actin-nucleation factor which regulates actin dynamics in many developmental processes, such as shape determination, intracellular transport, and locomotion. Both the dorsal-ventral and anterior-posterior axes of Drosophila egg and embryo are affected by spri, through its actions as a conserved maternal effect gene. Previous studies of spri have been focused on its function during development of Drosophila oogenesis, especially in domain interactions. In our current study, we demonstrate that Spri has a ubiquitous expression pattern being expressed in heart cells. Cell division of timinn (even-skipped and seven up) heart cells are all affected in the absence of spri. Also, we find that spri may interact with Dorsocross, tin and panmyer by genetic interaction analysis. spri mutant phenotype was compared with that of CyoA mutants, which show mitosis 16 is blocked during endodermogenesis. Our results suggest that in spri mutant embryos, most Svp-positive progenitors cannot separate after cycle 15, and Tin-positive progenitors cannot divide after cycle 16. Based on these findings, we conclude that Spri plays a crucial role in the genetic hierarchy controlling dorsal vessel formation and has a function in cell division during heart tube morphogenesis.

568A

**Parthenogenesis in a Drosophila Yemanuclein-alpha meiosis I defective mutant.** Oumissa Ait-Ahmed1, Regis Meyer1,2, Ahmed Algazeery1, Michelle Capri1. 1) Inst de Genetique Humaine, CNRS UPR 1142, Montpellier Cedex, France; 2) current address: Cell Cycle and Cancer Biology OMRF Oklahoma, USA.

We identified yemanuclein-alpha (yem-alpha) in a screen for genes specifically expressed in the female germ line. It encodes an oocyte specific DNA binding protein [1]. Yem-alpha is the ampliplet sequence of the Ubn/HPC2 family of proteins that have recently been shown to be involved in the HIRA mediated chromatin remodeling complexes in Humans and Yeastis (2-3). Yem-alpha biological function remained elusive for nearly two decades. The first mutant allele of yem-alpha (yem1) was obtained in a screen for female sterile mutations (Meyer, Delaage, Rosset, Capri and Ait-Ahmed, submitted). We report the experiments performed using this allele to explore its meiotic role. The yem1 point mutation (V478E) affects chromosome behavior at meioses I. At metaphase I yem1 oocytes display defects in aligning on the meiotic spindle suggesting kinetochore dysfunction. In a recombination defective context, the yem1 oocytes undergo precocious anaphase and female sterility is partially suppressed. This results in the development of exceptional progeny. Surprisingly these progeny do not bear the paternal chromosome, therefore they are parthenogenetic. The analysis of their X chromosome markers shows that these progeny are formed from diploid eggs that inherited the two maternal homologues. The cytological and the genetic data combined suggest the possibility that these eggs are diploid as a result of kinetochore defects induced by yem1 mutation. Indeed kinetochore dysfunction may result in a single equational division as a consequence of meiosis I skipping. Apparently during female meiosis I Yem-alpha was found to colocalize with CID, the Drosophila histone H3 variant specific for the kinetochore. 1) Aït-Ahmed et al. The yemanuclein-alpha: a new Drosophila DNA binding protein specific for the oocyte nucleus. Mech Dev 1992, 69-80. 2) Balaji et al Mol Biosyst 2009, 269-275. 3) Banumathy et al: Mol Cell Biol 2009, 758-770.

569B

**Nutrient Restriction Affects Polarized Transport in the Drosophila Ovary.** Katherine M. Burn, Lynn Cooley. Gen, Cooley Lab, Yale Sch Med I-359, New Haven, CT.

*Drosophila melanogaster* deprived of a protein-rich diet have low fecundity, due to slowed stem cell divisions, slowed egg chamber development and egg apotosis of egg chambers just prior to the onset of vitellogenesis (stage 7). However, little is known about the consequences of nutrient deprivation on polarized transport in previtellogenic (stage 7 and younger) egg chambers. Recent data from our lab show that previtellogenic egg chambers from flies deprived of a protein rich food source display cortically condensed microtubules (MT) and abnormal cytoplasmatic aggregation of Ypsilon schachtel (Yps), a member of the oskar mRNP complex. In order to understand the composition and function of these foci, we performed immunolocalization studies with known markers for cytoplasmic bodies. We found Yps foci colocalize with known processing body components Decapping protein 1 (Dcp1) and the 5′-3′exoribonuclease Pacman, but not with stress granule components phospho-eukaryotic initiation factor 2 (p-eIF2 ) and Staufen, or the autolysosome marker Lysotracker. Consistent with the compositional similarity of these puncta with processing bodies, these foci are devoid of ribosomes, and application of the translational inhibitor cycloheximide to egg chambers diminished Yps foci. We have additionally found that the starvation-induced phenotype is rapidly reversible by culturing egg chambers with insulin, and that this recovery is disrupted by the addition of the PI3K inhibitor wortmannin. These data suggest that specific transport of maternal components from nurse cells to the oocyte is highly regulated before vitellogenesis and may be influenced by environmental conditions. We are currently investigating the role of the MT motors Dynein and Kinesin in Yps foci dynamics as well as MT organization. Additionally, we are investigating if the germline cells respond to insulin signaling independent of somatic follicle cells, and if canonical insulin signaling modulates this response.

570C

**Analysis of Drosophila melanogaster Tudor: logic of a dynasty.** Remi-Xavier Coux1,2, Sophia Ju-Yu Wang1, Ying Huang1, Rui-Ming Xu1,4, Ruth Lehmann1. 1) Developmental Genetics Program, The Skirball Institute, Howard Hughes Medical Institute, NYU School of Medicine, New York; 2) Université Paris Diderot - Paris VII, GC2ID Doctoral School, Pol, Paris , France; 3) Structural Biology Program, The Skirball Institute, NYU School of Medicine, New York; 4) Institute of Biophysics, Chinese Academy of Sciences, Beijing, China.

Drosophila Tudor, the founder of the tudor domain family, contains 11 tudor domains and is a component of nuage and polar granules. Embryos lacking maternally contributed Tudor protein have defects in abdomen patterning, germ plasm assembly and germ cell formation. The Tudor domain is a protein motif that consists of four β-strands folded into a barrel-like structure, and binds proteins that contain symmetrically dimethylated Arginines (sDMAs) through an aromatic cage. Previously, we have shown that there is a requirement for the 5′-3′-5′ termination Tudor domains in germ cell formation, Tudor localization and polar granule architecture. Recent studies showed that Aubergine (Aub), a germline-specific Argonaute protein is symmetrically dimethylated and associates with Tudor through its sDMa modification. To test if individual Tudor domains play specific roles in germ cell formation and in Aub association, we mutated aromatic residues in individual Tudor domains. Aub localization and stability are affected similarly when the aromatic cage in Tudor domain D6 or D10 is mutated but only D10 affects germ cell formation. Our lab is now using structural biology approaches along with developmental biology experiments to figure out the role of Tudor and tudor domain proteins in germ plasm assembly.

571A

**Defining the maternal effect lethal phenotype of E(var)3-9.** Claire E. Dolan, Karen S. Weiler. Department of Biology, West Virginia University, Morgantown, WV.

Mutations of the E(var)3-9 gene were isolated as dominant enhancers of the variegation of Im(l)w^m (i.e. PEV), suggesting that the E(var)3-9 protein acts to antagonize heterochromatin and/or promote a euchromatic chromatin state compatible with gene expression. However, E(var)3-9 mutations also exhibit a recessive female sterile phenotype.
Drosophila oocyte development begins in an egg chamber that contains a cyst of 16 interconnected cells, which are derived from the incomplete cell divisions of a progenitor cell. Characterization of RNA targets of the nuclear RNA-binding protein Lark during oogenesis. in understanding how abnormal cell polarity contributes to certain cancers. mutagenesis screen was performed on the right arm of chromosome 3 (Morris, Navarro, and Lehmann, 2003). Here we present our work on the phenotypic characterization of 38-conserved between Drosophila and other eukaryotes; however, many more remain to be identified. In order to uncover new genes involved in these processes, a genetic One of these cells will become the oocyte and the others will become nurse cells. The oocyte is specified and maintained by the asymmetric localization of oocyte-specific factors, either during cell division, or through intracellular localization. Drosophila oogenesis is an excellent model system to study cell polarity establishment during development. Drosophila oocyte development begins in an egg chamber that contains a cyst of 16 interconnected cells, which are derived from the incomplete cell divisions of a progenitor cell. One of these cells will become the oocyte and the others will become nurse cells. The oocyte is specified and maintained by the asymmetric localization of oocyte-specific factors along a polarized microtubule cytoskeleton, spread across the cyst. Important genes involved in regulating cell polarity and cell fate specification have been found and are conserved between Drosophila and other eukaryotes; however, many more remain to be identified. In order to uncover new genes involved in these processes, a genetic mutagenesis screen was performed on the right arm of chromosome 3 (Morris, Navarro, and Lehmann, 2003). Here we present our work on the phenotypic characterization of 38-2; one of the mutants isolated from the screen. 38-2 mutant germ line clones initially specify an oocyte, but later this cell reverts back to a nurse cell fate. This failure to maintain an oocyte fate is also seen in certain eglington and par family mutant ovaries. These genes are known to be involved in both microtubule transport and cell polarity establishment. Deficiency mapping by lethality is currently being used to determine the location of the mutation and its gene identity. The identification of genes isolated in our screen could help in understanding how abnormal cell polarity contributes to certain cancers.

572B
Isolation of a novel regulator of the actin cytoskeleton. Tânia C. Ferreira, André Rosa, Rui Martinho. Instituto Gulbenkian de Ciencia, Oeiras, Portugal.
We are interested in tissue morphogenesis and cytoskeleton regulation. Through a 2R maternal screen we have isolated mutants for apke, RhoGEF2 and scraps (Marques et al. 2008). We have also isolated another complementation group whose females after induction of germ-line clones laid eggs significantly smaller than wild type. We named this complementation group "curto", which means "short" in Portuguese. curto complementation group contained two alleles: curto1 and curto2. The short egg phenotype was reminiscent of the phenotypes previously described for actin cytoskeleton-related mutants (Cooley et al., 1992; Castrillon and Wasserman, 1994), which have been associated with dumping defects during oogenesis. Consistently, curto mutants also showed significant dumping defects. Furthermore, zygotic mutants of curto showed defects in embryonic dorsal closure and wound healing. Reinforcing the role of curto in actin-related processes, capping protein B is a dominant suppressor of curto zygotic mutants embryonic lethality. Yet, despite these phenotypic similarities, we also detected significant differences between curto and other known actin cytoskeleton-related mutants: 1) cytokinesis was normal during oogenesis. 2) curto mutants did not show any loss of tissue integrity in follicular or larval wing disc epithelia. We hypothesize that Curto is only required for a subset of actin cytoskeleton functions, possibly the ones mostly related with the generation of force and cell-shape changes.

573C
The Identification and Characterization of Novel Genes Involved in Cell Polarity Establishment. Sarah E. Kleinsorge, Caryn Navarro. Department of Medicine/Genetics Program, Boston University School of Medicine, Boston, MA.
Cell specification and differentiation are important processes in development. Abnormal cell fate specification, due to a loss of cell polarity, can lead to defects in tissue organization, stem cell differentiation and carcinogenesis. One way to establish and maintain cell fate, is to change cell polarity through the asymmetric partitioning of cell specific factors, either during cell division, or through intracellular localization. Drosophila oogenesis is an excellent model system to study cell polarity establishment during development. Drosophila oocyte development begins in an egg chamber that contains a cyst of 16 interconnected cells, which are derived from the incomplete cell divisions of a progenitor cell. One of these cells will become the oocyte and the others will become nurse cells. The oocyte is specified and maintained by the asymmetric localization of oocyte-specific factors along a polarized microtubule cytoskeleton, spread across the cyst. Important genes involved in regulating cell polarity and cell fate specification have been found and are conserved between Drosophila and other eukaryotes; however, many more remain to be identified. In order to uncover new genes involved in these processes, a genetic mutagenesis screen was performed on the right arm of chromosome 3 (Morris, Navarro, and Lehmann, 2003). Here we present our work on the phenotypic characterization of 38-2; one of the mutants isolated from the screen. 38-2 mutant germ line clones initially specify an oocyte, but later this cell reverts back to a nurse cell fate. This failure to maintain an oocyte fate is also seen in certain eglington and par family mutant ovaries. These genes are known to be involved in both microtubule transport and cell polarity establishment. Deficiency mapping by lethality is currently being used to determine the location of the mutation and its gene identity. The identification of genes isolated in our screen could help in understanding how abnormal cell polarity contributes to certain cancers.

574A
Characterization of RNA targets of the nuclear RNA-binding protein Lark during oogenesis. Gerard P. McNeil1,2, Kirk Haltouderfde1, Christopher Ferrarr1. 1) Department of Biology, York College/CUNY, Jamaica, NY; 2) Program in Biology at the Graduate Center, CUNY, NY, NY.
From the formation of the female egg to the first three hours after fertilization, Drosophila development is controlled by maternal gene expression. During oogenesis, maternally expressed gene products control development of the oocyte and establish the body axes. Over the years many maternally-acting genes have been identified and characterized. One of these genes, lark, encodes a nuclear RNA-binding protein that has been shown to be essential for oogenesis. Elimination of the lark maternal component results in defects in the organization of the actin cytoskeleton resulting in a "dumping" defect and female sterility. Since Lark is an RNA-binding protein localized to the nucleus, it likely functions by regulating RNA metabolism through either splicing or nuclear-cyttoplasmic transport. To determine the mechanism of regulation of Lark, we have identified 38 potential RNA targets using a Ribonanics-based approach, one of which, Dmocin, encodes an actin-binding protein. Preliminary evidence shows that Dmocin protein localization is regulated by Lark, likely through regulation of splicing in the nurse cell nuclei. We present results of using an RT-PCR approach to identify potential defects in RNA splicing of Dmocin and other potential RNA targets in lark mutant ovaries.

575B
Argonaute 1 regulates germl cell division and oocyte determination in Drosophila melanogaster. Mohd Ghows Mohd Azzam, Ji-Long Liu. MRC Functional Genomics Unit, Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, OX1 3QX, United Kingdom.
Argonaute 1 (Argo1) is a member of the Argonaute/Piwi protein family that involves in small RNA-mediated gene regulation. In Drosophila melanogaster, Ago1 plays a specific role in microRNA biogenesis and functions. Previous studies have demonstrated that Ago1 regulates the fate of germline stem cells. However, the function of Arg1 in other aspect of oogenesis is still elusive. Here we study the function of Ago1 in developing egg chambers. We find that Ago1 protein is enriched in the oocytes and also expressed in the cytoplasm of follicle cells. Clonal analysis of multiple ago1 mutant alleles has shown that many mutant egg chambers contain only 8 nurse cells without an oocyte. Our results suggest that Ago1 plays a role in cytolysis of oocyte and oocyte determination.

576C
Investigating the role of Spindle-E in germline development. Kristen Mary Ott, Tram Nguyen, Caryn Navarro. Genetics and Genomics, Boston University Medical Center, Boston, MA.
A large portion of the genome of most organisms consists of transposable elements (TE) which, when active, can excise and insert back into the genome at random sites (transpose). Transposition can lead to genomic instability, which can be detrimental to the organism. This is especially true in the germline, where damaged DNA will be passed on to offspring causing disease and possibly death. In Drosophila, as well as mammals, small non-coding RNAs (piRNAs - Piwi-interacting RNAs) suppress transposon expression in the germline. Many genes have been identified as playing a role in piRNA generation, but their exact function remains unknown. Our research focuses on one of these genes: Drosophila spindle-E (SpnE). SpnE contains two highly conserved domains, a Tudor domain and a DEAH box RNA helicase, and is necessary for the generation of most piRNAs in the ovary. In addition, snf8 mutants lay eggs with severe developmental defects. Here, we show that loss of snf8 decreases the levels of both the piRNA pathway protein Aubergine (Aub) and the CPEB homologue oo18 RNA binding protein (Orb) in Drosophila ovaries. To determine if the snf8 mutant phenotype is due to the loss of Aub and Orb, we created a line of flies that are mutant for both aub and orb. We find that the phenotype of the double mutant ovaries resembles that of snf8 mutants. However, TE expression is not upregulated to the same extent as in snf8 mutants. Additionally, we do not detect a change in TE expression in orb single mutant ovaries. Therefore, our data indicates the snf8 oogenesis phenotype may not solely be due to an increase in TE levels and Snf8 may have additional functions during ovoary development. In addition to these results we will also report on our progress toward creating a transgenic HA-tagged version of wild-type Snf8 as well as constructs carrying point mutations within the Tudor domain or the DEAH
box helicase domain. These constructs will be used to perform a structure/function analysis of SpnE and help gain insight into its role in the piRNA pathway and oogenesis.

577A

Identification of Tramtrack69 Targets During Drosophila Dorsal Appendage Tubulogenesis. Nathaniel Peters, Celeste Berg. Department of Genome Sciences and the Molecular and Cellular Biology Program, University of Washington, Seattle, WA.

Tubes are vital for proper organ/tissue function in metazoans and exhibit a myriad of forms, yet the fundamental molecular mechanisms of tube formation are well conserved. During the late stages of Drosophila oogenesis, subsets of follicular epithelial cells that surround the oocyte undergo morphogenesis to form Dorsal Appendage (DA) tubes. DA tube formation provides an appealing model for studying epithelial tube formation because it occurs in the absence of cell division and apoptosis, permitting us to focus specifically on the role of coordinated cell movement and shape change in this process. Although Tramtrack69 (TTK69) is an essential transcription factor during development, the twin peaks mutation is a hypomorphic ttk69 allele that specifically affects TTK69 production during DA tubulogenesis. TTK69 loss in DA-forming follicle cells causes improper regulation of cell dimensions and migration, and the eventual formation of severely shortened DAs. To identify targets and downstream effectors of TTK69 during DA tubulogenesis, we compared gene expression profiles in wild type and twin peaks via microarrays. Our analysis suggests that genes involved in cytoskeletal regulation, G-protein-coupled receptor signaling, and cytochrome P450-mediated oxidation, are influenced by TTK69 in this context. To confirm candidate-gene expression results from our arrays, we are using in situ hybridization and we have noted several intriguing follicle-cell expression patterns that also agree with our array results. To establish functional links between TTK69 and our array candidates, we are using RNAi and over-expression constructs, driven by follicle-cell-specific GAL4 drivers, and subsequently screening for DA defects. Knockdown of cyp12D1 mRNA results in notable DA morphology defects and we are testing other array candidates from the P450 superfamily. Identifying the downstream targets of TTK69 during DA tubulogenesis will provide a more thorough understanding of how regulation of cell shape and movement are involved in the formation of cellular tubes.

577B

Female sterility and compromised eggshell integrity of drop-dead mutants. Tayler Diane Sheahan, Laura E. Korthauer, Edward M. Blumenthal. Biological Sciences, Marquette University, Milwaukee, WI.

Mutations in drop-dead (drd) result in female sterility. We have previously reported that drd expression is not required in the germline for fertility. We now find that eggs laid by females homozygous for the strong alleles drd" or drd' are fertilized but arrest before gastrulation. Knockdown of drd by RNAi with the pan-follicle cell Gal4 drivers CY2 or T155 also results in sterility and early developmental arrest. Given the role of the follicle cells in eggshell formation, we tested whether drd affects the integrity of the outer chorion or the inner vitelline membrane (VM) of the eggshell. Western blot analysis of chorion and VM protein solubility was conducted on egg chambers and laid eggs in the presence or absence of reducing agents to examine the formation of the disulfide and non-reducible/cross-linkable disulfide bonds that stabilize the eggshell during late oogenesis and ovulation. Results demonstrate that the solubility and cross-linking of chorion protein s36 is unaltered in drd mutants. Similarly, the VM proteins sV17 and sV23 are insoluble in the absence of reducing agents in late oogenesis egg chambers of mutant and control females, showing that disulfide cross-links are unaffected by drd. However, the large majority of both sV23 and sV17 in laid eggs from mutant females is soluble in the presence of reducing agents, while in control eggs these proteins are totally insoluble due to the formation of non-reducible/cross-linkable bonds. To analyze VM permeability, laid eggs were dechorionated and stained with neutral red. Mutants displayed both increased fragility and permeability, demonstrative of the compromised state of the VM. Dechorionation with bleach resulted in the bursting of 24% of eggs from mutant females, compared with 3% eggs from heterozygous controls. Of the surviving eggs, 43% of the mutants were permeable to neutral red, compared to 3% of controls. We conclude that drd expression in the follicle cells is necessary for embryonic development and that drd is required for the normal integrity and covalent cross-linking of the VM. Supported by 1R15 GM080682 to EMB.

579C

Age-dependent requirement for Upd3 in oogenesis. Claire M.-P. Venard, Liqun Wang, Travis Sexton, Douglas A. Harrison. Department of Biology, University of Kentucky, Lexington, KY.

The Janus Kinase (JAK) transduction cascade is one of the main conserved eukaryotic signaling pathways. In Drosophila, there is only one known receptor, one JAK, and one Signal Transducer and Activator of Transcription (STAT). Despite the diverse functions of this signaling pathway in flies, only the proteins of the Unpaired family, Upd, Upd2, and Upd3, are known to be ligands of the JAK/STAT receptor. During oogenesis, JAK activity is required in the germanium for specification of the stalk cells that separate egg chambers and for maintenance of somatic stem cells. In the vitellarium, JAK/STAT activity is required in a graded fashion for specification of follicular cells. In the egg chamber, proper JAK/STAT signaling is required for the border cell migration and specification. At the end of the egg chamber development, the border cells give rise to the micropyle. upd is expressed in the most posterior somatic cells in the germarium and in the polar cells of the vitellarium. Interestingly, upd3 is co-expressed with upd in the ovarian polar cells. Given the limited sequence identity between Upd and Upd3, we would like to understand the role of Upd3 during oogenesis. Lines mutant for upd3 were generated in a P element mobilization screen. Young upd3 mutant females are fertile and present mostly normal eggs. However, as these females age, they exhibit a higher frequency of defects than wild-type females, including ovarioles with fused and degenerated egg chambers. Egg chamber fusions may occur as a consequence of reduced follicle cell number or improper specification of stalk cells. We are currently investigating the effect of upd3 mutation on border cell specification, micropyle development, and egg viability.

580A

Mapping and Characterization of baal, a mutation in a novel gene that results in Drosophila Sterility marked by Germ Cell Loss. Qiao Zhang, Michael Busczak. UT Southwestern Medical Center, 6000 Harry Hines Blvd, Dallas, TX.

Here we present our initial characterization and mapping of a female and male sterile mutation we refer to as baal. Young baal mutant females exhibit egg chamber degeneration beginning at stage 5 or 6 of oogenesis. However after two weeks, mutant ovaries are mostly depleted of germ cells with only a small number of largely inactive and undifferentiated germ cells remaining in the germarium. In males, germ cells are mostly lost within one week. Using clonal analysis, we found that baal functions cell autonomously in germ cells and that female baal mutant germ line clones have weak GSC loss and strong egg chamber degeneration phenotypes. We used mitotic and deficiency mapping to map the baal locus to a 42 Kb region on 2R chromosome. Genomic and cDNA rescue experiments revealed that baal mutations disrupt a novel gene within the region. We further confirmed that baal functions within germ line cells through Nos-gal4::VP16 driven cDNA rescue of the male and female sterile phenotypes. These tagged rescuing transgenes localized to the nuclei of germ line cells. We have begun to test whether baal genetically interacts with other genes that function during gametogenesis or encode products that localize to the nucleolus. Surprisingly we find that baal and bam double mutant females have a cystic tumorsome phenotype with branched fusomes within their germaria, indicating these germ line stem cells still undergo differentiation in the absence of bam. Over time, baal and bam double mutants display a severe germ cell loss phenotype. These preliminary findings suggest baal promotes germ cell survival and differentiation in both males and females, perhaps through a mechanism that involves the regulation of nucleolar function.

581B

The awk/shark pathway: toward understanding dorsal appendage morphogenesis. Sandra G. Zimmerman, Celeste Berg. Genome Sciences, University of Washington, Seattle, WA.

Tubulogenesis, the morphogenesis of biological tubes, is required for the development and function of the gut, neural tube, and other organs and is evolutionarily conserved. A useful model for the study of tubulogenesis is dorsal appendage (DA) formation in the Drosophila egg chamber during oogenesis. The DAs are specialized eggshell structures that
facilitate embryonic gas exchange. They are made by two patches of ~75 follicle cells, each composed of roof and floor cells that reorganize from a flat epithelium into tubes, extend anteriorly, form paddle-shaped structures at the anterior tips of the tubes, and secrete chorion into the tube lumens. During anterior extension of the DA tubes, roof and floor cells extend filopodia-like extensions towards their substrate; the squamous stretch follicle cells (SCs). Likewise, the SC's project cellular extensions toward the migrating DA-forming cells. In bullwinkle (bwk) mutants, patterning of the DA primordium occurs normally but the egg chamber forms moose-antor shaped DAs. A modifier screen identified shark, which encodes a tyrosine kinase, as a strong enhancer of the bwk phenotype. shark is required in the SCs for proper DA morphogenesis and shark mRNA localizes in distinct foci or patches at stage 10. These foci are often associated with SC nuclei. After stage 10, the foci are no longer readily visible. The nature of the signal from the SCs to the DA forming cells, the purpose of the cytoplasmic extensions, and whether shark mRNA localization is required for DA morphogenesis are unknown. To understand the nature of the bwk/shark pathway, we are using candidate and proteomic approaches to identify proteins that interact with shark. We are working to determine whether shark mRNA localization and the cytoplasmic extensions play a role in this process. The answers to these questions will shed light on the fundamental principles underlying tubulogenesis.

583A

Differential requirements for components of the apical PAR complex in subcellular morphogenesis. Tiiffani A. Jones, Mark M. Metzstein. Human Genetics, University of Utah, Salt Lake City, UT.

Cell morphology is key to cell function. A particular morphology we focus on is branched networks of cellular tubes. Tracheal terminal cells are an example of this: single terminal cells undergo extensive outgrowth, subcellular branch and tubulogenesis. Wild-type terminal cells initiate branching from a central branch containing the cell body. Subsequent side branches continue to bifurcate, with a general reduction in the diameter of successive branches. Wild-type cells also contain a single continuous, air-filled lumen, which extends through the central branch and into all side branches. We have been studying the role of the apical PAR-polarity complex, consisting of par-6, aPKC, and baz, in terminal cell development. We have identified two previously unidentified roles for apical PAR-complex members in terminal cell branch and tubulogenesis. First, the apical PAR-polarity complex is required for establishing subcellular branch points in a branching cell. All complex members tested and the known physical interactions between them are required for this process. Second, the complex members Par-6 and aPKC, but not Baz, are required for subcellular lumen formation. However, surprisingly, in this case Par-6 and aPKC appear to be acting independently from each other. Specifically, we have found no physical interaction between Par-6 and aPKC is required for lumen formation and the proteins have different localization domains within the terminal cell. These results suggest a novel mechanism by which the polarity proteins Par-6 and aPKC, acting independently, mediate subcellular lumogenesis.

583B

Role of Notch and its targets in regulating myogenesis. Guillaume Pézron, Sarah Bray. Dpt of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, United Kingdom.

Notch signalling, a cell communication pathway conserved throughout the animal kingdom, is involved in many developmental processes and linked to many diseases including cancers. While Notch signalling is well known for its function in cell fate control, it has also been implicated in regulating cell morphology and behaviour. For example, Notch is required in Drosophila for the formation of an actin-myoinsin fence between dorsal and ventral compartments in the wing, for myogenesis, for correct axon connectivity in the nervous system and for the migration of border cells in the ovary. However, although the regulation of cell architecture and behaviour is a key process during morphogenesis and tissue formation, it is unclear how Notch exerts its effects on these processes. Studies on Notch signalling have mainly focused on cell fate control and most identified Notch target genes are nuclear effectors. However, genome wide expression array and chromatin immunoprecipitation analysis revealed a significant enrichment in genes associated with cell morphology (Krejci et al., 2009). The identification of these new Notch targets prompted us to analyse their function in morphogenetic processes that involve Notch. By using RNAi knock-down to ablate gene functions, we have identified new Notch targets involved in adult myogenesis and we will present data on their regulation by Notch as well as on their function in this process.

583C

Temporal and spatial requirements for drop-dead expression. Christine L. Sansone, Edward M. Blumenthal. Biological Sci, Marquette Univ, Milwaukee, WI.

Flies mutant for drop dead (drd) show a range of phenotypes, including shortened lifespan, neurodegeneration, enlarged crop, small body size, and female infertility. With the severely allele drd, homoygotes have a median survival of four days and all die within two weeks of eclosion. In order to sort out the connections among these phenotypes, determine the cause of death of mutant flies, and elucidate the molecular function of DRD, we aim to identify the critical time and location for drd expression. We constructed a UAS-drd transgene and obtained RNAi transgenes against drd for directed rescue and knockdown, respectively. To determine when drd expression is necessary for adult survival, ubiquitous knockdown and rescue of drd under the control of hsp70-GAL4 was induced at different points in the life cycle. Manipulation of drd expression in adults or larvae had no effect on adult survival. However, knockdown of drd beginning with wandering 3rd instar larvae and continuing through metamorphosis resulted in profound adult lethality. Therefore, our results indicate that drd expression is necessary during metamorphosis or at eclosion but not continuously during adulthood. To establish where drd expression is necessary, tissue-specific knockdown and rescue of drd under the control of various Gal4 drivers was performed and the lifespan of the adult flies was determined. We find that expression of drd within the nervous system is not required, as knockdown with the neuronal and glial drivers elav-Gal4 and repo-Gal4 had no effect on adult lifespan. In contrast, three Gal4 drivers that identify caused knockdown-induced adult lethality: breathless, Dros62, and DJ1717. Of these three, only DJ62 was also capable of completely rescuing adult survival by driving expression of UAS-drd on a mutant background. We are currently determination the expression patterns of these three drivers during metamorphosis, and preliminary data suggest that these patterns are not as restricted as those previously reported for these drivers at other developmental stages. Supported by IR15 GM080682 to EMB.

586A

Secondary cells in the male accessory gland contribute to the post mating response in Drosophila melanogaster. Jessica Sitnik1, Dragan Gilgerov2, Robert Macek2, François
Identifying Doublesex targets with DamID-seq.

Emily Clough, Brian Oliver. LCBD/NIDDK/NIH, Bethesda, MD.

Doublesex (dsx) is a sex-specifically spliced DMRT-family transcription factor that regulates almost all aspects of somatic sexual development and non-autonomously directs germ cell development. Although dsx is an essential regulator of sexual differentiation, few direct targets are known. We are using DNA adenine methyltransferase identification (DamID) to identify Dsx targets in vivo and are adapting the DamID protocol for use with Illumina deep sequencing technology to identify the positions where Dsx protein contacts the genome. The DamID approach involves tagging Dsx isoforms with the GATC recognition sequence. Wherever the Dam-Dsx fusion protein contacts the genome, the local surrounding GATC sequences are methylated by the Dam molecule. To control for potential non-specific binding, we are using a single copy Dam transgene and mating to wildtype flies to ensure that the DamID signal is specific to dsx.

CHARACTERIZATION OF FLIES EXPRESSING UAS-Dam

We observed several notable phenotypes in flies carrying a UAS-Dam transgene. Males homozygous for this mutation lay fewer eggs and become receptive to remating sooner than those of their heterozygous siblings, suggesting that the secondary cells are necessary for these aspects of the PMR. We are continuing to investigate the role of the iab-delta5 mutation on PMR to determine how the secondary cells may interact with or play a role in known ACP related pathways.
When DSX is acting to influence sex-specific development. Our second approach is a genetic interaction screen. Genes identified in this screen will be either direct or indirect targets of DSX. To understand how sex-specific development is regulated by DSX, it is essential to identify the targets of DSX and understand how the male and female forms of DSX function as a moderator of Sxl expression to allow for proper Notch expression and signaling in monomorphic organ development.

The role of doublesex in the development of somatic sexual identity. Cale D. Whitworth, Mark Van Doren. Dept Biol, Johns Hopkins Univ, Baltimore, MD.

Doublesex is a founding member of the highly conserved DMRT (doublesex and mab-3 related transcription factor) family of genes that are known to regulate aspects of sexual development in diverse species. In Drosophila melanogaster, a single dsx transcript is alternatively spliced to allow production of male- and female-specific DSX proteins (DSXM and DSXF). These proteins have identical DNA binding domains yet distinct C-terminal domains. It is not completely clear how these C-termini regulate distinct transcriptional programs. To understand how sex-specific development is regulated by DSX, it is essential to identify the targets of DSX and understand how the male and female forms of DSX differentially regulate these targets. We have undertaken a two-pronged approach to better characterize the role of dsx in sexual development. Our first approach is chromatim immunoprecipitation followed by sequencing (ChIP-seq) to identify genomic loci directly bound by DSX. We utilized recombinography to reconstruct the endogenous dsx locus and insert an epitope tag that will be present in both male and female DSX isoforms. Flies carrying this construct are being used to perform ChIP-seq at important developmental stages when dsx is acting to influence sex-specific development. Our second approach is a genetic interaction screen. Genes identified in this screen will be either direct or indirect targets of DSX or co-regulatory proteins required for DSX's proper function. Thus far, the genetic interaction screen has yielded 12 deficiencies that modulate the phenotype of dsxD+/XX adult flies. Here we show that spalt major (salm) genetically interacts with DSXM to promote development of male external genitalia. Interestingly, salm also genetically interacts with DSX to promote female-specific gonad morphology. Together these two complementary data sets will provide a detailed description of how dsx regulates sexual development. These experiments will also shed light on the mechanisms by which the highly conserved DMRT family of proteins influences sexual development in diverse species.

Hrph48 functions as a modulator of Sxl expression to allow for proper Notch expression and signaling in monomorphic organ development. Offer Gerlitsh, Yaron Suissa, Yossi Kalifa, Tama Dinur, Patricia Graham, Girish Deshpande, Paul Scheid. Developmental Biology and Cancer Research, IMRIC, The Hebrew University-Hadassah Medical School, Jerusalem, Israel; 2) Department of Molecular Genetics, Weizmann Institute of Science, Rehovot Israel; 3) Department of Molecular Biology, Princeton University, Princeton, NJ 08540, USA.

During metazoan development, a small number of signaling pathways are repeatedly used to orchestrate diverse processes such as cell division, cell fate specification and survival. Temporal and spatial regulation of these pathways underlies the final cellular makeup, size and shape of organs. In Drosophila melanogaster, the master switch gene Sex-lethal (Sxl) orchestrates all aspects of female development and behavior by modulating gene expression. Recently, Sxl was shown to downregulate Notch signaling to accomplish sex-specific patterning. Paradoxically however, Sxl activity is essential in every female cell to prevent the activation of the male-specific dosage compensation system and thus to ensure the proper level of X-linked gene expression. This raises a key question as to how, during female development, Notch signaling escapes the negative impact of Sxl in monomorphic tissues. We have uncovered a novel mechanism where Hrph48, an abundant essential IntrRNP, functions to restrict Sxl expression in monomorphic tissues and thus allows for proper development. Phenotypic consequences of the partial loss of hrph48 resemble that of Notch but are more pronounced in females than in males. Likewise, Notch levels are drastically diminished only in females. Interestingly, monomorphic female tissues including wing, eye and antennal discs display considerable increase in Sxl amounts. Lastly, female-specific attenuation of Notch signaling is rescued upon simultaneous removal of Sxl. Our findings bring into focus the critical role played by general homeostatic factors in specification of diverse cell fates and morphogenesis.

The role of Drosophila doublesex in the development of a sexually dimorphic gonad. Erin Jimenez, Mark Van Doren. Johns Hopkins University, Baltimore, MD.

Sex determination pathways are diverse throughout the animal kingdom, but converge upon conserved genes that encode products that regulate sexual dimorphism. One such downstream factor across many diverged sex determination pathways is the Drosophila doublesex (dsx) gene. The role of doublesex is conserved in different insects and dsx homologs play roles in sexual differentiation in a diverse array of metazoans such as, frogs, fish, birds, mice, and man. In Drosophila, nearly all manifestations of sexual dimorphism between males and females are regulated by doublesex, yet there are only three known direct targets of DSX, which cannot account for the differences in regulation by DSX in sexually dimorphic tissues. While many tissues are regulated by DSX to manifest sexual dimorphism, the Drosophila gonad represents an excellent model to dissect how DSX acts on a particular time and place to promote development of a sexually dimorphic tissue. In dsx mutant gonads, either a male or female niche is observed half the time in either sex. Thus, we hypothesize that DSX does not independently regulate the genes required to form the niche. Rather, the cells of the niche make a decision based on a non-autonomous signal that instructs which niche to form in the absence of DSX. This model is similar to what is observed in mammalian sex determination of the gonad. In mammals, the sex determination pathway and non-autonomous signals (Fgf9 and Wnt4) are required to establish a male or female gonad. Because it appears that the choice of the niche is regulated both autonomously, by dsx, and non-autonomously, through cell signaling in the gonad, this research seeks to identify the non-autonomous signal coming from the cells of the gonad that influences the decision to form the correct niche in the appropriate sex. Since the formation of the gonad may represent processes that are conserved from flies to man, this kind of research will provide insight into conserved genes that regulate developmentally similar pathways whose outcome generates major differences observed between the sexes.

Possible roles in spermatic mitochondrial shaping for tests-specific paralogs of ATP synthase subunits. Tessa Campbell, Casie Genetti, Chris Lima, Taylor Gunnell,
zombie is involved in restricting spermatogonial proliferation intrinsically. Di Chen1,2, Bangxia Suo1, Shaowei Zhao1,2, Qing Gong1,2, Zhaohui Wang1. 1) Institute of Genetics and Developmental Biology, Chinese Academy of Science, Beijing; 2) Graduate School of the Chinese Academy of Science. 

Spermatogenesis, the highly conserved biological process, represents an excellent model system for the study of stem cell maintenance, germline-soma interaction, cell proliferation, and cell differentiation. In order to identify more factors regulating germ cell development, we conducted an EMS screen, through which we got an interesting mutant line homozygous viable but with tiny testes full of un-differentiated germ cells. We mapped this mutation to a gene predicted to encode an RNA binding protein. We designated this new gene as zombie (zom). Based on the morphological examination, marker gene expression, cell division pattern, and fusome morphology, we concluded that the undifferentiated germ cells accumulated in zom mutant testes were spermatogonia. Further clonal analysis, germline or somatic specific rescue, and Rnaai experiments indicated that zom was an intrinsic factor for regulating spermatogonial proliferation. Next, we performed in situ hybridization and found that zom was mainly expressed in spermatocytes.

Given the phenotypic similarity between zom and nmd, we tried genetic tests and found that zom genetically interacted with nmd. Taken together, we isolated a previously uncharacterized gene that plays an essential role in restricting spermatogonial proliferation.

Role of CG4701 in mitochondrial morphogenesis during Drosophila melanogaster spermatogenesis. Dylan Coughtrey, Leah Smith, Hunter Stone, Bevin English, Karen G. Hales. Department of Biology, Davidson College, Davidson, NC.

CG4701 encodes an AAA ATPase protein that helps to regulate mitochondrial morphogenesis in Drosophila melanogaster spermatogenesis. Homozygotes for a mutation in CG4701 exhibit the formation of unusually large, vacuolated Nebenkerne at the onion stage. Multiple nuclei surround each Nebenkern, suggesting failure of cytokinesis. These defects result in improper spermatogenesis and, subsequently, male sterility. CG4701 falls into the same family as other AAA ATPase-encoding genes such as spastin and katanin, and is a testis-specific paralog of the essential gene nmd. Like CG4701, mutations in nmd result in male sterility, though the two genes show distinct expression patterns within the testes. Our research seeks to fully characterize the CG4701 phenotype, determine how the gene product interacts with other molecules involved in spermatogenesis, and compare its function to that of nmd. Localization of the CG4701 protein is analyzed through creation of CG4701-RFP transgenic flies. GFP-tagged versions of other critical proteins in spermatogenesis, such as β tubulin and anillin, will allow us to examine their localization in a CG4701 mutant background. The creation of germline clones homozygous for mutant alleles of both CG4701 and nmd allows assessment of redundancy vs. overlapping but distinct roles.

Structural heterogeneity of the 26S proteasome in Drosophila spermatogenesis. Jing Dai, Xiazhenn Li, Long Qian Wang, John Belote. Biology Department, Syracuse University, Syracuse, NY.

The 26S proteasome is a large protease that plays a major role in regulated intracellular proteolysis in eukaryotes. It consists of a 20S core particle (CP) made up of α-type and β-type subunits, and a 19S regulatory particle (RP) at one, or both, ends of the CP. In D. melanogaster, six of the 14 CP, and six of the 19 RP, subunits are encoded by two or three paralogous genes, with the “extra” copies showing testis-specific expression. To investigate the functional significance of these testis-specific subunits, we have examined their expression patterns using GFP tagged transgenes, and, for a subset of these, have created mutant alleles and examined their spermatogenic defects. Previous work focused on the testis-specific CP subunits [1-3]. Their expression showed similar, but non-identical, patterns. In every case the GFP signal becomes prominent following meiosis, and accumulates in “speckles” near the actin cones of the individualization complex (IC). Some showed spermatid nuclear expression and most were seen in mature sperm. A null allele of one of these, Pros/g3026T, is male-sterile with defective sperm individualization [2,3]. Here we report on results with the testis-specific RP subunits Rpt3R, Rpt4R, Rpt6R, Rpn12R, Rpn13R and Uch3R. The conventional (i.e., non-testis-specific) RP subunits are somatically expressed and in early spermatogenesis, but fade away during spermatid elongation. As was the case with the 20S subunits, the testis-specific RP subunits become apparent following meiosis and are seen at the individualization stage along the elongated bundle and in nuclei, with nucleate punctate expression in the IC. A null mutant of the Rpt3R gene is male sterile with defects in actin cone organization and defective individualization. The evidence to date suggests an important role of the testis-specific proteasome is protein in spermatid individualization, perhaps by regulating the spermiogenic function of the apoptosis machinery [2,3]. [1] Ma, et al. (2002) Insect Mol. Biol. 11:624. [2] Zhong & Belote (2007) Development 134:3517. [3] Belote & Zhong (2009) Heredity 103:23.
from homozygous ms(2)1400 males indicate aberrant membrane wrapping within the Nebenkern. We mapped the mutation to a region that includes two candidate genes. One candidate gene is highly expressed in the testis and encodes an unusually large paralog of ATP synthase subunit d, the standard versions of which associate with the ATP synthase peripheral stalk. This is an intriguing observation given that ATP synthase dimerization via the peripheral stalk is reported in other organisms to affect mitochondrial morphology and cristae structure. We report molecular analysis of the candidate gene and progress of a rescue experiment, as well as close examination of ms(2)1400, for double mutant phenotypes confirming that the primary defect is in membrane unfurling and disentanglement prior to the elongation stage.

601A
A transcription factor Samuel is involved in the regulation of germ cell proliferation in spermatogenesis. Hiroyuki Kose1, Masataka Okabe2, Kozo Matsumoto3, Hiromi Yasuh1. 1) Dept of Life Science, International Christian University, Tokyo, Japan; 2) Dept. of Anatomy, The Jikei University School of Medicine, Tokyo, Japan; 3) Dept. of Animal Medical Sciences, Kyobo Sangyo University, Kyoto, Japan; 4) National Institute of Genetics, Mishima, Japan.

The maintenance of tissues, which requires continuous turnover of differentiated cells, depends on stem cells and neighboring stromal cells. While much progress has been made for molecular mechanisms of stem cell maintenance, less is known about how the stem cells regulate the differentiation steps of the stem cell progeny. The cytoplasm cell, a somatic cell crucial for the development of the Drosophila male germ line, is ideal for studying such regulatory activities of stromal cells. We are analyzing the role of transcription factor Samuel, which is expressed in the cyst cell nucleus and exerts a non-autonomous effect on the behavior of the male germ line; spermatagonia in samuel mutant testis fail to properly exit mitotic phase thus causing extra cell division. We found that samuel mutant cyst cells exhibit prolonged activation of Notch signaling. Artificial activation of Notch in cyst cells caused overproliferation of the germ cells similar to samuel loss of function, and suppressing Notch signaling in the cyst cell, but not in the germ cell, resulted in reduced male fertility. We propose that Samuel acts in the cyst cell to negatively regulate Notch signaling pathway, which in turn is required for the production or transmission of mitosis-promoting signal to the germ line.

602B
The role of the bromodomain protein IBRD-1 during spermatogenesis. Katja Leser1, Bridlin Barckmann1,2, Xin Chen1, Margaret T. Fuller3, Renate Renkawitz-Pohl1, Christina Rathe1. 1) Developmental Biology, Philipps-Universität Marburg, 35043 Marburg, Germany; 2) Institute of human genetics, Montpellier F-34396, France; 3) Johns Hopkins University, Baltimore, MD 21218-2685, USA; 4) Stanford University School of Medicine, Stanford,CA 94305, USA.

In Drosophila spermatogenesis high amounts of genes are transcribed, which requires a tightly regulated gene activity. The mRNAs of proteins needed for post-meiotic chromatin reorganization (e.g. ProtB and Mst77F) are transcribed pre-meiotically and are translationally repressed for several days. It is known that transcription of these mRNAs depends on testis specifically expressed TAFs (ITAFs). By performing ChIP experiments we could show that protB and Mst77F are direct target genes of ITAFs. As it is very likely that beside ITAFs further regulators are involved in the synthesis of the repressed mRNAs, we aimed to identify new candidate proteins acting together with ITAFs to activate genes encoding proteins relevant for spermatid differentiation. We identified a protein with two predicted bromodomains specifically expressed in the male germline. We named this protein tIBRD-1, short for testis-specifically expressed bromodomain containing protein-1. Within the testis, tIBRD-1 is expressed pre-meiotically and colocalizes with ITAFs. By studying tIBRD-1 localization in ITAF mutants, we found that the correct localization of tIBRD-1 depends on the presence of ITAFs. We generated a third-1 mutant via a P-element jumpout experiment and could show that the lack of tIBRD-1 leads to male sterility and a disturbed post-meiotic phenotype. As ProtB and Mst77F are still expressed, it is unlikely that sterility of third-1 mutants is due to chromatin reorganization defects. We suppose that ITAFs and tIBRD-1 act together to regulate transcription of a so far unknown subgroup of genes encoding for proteins required for spermatid differentiation. In which way tIBRD-1 interacts with ITAFs and how these interactions influence the transcription in spermatocytes is subject of further studies.

603C

THO complex is a conserved multi-subunit protein complex that functions in the formation of export-competent ribonuclear protein (mRNP). Although the complex has been extensively studied at the single cell level, yet its exact role at the multicellular organism level have been poorly understood. Here we isolated a novel Drosophila male sterile mutant, called garmcho. Positional cloning indicated that garmcho encodes a subunit of Drosophila THO complex, THOC5. thoc5 mutant fly showed a typical meiotic arrest phenotype during spermatogenesis. In mutant spermatocytes, nuclear structure such as nucleolus and chromatin structure is severely disorganized. A functional GFP tagged fusion protein, THOC5::GFP, revealed its unique localization pattern near the nucleolus. One of the Drosophila tests-specific TAFs (ITAFs), so which is required for the expression of genes responsible for meiotic entry and sperm differentiation was severely mislocalized in thoc5 mutant testis. In addition, the distribution of dJ transcript, a target mRNA of ITAFs, was abnormal whereas its nuclear export itself is not significantly affected in the mutant testis. Taken together, our study suggests that Drosophila THO complex is required for the establishment or maintenance of nuclear integrity in spermatocytes.

604A
Characterizing the function of nmd in mitochondrial morphogenesis and in cytokinesis during spermatogenesis in Drosophila melanogaster. Sarah Catherine Pyfrom1, Leah Smith1, Samantha Lightcap2, Helen White-Cooper2, Karen G. Hales1. 1) Department of Biology, Davidson College, Box 7118, Davidson, NC, 28035; 2) School of Biosciences, Cardiff University, Cardiff, Wales, UK.

The Drosophila melanogaster essential gene nmd codes for a protein in the AAA ATPase family; other proteins within the same subfamily include spastin and katanin, which are involved in microtubule cleavage. Visualization of GFP-tagged Nmd has localized the protein to the mitochondria and centrosome at all stages of spermatogenesis. Male homozygous or transheterozygous for nmd hypomorphic alleles are sterile. Rescue experiments using the nmd-GFP transgene have restored the wild type phenotype in homozygous nmd males. The nmd* allele—associated with a P-element insertion upstream of nmd—shows failure of mitochondria to associate with the meiotic spindle, and absent or small Nebenkern during the onion stage. This phenotype may result from decreased quantities of the wild type protein caused by the P-element insertion in the 5' UTR. A new allele, nmd2973, contains a missense mutation in the ATP-binding domain and results in failure of cytokinesis during meiosis I and/or meiosis II. In this allele, mutant Nmd's putative ATP hydrolysis defect does not impair mitochondrial aggregation, but we may speculate it may interfere with a cytokinesis signal from the the central spindle. Transheterozygotes which contain both nmd2707 and nmd* show the nmd2707 phenotype. Subsequent experiments include assessing the functions of Nmd by using fluorescently-tagged versions of know genes (β-tubulin, Unc and Anillin) to visualize how mutations in nmd affect microtubules, basal bodies and cytokinesis as well as testing the different nmd alleles for temperature sensitivity.
**POSTER: Gametogenesis and Organogenesis**

See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

605B


Prenylation is the post-translational addition of a hydrophobic membrane anchor required of specific proteins that possess a C-terminal CAAX motif (C=cysteine, a=aliphatic, X=almost any amino acid residue). First, the prenyl group is attached to the CAAX motif cysteine by a prenyl transferase. Second, a prenyl protease cleaves the terminal three (-AAAX) amino acids, and finally the terminal cysteine is methylated. Zmpste24 is a prenyl protease that is implicated in a number of human diseases with overlapping symptoms (Hutchinson-Gilford Progeria, Restrictive Dermopathy, Mandibuloacral dysplasia). These diseases have in common a failure to properly process a nuclear structural protein called an A-type lamin, which exhibits transient prenylation under normal circumstances. Zmpste24 is very highly conserved, and apart from functioning in lamin processing, it is also responsible for processing a mating-type pheromone in yeast. Drosophila, uniquely, has triplicated its Zmpste24 homolog. We have undertaken a genetic and molecular characterization of these three genes, as part of a larger initiative to study lamin processing and disease etiology. We have removed all three genes from the Drosophila genome using ends-out gene targeting. Loss of all three Zmpste24 genes in flies does not affect viability but does affect male fertility, indicating a deep evolutionary connection between prenylation and gamete biology.

606C

**Loss of Function Alleles of the p50 Subunit of Dynactin Reveal a Role for Cytoplasmic Dynein in the Post-Meiotic Differentiation of Drosophila Sperm.** Stephanie Skelly, Tatsuhiyo Noguchi, Marta Lenartowska, Jason Duncan. 1) Department of Biology, Willamette University, Salem, OR; 2) Laboratory for Morphogenetic Signaling, Center for Developmental Biology, RIKEN Kobe, Japan; 3) Institute of General and Molecular Biology, Laboratory of Developmental Biology, Nicolaus Copernicus University, Torun, Poland.

Spermatogenesis is a dynamic and complex process that encompasses mitosis, meiosis and extreme cellular morphogenesis to produce mature sperm. We have generated a series of loss-of-function mutations in the p50 subunit of dynactin, a complex required for cytoplasmic dynein function, that have allowed us to identify a role for cytoplasmic dynein during the post-meiotic differentiation of sperm. The p50 mutants have we isolated exhibit various degrees of male sterility. Gross examination reveals that mutant testes are engorged with sperm at their proximal end while the seminal vesicle is largely devoid of sperm. Analysis of early differentiating spermatid cyts reveals that nuclei are not in tight register with the cyst head cell but are instead spread along the length of the cyst, indicative of defects in sperm nuclei clustering. The actin cones of the individualization complex are also grossly misaligned and dispersed, moving asynchronously down the cyst. Transmission electron microscopy of transverse sections through each of the tails of mutant spermatid cysts reveals that the size of the major and minor mitochondrial derivatives is variable, although the structure of the axoneme appears normal. In addition, the orientation of both the axoneme and mitochondrial derivatives are disrupted and randomly arranged in mutant cysts. Finally, multiple axonema within a cyst are routinely encased within a single membrane. Collectively, our p50 mutants exhibit phenotypes consistent with defects in post-meiotic differentiation of sperm.

607A

**Drosophila Z2-5584 line carries a mutation in a nuclear mRNA export gene (rae1) responsible for male sterility.** Silvia Volpi, Barbara T. Wakimoto, Giorgio Prantera. 1) Dept. Agrobiology & Agrochemistry, Univ Tuscia, Viterbo, Italy; 2) Dept. of Biology, University of Washington, Seattle, WA 98195 USA.

In Drosophila melanogaster spermatogenesis, several genetic pathways independently or coordinately act to orchestrate all the meiotic and post-meiotic events. Here, we report the identification of the gene responsible for the phenotype of the (EMS)-induced recessive male sterile Z2-5584 line (Zuker Collection). Living testes of Z2-5584 homozygotes show the same severe phenotype as hemizygotes, suggesting the presence of a null mutation in this Z-line. Immunofluorescence assays revealed early defects of nuclear envelope morphology and chromosome condensation in primary spermatocytes. Upon meiosis, these mutants display a unique behavior since they execute a strongly impaired first meiotic division and skip the second one, resulting into onion stage spermatids that, nonetheless, attempt a highly defective spermiogenesis. A combined strategy of traditional gene mapping techniques, based on complementation analysis, and DNA sequencing, allowed us to identify the locus affected by EMS treatment in rae1 (ribonucleic acid export) gene. Rae1 is part of a ribonucleoprotein (RNP) complex and it has been shown to be required for nuclear mRNA export and mitotic spindle assembly from yeast to mammals. The missense mutation in Z2-5584 line brings about a substitution of an evolutionarily highly conserved amino acid on the third putative WD40-repeat domain. We observed a statistically significant higher mitotic index in hemizygotes versus wildtype, but neither mitotic anomalies in larval brains nor effects on development and viability. As a whole, these results lead us to speculate that the domain affected by the mutation in RAE1 protein is specifically required for meiotic cell cycle, thus providing the first evidence of the RAE1 involvement in Drosophila male germ line differentiation, where its function is strictly necessary to ensure male fertility.

608B

**The cholesterol trafficking protein NPC1 is required for Drosophila spermatogenesis.** Chaow Wang, Zhiguo Ma, Xun Huang. 1) Key Laboratory of Molecular and Developmental Biology,Institute of Genetics and Developmental Biology,Chinese Academy of Sciences,Beijing, China; 2) Graduate School of Chinese Academy of Sciences,Beijing, China.

Niemann Pick C (NPC) disease is a lethal neurodegenerative disorder affecting cellular sterol trafficking. Beside neurodegeneration, NPC patients also exhibit other pleiotropic conditions, indicating that NPC is required for other physiological processes. Previous studies indicated that the sterol shortage and the subsequently the shortage of steroid hormones (for example, ecdysone in Drosophila) are likely the cause of NPC disease pathology. Previously we have reported that mutations in Drosophila npc1, one of the two NPC disease-related genes, lead to male infertility. Here we found that npc1 mutants have multiple defects during spermatogenesis, including cytokinesis, and individualization. Interestingly, we found that ecdysone, the steroid hormone response for the larval lethal phenotype in npc1 mutants, is not required for the spermatogenesis. However, 7-dehydrocholesterol can partially rescue the male infertility of npc1 mutants, suggesting that the sterol shortage is responsible for the spermatogenesis defects. Together, our study reveals a sterol-dependent, ecdysone-independent function of NPC1 in Drosophila spermatogenesis.
POSTER: Immunity and Pathogenesis
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

609C
A screen for modifiers of a tyrosine kinase induced blood cell phenotype identifies new players in the cellular immune response and serves as a model for leukemia. Ines Anderf1,2, Jesper Kronhamn3, Annil Klein1, Dan Hultmark1,2. 1) Institute of Medical Technology, University of Tampere, Tampere, Finland; 2) Department of Molecular Biology, Umeå University, Umeå, Sweden.

The activation of the cellular immune response in Drosophila is not yet well understood. We have previously shown that the overexpression of the receptor tyrosine kinases ALK and PVR in larval sessile and circulating blood cells induces an activated blood cell phenotype that is reminiscent of leukemia. Even though ALK is not expressed in Drosophila larval blood cells, we employ its phenotype to model the role of receptor tyrosine kinase signaling in the activation of cellular immunity and human leukemia. Moderate overexpression of ALK with a blood cell driver leads to the formation of melanotic nodules primarily around the eyes and in the abdomen of adult flies. We used this phenotype to perform an unbiased screen for suppressors and enhancers of this phenotype. We identified 48 modifier regions that comprise approximately 1500 genes and have mapped most regions to the single gene level with the help of RNAi lines and mutants. We are currently applying further tests to elucidate the mechanisms of how these genes affect the activation of the cellular immune response.

610A
PKD2 and DmelCed-12 act in parallel pathways during apoptotic cell clearance. Nathalie C. Franc1,2, Emeline Van Goethem3, Christina Bakatselou2, Hui Xiao1. 1) Present address: The Department of Genetics, The Scripps Research Institute, La Jolla, CA; 2) Medical Research Council Cell Biology Unit. MRC Laboratory for Molecular Cell Biology and Anatomy and Developmental Biology Department, University College London, London, UK; 3) Present address: Institut de Pharmacologie et Biologie Structurale, Toulouse, France.

Apoptosis, a genetically programmed cell death, allows for homeostasis and tissue remodeling of all multi-cellular organisms. Phagocytes swiftly recognize, engulf and digest apoptotic cells. Yet, to date the molecular mechanisms underlying phagocytosis are poorly understood. To delineate the molecular mechanisms of apoptotic cell clearance in Drosophila, we have carried out a deficiency screen and have identified three overlapping phagocytosis-defective mutant alleles, which all delete the fly homologue of the C. elegans gene, DmelCed12 (also known as DmelmElmo). As for its C. elegans and mammalian homologues, ced-12 and elmo, respectively, we found that DmelCed12 is required for apoptotic cell clearance. Its loss-of-function, however, did not solely account for the phenotypes of all three deficiencies, as zygotic mutations and germ line clones of DmelCed-12 had weaker phenotypes. Using overlapping and nearby deficiencies with a genome-wide RNAi screen in S2 cells, we have found that the polycystic kidney disease 2 gene, Pkd2, which encodes a calcium-activated cation channel of the Transient Receptor Potential (TRP) channel family, is also required for phagocytosis of apoptotic cells. We have observed genetic interactions between Pkd2, simu, drpr, ryo-r44F and uta, a gene encoding a MORC-repeat containing molecule, which we recently found to be implicated in calcium homeostasis during phagocytosis. However, we found no genetic interaction between DmelCed-12 and simu. These results demonstrate a role for DmelCed-12 and Pkd-2 in apoptotic cell clearance and argue that Pkd2 functions in the DRPR/UTA pathway to regulate calcium homeostasis, while DmelCed-12 functions in a parallel pathway.

611B
PKD2 and DmelCed-12 act in parallel pathways during apoptotic cell clearance. Nathalie C. Franc1,2, Emeline Van Goethem3, Christina Bakatselou2, Hui Xiao1. 1) Present address: The Department of Genetics, The Scripps Research Institute, La Jolla, CA; 2) Medical Research Council Cell Biology Unit. MRC Laboratory for Molecular Cell Biology and Anatomy and Developmental Biology Department, University College London, London, UK; 3) Present address: Institut de Pharmacologie et Biologie Structurale, Toulouse, France.

Apoptosis, a genetically programmed cell death, allows for homeostasis and tissue remodeling of all multi-cellular organisms. Phagocytes swiftly recognize, engulf and digest apoptotic cells. Yet, to date the molecular mechanisms underlying phagocytosis are poorly understood. To delineate the molecular mechanisms of apoptotic cell clearance in Drosophila, we have carried out a deficiency screen and have identified three overlapping phagocytosis-defective mutant alleles, which all delete the fly homologue of the C. elegans gene, DmelCed12 (also known as DmelmElmo). As for its C. elegans and mammalian homologues, ced-12 and elmo, respectively, we found that DmelCed12 is required for apoptotic cell clearance. Its loss-of-function, however, did not solely account for the phenotypes of all three deficiencies, as zygotic mutations and germ line clones of DmelCed-12 had weaker phenotypes. Using overlapping and nearby deficiencies with a genome-wide RNAi screen in S2 cells, we have found that the polycystic kidney disease 2 gene, Pkd2, which encodes a calcium-activated cation channel of the Transient Receptor Potential (TRP) channel family, is also required for phagocytosis of apoptotic cells. We have observed genetic interactions between Pkd2, simu, drpr, ryo-r44F and uta, a gene encoding a MORC-repeat containing molecule, which we recently found to be implicated in calcium homeostasis during phagocytosis. However, we found no genetic interaction between DmelCed-12 and simu. These results demonstrate a role for DmelCed-12 and Pkd-2 in apoptotic cell clearance and argue that Pkd2 functions in the DRPR/UTA pathway to regulate calcium homeostasis, while DmelCed-12 functions in a parallel pathway.

612C
Phagocytic Ability Changes With Age In Adult Drosophila. Lucas A. Horn, Jeff Leips, Michelle Starz-Gaiano. Biological Sciences, University of Maryland Baltimore County, Baltimore, MD.

Immunosenescence, a decline in the ability of the immune system to clear infections with age, is exhibited in most multicellular organisms, including humans. While physiological changes in the immune system with age are well-known, very little is known about the genetic basis of such changes. To identify genes contributing to natural variation in immunocompetence, we have screened a number of natural genotypes for their ability to clear an artificial infection and identified significant differences among lines in the way that age influences clearance ability. The immune response of Drosophila has two main components, clearance of bacteria by phagocytic blood cells and production of antimicrobial proteins, and age-related changes in either or both of these components underlie general immunosenescence. This study focuses on phagocytosis because engulfment ability declines with age in many organisms and recent studies suggest that it may be the most important component of the innate immune response for clearing infection. We developed a protocol to measure the phagocytic ability of adult blood cells, and found striking changes with age. The protocol involves injecting fluorescently labelled bacteria, followed by dissection of the heart, staining for blood cells and then counting the number of phagocytic events per cell. Our results reveal that the blood cells of five week old individuals contain more phagocytic events than blood cells from one week old individuals, and that this is consistent among different genotypes. Because the assay shows a snapshot in time of phagocytosis in the blood cells, the disparity between ages could be a result of altered phagocytic rate or changes in the ability of the blood cells to destroy bacteria once engulfed. Here we report our results from a series of experiments supporting the latter hypothesis. Moreover, we describe how phagocytosis contributes to overall infection clearance ability among genetic lines. Thus, this project will contribute to our understanding of the cellular and genetic bases of immunosenescence.

613A
Evolution of Toll-Mediated Innate Immunity in Drosophila. Scott Lindsay, Steven Wasserman. Cell & Developmental Biology, Univ. California, San Diego, La Jolla, CA.

In animals ranging from Drosophila to humans, Toll receptors act through Rel family transcription factors to trigger innate immune responses to particular classes of pathogens. To begin exploring how this pathway has evolved in the context of different ecological niches, we have focused our studies on four Drosophila species: amanuasae, melanogaster, mojavensis, and viridis. Using infection with the entomopathogenic fungus Beauveria bassiana and 'next-generation' RNA sequencing, we have carried out a global comparative analysis of the Toll pathway. These studies reveal not only conserved sets of innate immune effectors and core upstream regulatory elements, but also significant divergence in the immune repertoire, including species-specific effectors and significant variation in the composition of effector gene families.

614B
Protease targets and trafficking receptors of the Nec serpin-family inhibitor in the innate immune response. Veronika Miktova, Sandra Soukop, Arantza Sanz-Parra, David
A genome-wide approach to characterize new genes involved in the maintenance of hematopoietic progenitors in the *Drosophila* Lympg Gland. Justine Opavony1, Delphine Pennetier1, Ismael Morin1, Sebastien Djean1, Alain Vincent1, Michèle Crozatier1. 1) Centre de Biologie du Développement, UMR 5547 CNRS, University of Toulouse 3, Toulouse, France; 2) Biostatistic Platform, Genopole, Toulouse, France.

The *Drosophila* lympg gland is a model system for studying hematopoiesis and blood cell homeostasis. This specialized organ is composed of a medullary zone (MZ) containing pro-hemocytes, a cortical zone (CZ) containing differentiated hemocytes and the “Posterior Signalling Center” (PSC). The PSC, a small group of specialized cells, which express the transcription factor Col/Kn, controls hemocyte homeostasis in the lymph gland in a non-cell autonomous manner. The role of the PSC is reminiscent of the “niche”, the micro-environment of hematopoietic stem cells in vertebrates. Several signalling pathways, such as JAK/STAT, Wingless, ROS and Hedgehog, have been shown to be necessary to maintain the pool of pro-hemocytes. How they act and interact with each other remains however little understood. Interestingly, we recently established that Col function during larval development also controls the PSC size, which impacts on the hemocyte homeostasis. By genome-wide transcriptomic analyses of dissected LG from different mutant backgrounds, we identified new genes specifically expressed in the MZ. Functional studies indicate that they are cell-autonomously required to maintain the pro-hemocyte state. Through the analysis of their variation in expression in different signalling mutants, the establishment of a regulatory network controlling the pro-hemocyte state is in progress.

Disappearing Phenotypes: The contribution of diet to immune robustness. Moria C. Chambers, David Schneider. MicroBio & Immunology, Stanford Univ, Stanford, CA.

Previous research established that immune responses can change depending on environmental factors including sleep, mating status, temperature and diet. While this has influenced the field by causing careful control of these factors within a given lab, the fact remains that each lab has a very different environment and immune phenotypes may have limited robustness under varying conditions. Specifically, we are studying the robustness of immune phenotypes during infection with *Listeria monocytogenes* with respect to the nutrition the fly receives. We hypothesize that diet will have a profound effect on the stability of immune phenotypes. The robustness, or lack thereof, will impact both the practical implementation of immunology screens and the interpretation of the results from any given experiment.

**POSTER: Immunity and Pathogenesis**

See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

Gubb. Functional Genetics, CICbioGUNE, Deroio, Bizkaia, Spain.

The Necrotic (Nec) serpin regulates the innate immune response upon fungal or Gram-positive infection in Drosophila. Nec is expressed in fat body and sequestered into the haemolymph where it regulates the extra-cellular protease cascade that activates the Toll pathway. Transgenic Nec has a broad inhibitory range in vitro, with highest affinity for porcine pancreatic elastase. Identifying its target protease(s) in vivo has been however extremely difficult, as Nec is promiscuous and the fly genome has a couple of hundred serine proteases, plus any pathogen encoded proteases that may be introduced following immune challenge. We have recently demonstrated that Nec is rapidly removed from circulating haemolymph by endocytosis in garland and pericardial cells and then degradedated in the lysosomes. This uptake is mediated by two trafficking receptors of the LDLR family, LpR1 and LpR2. Our aim is to identify the target protease of Nec and the part of proteolytic pathway that it regulates. We will also study Nec together with associated proteins in *in vitro* conditions after immune challenge and then we will identify interacting proteins by mass spectrometry. Knowing the interaction partners of Nec should help better understanding of its exact physiological role in immunity. Combining genetic and biochemistry, we hope to identify which of the LpR receptors are responsible for uptake of the native Nec monomer, the Nec protease complex in garland and pericardial cells.

615C

**A genome-wide approach to characterize new genes involved in the maintenance of hematopoietic progenitors in the *Drosophila* Lympg Gland.** Justine Opavony1, Delphine Pennetier1, Ismael Morin1, Sebastien Djean1, Alain Vincent1, Michèle Crozatier1. 1) Centre de Biologie du Développement, UMR 5547 CNRS, University of Toulouse 3, Toulouse, France; 2) Biostatistic Platform, Genopole, Toulouse, France.

The *Drosophila* lymph gland is a model system for studying hematopoiesis and blood cell homeostasis. This specialized organ is composed of a medullary zone (MZ) containing pro-hemocytes, a cortical zone (CZ) containing differentiated hemocytes and the “Posterior Signalling Center” (PSC). The PSC, a small group of specialized cells, which express the transcription factor Col/Kn, controls hemocyte homeostasis in the lymph gland in a non-cell autonomous manner. The role of the PSC is reminiscent of the “niche”, the micro-environment of hematopoietic stem cells in vertebrates. Several signalling pathways, such as JAK/STAT, Wingless, ROS and Hedgehog, have been shown to be necessary to maintain the pool of pro-hemocytes. How they act and interact with each other remains however little understood. Interestingly, we recently established that Col function during larval development also controls the PSC size, which impacts on the hemocyte homeostasis.

**Globally, our study uncovers a new mechanism of virulence of *P. entomophila* and demonstrates that this bacterium has multiple strategies to exert its pathogenesis.**

**Disappearing Phenotypes: The contribution of diet to immune robustness.** Moria C. Chambers, David Schneider. MicroBio & Immunology, Stanford Univ, Stanford, CA. Previous research established that immune responses can change depending on environmental factors including sleep, mating status, temperature and diet. While this has influenced the field by causing careful control of these factors within a given lab, the fact remains that each lab has a very different environment and immune phenotypes may have limited robustness under varying conditions. Specifically, we are studying the robustness of immune phenotypes during infection with *Listeria monocytogenes* with respect to the nutrition the fly receives. We hypothesize that diet will have a profound effect on the stability of immune phenotypes. The robustness, or lack thereof, will impact both the practical implementation of immunology screens and the interpretation of the results from any given experiment.

617B

Entomopathogenic bacterium *Pseudomonas entomophila* causes a global translation inhibition in *Drosophila melanogaster* gut triggered by the host oxidative stress response. Svetla Chakrabarti, Nicolas Buchon, Bruno Lemaître. Global Health Institute EPFL/SV/GHI, Lausanne, Switzerland.

Understanding the relationship between pathogens and their hosts is a major challenge and has important medical implications. During the course of evolution, pathogens have evolved a host of strategies to avoid or evade the host immune response. This includes the use of specific proteases, plus any pathogen encoded proteases that may be introduced following immune challenge. We have recently demonstrated that Nec is rapidly removed from circulating haemolymph by endocytosis in garland and pericardial cells and then degradedated in the lysosomes. This uptake is mediated by two trafficking receptors of the LDLR family, LpR1 and LpR2. Our aim is to identify the target protease of Nec and the part of proteolytic pathway that it regulates. We will also study Nec together with associated proteins in *in vitro* conditions after immune challenge and then we will identify interacting proteins by mass spectrometry. Knowing the interaction partners of Nec should help better understanding of its exact physiological role in immunity. Combining genetic and biochemistry, we hope to identify which of the LpR receptors are responsible for uptake of the native Nec monomer, the Nec protease complex in garland and pericardial cells.

**Drosophila immune responses to entomopathogenic nematodes and their symbiotic bacteria.** Ioannis Eleftherianos, Julio Cesar Castillo. Department of Biological Sciences, Institute for Biomedical Sciences, The George Washington University, Washington, D.C.

*Drosophila* is an excellent model for studying host-pathogen interactions and immunity. The bacteria *Photorhabdus* and *Xenorhabdus* live in a “symbiosis of pathogens” with the entomopathogenic nematodes, *Heterorhabditis* and *Steinernema*, respectively, which invade and kill insects. Unlike other animals associated with bacterial symbionts, these nematodes are viable in the absence of their symbiotic bacteria. Consequently, each partner of this symbiotic/pathogenic relationship can be separated and studied in isolation or in combination, thus enabling pathogenesis and symbiosis to be investigated separately or together. We recently began to use the powerful genetics of *Drosophila* to dissect the molecular and evolutionary basis of insect immunity, bacterial symbiosis/pathogenicity and nematode parasitism, and to understand the basic principles of the complex interactions between these important biological processes. The great advantage of this unique tripartite system is that all three players (insect, bacterium, and nematode) in the interaction can collaborate in different ways, which means that we can study the specific contributions of each player and their interactions.

619A

**Drosophila immune responses to entomopathogenic nematodes and their symbiotic bacteria.** Ioannis Eleftherianos, Julio Cesar Castillo. Department of Biological Sciences, Institute for Biomedical Sciences, The George Washington University, Washington, D.C.

*Drosophila* is an excellent model for studying host-pathogen interactions and immunity. The bacteria *Photorhabdus* and *Xenorhabdus* live in a “symbiosis of pathogens” with the entomopathogenic nematodes, *Heterorhabditis* and *Steinernema*, respectively, which invade and kill insects. Unlike other animals associated with bacterial symbionts, these nematodes are viable in the absence of their symbiotic bacteria. Consequently, each partner of this symbiotic/pathogenic relationship can be separated and studied in isolation or in combination, thus enabling pathogenesis and symbiosis to be investigated separately or together. We recently began to use the powerful genetics of *Drosophila* to dissect the molecular and evolutionary basis of insect immunity, bacterial symbiosis/pathogenicity and nematode parasitism, and to understand the basic principles of the complex interactions between these important biological processes. The great advantage of this unique tripartite system is that all three players (insect, bacterium, and nematode) in the interaction can collaborate in different ways, which means that we can study the specific contributions of each player and their interactions.
be genetically manipulated and the availability of the genome sequences for the three organisms facilitates functional genomics comparisons, reverse genetics screens and systems biology-based approaches. Finally, such studies set the scene for revealing not only how pathogens evolve virulence but also how two pathogens can come together to exploit a common host.

620B

Pseudomonas aeruginosa RhlR is required to neutralize the cellular immune response in a Drosophila melanogaster oral infection model. Dominique X. Ferrandon1, Stefanie Limmer2, Samantha Haller3, Janice Lee2, Rhonda Feinbaum4, Christine Kocks4, Frederick Ausubel3, 4. 1) IBMC, CNRS UPR 9022, Strasbourg, France; 2) Department of Pediatrics, Harvard Medical School; 3) Department of Genetics, Harvard Medical School; 4) Department of Molecular Biology, Massachusetts General Hospital, Simchess Research Building CPZN7250, 185 Cambridge St., Boston, MA 02114, USA.

An in-depth mechanistic understanding of microbial infections necessitates a molecular dissection of host-pathogen relationships. D. melanogaster and Pseudomonas aeruginosa PA14 have both been intensively studied. Here, we analyze infection of Drosophila by PA14 using mutants in both host and pathogen. We show that orally ingested PA14 crosses the intestinal barrier and that it proliferates in the hemolymph. Unlike other oral infection models, flies succumb to systemic PA14 infection, i.e., bacteremia. Host defenses against ingested PA14 include an Immune deficiency (IMD) response that takes places in the intestinal epithelium, systemic Toll and IMD pathway responses, and phagocytosis of bacteria in the hemocoel by hemocytes. While phagocytosis and the local intestinal immune response act throughout the infection, there was a late onset of the systemic IMD and Toll responses. PA14 does not require its type III secretion system or other well-studied virulence factors such as the two-component response regulator GacA or the protease AprA for virulence in this oral infection model. In contrast, the quorum sensing transcription factor RhlR, but surprisingly not LasR (the LasR quorum sensing system function upstream of RhlR in vitro), plays a key role in circumventing the cellular immune response against PA14, possibly at an early stage when only a few bacteria are present in the hemocoel. These results illustrate the power of studying infection from the dual perspective of host and pathogen by revealing that RhlR plays a more complex role during pathogenesis than previously appreciated. Our work underscores the fact that the bacterial genetic circuitry that has been established in vitro may not be relevant in vivo.

621C

The Serine Protease Inhibitor SpnI is involved in immunity to fungal infections through the pattern-recognition signalling pathway. Susana Garcia-Sanchez, Ane Fullaondo, Arantza Sanz Parra, So Young Lee, David Gubb. CIC Biogune, Derio, Vizcaya, Spain.

Serpins are a large family of protease inhibitors with a conserved fold, but divergent functions in mammals and invertebrates. In flies, the Necrotic (nec) serpin has long been identified as a component of the protease cascades that is triggered in the haemolymph upon immune challenge. This extracellular cascade leads to the activation in the fat body of the transmembrane receptor Toll-1, which induces the transcription of anti-microbial peptides (AMPs) as a defense against pathogenic microorganisms. In an RNAi screen to discover novel Drosophila serpins with immunity-related functions, we identified Serpinl (Spnl, C04560). RNAi knockdown of the Spnl transcript activates expression of Drosomycin (Dsc), the main AMP induced through the Toll pathway after pathogen challenge. Over-expression of Spnl results in reduced levels of dsc after fungal, but not bacterial infection. Since the Toll receptor can be activated through two separate side-branches, we performed epistatic analysis of Drs-GFP activation to localize Spn1 in the pathways. For these experiments we isolated a null allele of Spn1, SpnlP1. Our epistatic analysis places Spnl downstream of the GNBP3 receptor of fungal cell-wall and upstream the Grass protease. Spn1 is upstream the Spre protease, which is common to both the danger-signalling branch (repressed by Nec) and the pattern recognition (PRR) branch. The Spnl-/- mutant shows decreased viability after B. bassiana infection, which demonstrates the necessity of this serpin for a proper modulation of immune response. Some of our data support a role of Spnl in the repression of immune response in the first hours after fungal infection. Co-immuno precipitation experiments are underway to discover the proteins that interact with Spnl during immune challenge and which modulate the fly defense mechanisms.

622A

Anthrax toxins cooperatively inhibit endocytic recycling by the Rab11/Sec15 exocyst. Annabel E. Guichard, Beatriz Cruz-Moreno, Adrianne Kurciyan, Ethan Bier. Dept Biol, Univ California, San Diego, La Jolla, CA.

Anthrax, a deadly infectious disease, is still a major threat to cattle in underdeveloped countries, and affects humans occasionally. *Bacillus anthracis*, the Gram-positive bacterium that causes anthrax, achieves infectivity through secretion of two toxic factors: Lethal Factor (or LF), and Edema factor (or EF), which enter most host cells to silence various aspects of the immune response, and cause fatal cardiovascular collapse in late stages of the disease. LF is a protease that cleaves and inactivates most MAPKs, while EF is a potent Adenylate Cyclase. Although anthrax is not known to affect insects, we showed previously using transgenic lines expressing either of these toxins that both EF and LF affect conserved *Drosophila* targets (PKA and MAPKKs, respectively) and induce expected developmental phenotypes. More recently, we used these lines to uncover novel activities of EF and LF: they affect the endocytic trafficking of the Notch ligand Delta and the homophilic adhesion protein E-cadherin, which normally co-localize at the adherens junctions in various epithelia. EF alters the distribution and levels of the small GTPase Rab11, which controls traffic through recycling endosome. To promote fusion of vesicles from the recycling endosome with the plasma membrane at specific sites, Rab11 interacts with its effector Sec15, which then recruits other components of the exocyst complex. We found that both LF and EF prevent formation of Sec15-rich vesicles, suggesting that the exocyst acts as a connecting point that implements the activities of these toxins, and mediate their effects on Notch signaling and cell-adhesion. We propose that these effects are relevant to the vascular leakage induced by anthrax toxins during late stage anthrax. Here we present additional mechanistic data on how EF and LF achieve synergism to inhibit the Rab11/Sec15 exocyst.

623B

The interactions of gut microbiota and the innate immune system in response to pathogenic infection. Virginia M Howick, Adam CN Wong, Brian P Lazzaro, Angela E Douglas. Cornell University, Ithaca, NY.

Drosophila melanogaster ingests beneficial, commensal, and pathogenic microbes. The fate of various microorganisms in the gut is determined by complex interactions between the microorganisms and the fly, and can be mediated by immune system function in the gut. We hypothesized that interactions between the resident microbiota and the gut immune system provide a protective mechanism against a range of pathogens. We tested this hypothesis through infection of axenic (germ-free) and conventionally reared flies with a spectrum of pathogenic bacteria and monitored survival, bacterial load, and gene expression. Our results indicate that the resident microbiota is actively involved in the protecting the fly against bacterial pathogens.

624C

Expression and specificity of a candidate immune recognition protein in Drosophila. Erin S. Keebaugh, Todd A. Schlenke. Dept Biol, Emory Univ, Altanta, GA.

We are interested in determining how metazoan hosts, such as Drosophila, can distinguish themselves from metazoan parasites in the absence of acquired immune system components. To gain a better understanding of non-self recognition between eukaryotes we study the interaction between fruitflies and one of their natural metazoan pathogens, parasitic wasps. The method by which Drosophila hosts kill parasitic wasps is melanotic encapsulation, where host hemocytes form a multi-layered capsule around the parasite and release free radicals to kill the entrapped parasite. As a first step in the encapsulation response, the host must be able to recognize the parasite as foreign. We are interested in identifying the immune receptors Drosophila use to identify parasitic wasps as non-self, and uncovering the evolutionary history of such genes as a first step towards understanding the selective forces parasites impose on their hosts in nature. Microarray analysis of Drosophila larva post-wasp attack identified several promising candidate immune receptors. We have found that one such candidate wasp egg receptor shows signs of recent and recurrent selective pressures in D. melanogaster and D. simulans. Expression analysis of this candidate immune receptor shows enriched expression in immune tissue and a wasp-specific regulatory response. We are currently investigating the encapsulation potential and hemocyte reaction of wasp-attacked D. melanogaster strains expressing mutant levels of this candidate receptor as well as determining the receptor’s binding specificity.
The role of low protein diets and nutrient signaling in the pathogenic survival of Drosophila. Jung-Eun Lee¹, Isaac Edery¹, Scott Fletcher¹. 1) Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI; 2) Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ.

Dietary restriction (DR), reduced nutritional uptake without causing malnutrition, has been proven to be the most powerful intervention extending life span from yeast to humans. As part of its beneficial effects, DR also enhances some aspects of rodent immunity. However, the functional consequences of such an enhanced immunity are controversial. On the other hand, moderate protein restriction without reducing the total calorie intake has been documented to provide a significant protection for mice against pathogenic infections such as hepatitis B virus or malaria. We sought to determine if protein restriction would also benefit pathogenic survival of Drosophila melanogaster via dTOR pathway that senses the availability of intracellular amino acids. Drosophila lab food consists of Baker’s yeast and sucrose, a main source for protein and carbohydrate, respectively. For 2 days, young female flies were kept on experimental food that contains a combination of 1, 3, or 9% of sucrose and Baker’s yeast. On the third day, they were infected with Pseudomonas aeruginosa. Their survival rates and bacterial growth were monitored over the next 3 days. We found that low yeast diets greatly improve survival over Pseudomonas infections and suppress bacterial growth, in vivo irrespective of the amount of sucrose in diets, suggesting evolutionarily conserved effects of protein restriction on innate immunity. Nutrient signaling mediates these diet effects: rapamycin, the best characterized inhibitor of target of rapamycin (TOR), ameliorates the poor pathogenic survival outcomes of flies on high yeast diets correlated with a high TOR activity. We are currently testing which aspects of Drosophila innate immunity are responsible for the beneficial effects of low yeast diets and rapamycin. This study will provide some insight into the genetic networks mediating the effects of protein restriction on boosting the ability of animals to combat pathogenic infections.

JNK/FOXO signaling regulates the expression of Drosophila peroxiredoxin V during immune response. Kyu-Sun Lee¹, Hye-Mi Ahn¹,², Dong-Seok Lee², Kweon Yu¹. 1) Aging Res Ctr, KRIBB, Daejeon, Korea. 2) College of Natural Sciences, Kyungpook National University, Daegu, Korea.

Innate immunity plays an important role in combating microbial infection in all animals. In Drosophila gut, Dual oxidase (Duox) produces sufficient amounts of ROS, a potential bacterial-killing molecule, to combat the infectious microbe. Concurrently, antioxidant systems are activated for the elimination of residual ROS in order to protect the host. Here we identified the Drosophila peroxiredoxin V (dPrxV) as an immune-related antioxidant enzyme for maintaining the intestinal redox homeostasis. We found that dPrxV was predominantly expressed in gut and induced by oral infection with Erwinia carotovora carotovora (Ec15) strains. In Drosophila gut, JNK/FOXO signaling was activated and the expression of dPrxV was significantly increased by infection with Ec15. The expression of dPrxV was also increased by gut-specific overexpression of Duox, while decreased by knockdown of Duox. Moreover, the activation of JNK/FOXO signaling in gut increased the dPrxV expression. These results suggest that JNK/FOXO signaling plays a critical role in regulating the expression of antioxidant system for protecting the host gut epithelial cells from oxidative stress.

The population genetics of Drosophila viruses in the wild. Darren J Obbard, Claire L. Webster, Ben Longdon. Institute of Evolutionary Biology and Centre for Immunity, Evolution and Environment, University of Edinburgh, Edinburgh, UK.

Population-genetic analyses have shown that antiviral RNAi genes such as Dicer-2, Argonaute-2 and R2D2 have highly elevated rates of protein evolution in multiple species of Drosophila, and that this evolution can be attributed to the action of natural selection. Although the selective agent remains unknown, the key role that these genes play in antiviral immunity makes viral pathogens a strong candidate driver, and it is known that a broad spectrum of RNA viruses actively suppress or block RNAi in many other systems. To explore whether the antiviral RNAi pathway is engaged in a reciprocal host-parasite arms-race with common viral pathogens, we have sequenced complete genomes of two of the most common horizontally-transmitted Drosophila viruses (Drosophila A Virus and Drosophila Nora Virus) from multiple Drosophila populations around the world. These data show that synonymous-site diversity is high in both DAV and Nora Virus, and that recombination is widespread. However, non-synonymous diversity is relatively low, and no viral genes are clear ‘hot-spots’ of adaptive protein evolution, perhaps suggesting that these viruses are not engaged in a tight reciprocal arms-race. An alternative hypothesis is that the rapid evolution in host genes is driven by the frequent need to adapt to the emergence of new viral pathogens, such as those acquired from other species. To help estimate a time-scale for the co-ancestry of extant viruses, we have also sequenced samples from endemic laboratory populations of Nora virus to estimate the mutation rate.


The housefly and flies in general are considered to be mechanical vectors of many kinds of pathogens such as bacteria, protozoa and viruses, whereas the mosquito serves as the biological vector for those pathogens. Mechanical vectors simply convey pathogens and are not essential for their development and life cycle. It has been shown that a large number of bacteria adhere to the surface of the housefly mouthparts, actively proliferate in the minute spaces of the labellum, and accumulate in the crop. In order to uncover the biological vector for those pathogens, we have also sequenced samples from endemic laboratory populations of Nora virus to estimate the mutation rate.
Hemolymph clotting and immunity. Ulrich Theopold1, Zhi Wang1, Pavel Dobes1,2, Pavel Hyrsl1,2. 1) Department of Molecular Biology & Functional Genomics, University of Stockholm, 10691 Stockholm, Sweden; 2) Department of Animal Physiology & Immunology, Institute of Experimental Biology, Masaryk University, 61 37 Brno, Czech Republic.

We are interested in the clotting of insect hemolymph. Clotting in Drosophila involves the precipitation of clotting factors and crosslinking of the clot. The latter process is mediated through the activity of the two enzymes transglutaminase and phenoloxidase. Our recent work shows that in addition to sealing wounds clotting also has a major function in protecting Drosophila larvae from infections. Larvae with reduced expression of either clotting factors or transglutaminase are more susceptible to infections with certain bacteria. The most significant effects were observed when we infected larvae using a natural infection model involving entomopathogenic nematodes and their associated bacteria. Our most recent results show that while single mutants in several previously characterized immune pathways (such as the imd pathway) behave similar to normal larvae, double mutants succumb faster to the infection. For example Bc/imd double mutants show immune defects. Using inhibitors of eicosanoid biosynthesis and knockdown lines specific for candidate genes involved in eicosanoid production we also provide evidence that lipid mediators might play a role in insect immunity. Finally, we also show that blood clots in mammals contribute to immunity.

631A The enemy of my enemy is my friend: Pseudomonas aeruginosa selected for resistance to lytic bacteriophage are less virulent to their Drosophila host. Heather Wilson1, Jason Behnke1, Aaron Moiser2, Nathaniel Cady2, Ing-Nang Wang1, Kurt McKeen1. 1) State University of New York at Albany, Biological Sciences; 2) State University of New York at Albany, College of Nanoscale Science & Engineering.

Pathogen fitness is determined not only by interaction with its primary host, but also interaction with competitors and hyperparasites as well as performance in non-host environments. Adaptation in response to these diverse selective pressures may be constrained by the pleiotropic effects of traits improving performance in one setting while reducing performance in another. Such trade-offs may be important in determining the precise nature of the interaction between a pathogen and its primary host, including patterns of pathogen virulence. To test this hypothesis we used experimental evolution to select populations of the opportunistic pathogen Pseudomonas aeruginosa for resistance to a hyperparasite, a type IV pilin-binding lytic bacteriophage (phage). Populations quickly evolved phage resistance and we determined that the likely mechanism of resistance was the selective loss of type IV pili. In infections established in a Drosophila melanogaster host, phage-resistant lines were less virulent and exhibited reduced within-host population growth than the ancestral phage-susceptible strain. The loss of type IV pili leads to loss of twitching motility and reduced biofilm formation, both of which are known P. aeruginosa virulence factors, and both of which were sharply reduced in our phage-resistant strains. In addition, phage-resistant strains showed reduced population growth in nutrient poor, but not nutrient rich, media. Overall our results indicate that pathogen adaptation in response to selection by a natural enemy, a lytic bacteriophage, may affect virulence in its primary host. Furthermore, the observed trade-off may be understood through the specific pleiotropic effects of the evolved mechanism of defense.

632B Ecdysone Control of Innate Immune Responses. Neal Silverman1, Florentina Rus1, Thomas Flatt2,3, Marc Tatar2. 1) Med/Div Infectious Dis, Univ Massachusetts Med Sch, Worcester, MA; 2) Ecology and Evolutionary Biology Brown University, Providence RI; 3) Institute of Population Genetics Department of Biomedical Sciences University of Veterinary Medicine Vienna.

In insects, the steroid hormone 20-hydroxy-ecdysone (20E) is well known for its role in regulating development and reproduction. In addition, previous studies have shown that 20E regulates the IMD pathway, one of the central innate immune response pathways in insects. In particular, 20E was required to create an environment conducive to the robust induction antimicrobial peptide (AMP) genes following microbial challenge. However, the molecular mechanisms by which this steroid hormone modulates immune responses remain unclear. Here we identify two distinct mechanisms by which 20E controls Drosophila immune responses. First, 20E regulates the expression of the peptidoglycan receptor PGRP-LC, in cultured cells and in vivo, and thereby has a profound effect on all IMD responses. Through a second mechanism, 20E regulates the expression of a subset of AMP genes (e.g. Diptericin, Metchnikowin or Drosomycin), independent of its control of the receptor PGRP-LC. We have identified three ecdysone-inducible transcription factors - br-c, Eip93F and Eip78C that play a critical role in IMD signaling. Depletion of any one of these factors nearly abolishes 20E-mediated expression PGRP-LC. However, br-c alone is required to mediate the PGRP-LC-independent effects of 20E. Consistent with the study of these ecdysone-inducible transcription factors in S2* cells, knock-down of br-c or Eip78C in adult flies caused significantly reduced expression of the AMP genes and increased susceptibility in response to bacterial infection. Together, these results show that 20E uses complex mechanisms to modulate innate immune responses, both in adults and in cultured cell lines, and these regulatory networks involve a subset of the ecdysone-inducible transcription factors. Moreover, these ecdysone-controlled regulatory circuits play a critical role modulating immune responses in the adult animal.

633C Imbalance Effects of fat metabolism to immune responses in Drosophila. Kyung Han SONG, David Schneider. School of Medicine, Department of Microbiology and Immunology, Stanford University, Stanford, CA.

My goal is to understand whether metabolic disruptions that occur during infections aid survival or contribute to pathology. I use an infection model in which I challenge fruit flies with Listeria monocytogenes. Preliminary work suggested that fatty acid biosynthesis decreases during infections. I followed up on this work by testing mutants in this biosynthetic pathway for immune phenotypes. Triacylglycerol is synthesized by the addition of fatty acids to a glycerol backbone by fatty acyl transferases. I tested three of these enzymes and found a difference in their immune behavior. Normally Drosophila melanogaster is able to control the extracellular growth of L. monocytogenes through two innate immune effectors, antimicrobial peptides and reactive oxygen intermediates. Mutations in the enzymes that convert monoaoylglycerol to diacylglycerol (CG4729, CG4753) did not affect extracellular growth of L. monocytogenes. However, mutations in the enzyme which converts diacylglycerol to triacylglycerol (mdy) permitted much more extracellular growth. And I monitor levels of triacylglycerol and it was lower than normal because mutation of mdy prevented diacylglycerol from being converted to triacylglycerol. Interestingly, mdy injected L. monocytogenes lost almost their triacylglycerol and were dead. We find that decrease of triacylglycerol is related to shorten life span and disruption of fatty acid biosynthesis can cause pathogenesis in the flies.
POSTER: Immunity and Pathogenesis
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

634A
Ninjurin A and Drosophila Immunity. Sarah M. Broderick, Andrea Page-McCaw. Cell and Developmental Biology, Vanderbilt University, Vanderbilt Medical Center, Nashville, TN.

Drosophila Ninjurin A is a two-pass transmembrane protein homologous to the Ninjurin family of proteins in mammals. Ninjurin A is up-regulated in response to a variety of stress stimuli and immune challenge in both mammals and Drosophila. The conservation of both the protein and expression patterns of Ninjurin A suggests that Drosophila is a good model to investigate the function of Ninjurin A in vivo. Previous studies in the lab using Drosophila S2 cell culture have shown that Ninjurin A over-expression signals a loss-of-adhesion in an Mmp1 dependent manner. To investigate the function of Ninjurin A in vivo we generated a null mutant by P-element mutagenesis. We have not observed any phenotypes in a Ninjurin A null mutant, and we are currently assaying a loss-of-function mutant knocked down simultaneously for the three Ninjurin family members. In wild-type animals we have observed dramatic increases in Ninjurin A expression in response to immune challenge in the blood cells and fat body of larvae. Using the binary GAL4-UAS system we have mimicked the up-regulation Ninjurin A in response to immune challenge in these immune response organs. The over-expression of Ninjurin A in the fat body (similar to the mammalian liver) was embryonic lethal. The over-expression of Ninjurin A using the hemolectin-GAL4 driver, which expresses in the hemocytes (similar to mammalian macrophages), led to melanotic masses and malformed lymph glands. Closer inspection of the malformed lymph glands suggested massive cell death may cause the malformation. Many mutants display melanotic masses in larvae including Toll gain-of-function mutants. Ninjurin A is also up-regulated in Toll gain-of-function mutants suggesting it is downstream of the Toll immune response pathway. We are currently taking advantage of the many GAL4 drivers available to drive expression of Ninjurin A in non-essential organs like the eye or wing to better understand the function of Ninjurin A in vivo.

635B
Differential activity of deubiquitinating enzymes regulate Imd ubiquitination and immune signalling. Marie-Odile Faivre-1,2, Elodie Engel1,2,3, Magda Mortier1,2,3, Emmanuel Taillebourg1,2, Dominique Thevenon1,2,3. 1) Inst. of life science research and technologies, CEA-Grenoble, Grenoble, France; 2) Inserm, Grenoble, France; 3) UJF, Grenoble, France.

Ubiquitination emerged as a key mechanism controlling either the activity or the stability of proteins in many cellular processes. While K48-polyUb chains direct proteins for proteasomal degradation, K63-polyUb modify protein activity and promote protein-protein interaction. As such, deubiquitinating enzymes (DUBs) can act as major regulators of cell signals although their functions or substrates are still poorly described. Ubiquitination notably regulates several steps of signal transduction to Nuclear Factors-kappaB (NF-kB) controlling stress and immune response. In Drosophila, the Immune deficiency (Imd) pathway is induced by Gram-negative bacteria peptidoglycans binding to the receptor PGRP-LC which associates with Imd, then inducing a kinase signalling cascade leading to the activation of the NF-kB like factor Rel and of antimicrobial peptide genes expression. To determine which DUBs would act on this pathway, we created a dsRNA library to silence each of the twenty-one ubiquitin specific proteases (USPs) and two ubiquitin and (three IUCHs) identified in the Drosophila genome. Screening this library in immune competent S2 cells detected three USPs down-regulating the Imd pathway, including USP36/Sony. Tissue directed gene extinction of each of these USPs provoked a constitutive deregulation of immune signalling in either the fat body or the gut or in both immune organs. Further biochemical analysis showed that Imd is ubiquitinated by both K48 and K63-polyUb chains and that USP36 stops signal transduction by hydrolysing K63-polyUb on Imd. Moreover, USP36 competes with one of the two other USPs identified in the screen to directly interact with Imd and differentially remove either K63- or K48-polyUb. Unexpectedly, while playing opposite biochemical function on Imd, both USPs downregulate Imd-dependent signalling suggesting that ubiquitin chains editing is required for signal transduction.

636C

In Drosophila, the Toll signalling pathway is known as a regulator of dorsal-ventral patterning during embryogenesis, and it also has important roles in regulation of immunity. Activation of Toll pathway results in the nuclear accumulation of Dif and Dorsal and activation of many different target genes. The current model is that Cactus binds to Dorsal and Dif in the cytoplasm and thereby inhibits their nuclear translocation. Cactus has been considered a strictly cytoplasmic protein that is degraded upon Toll signaling. We investigated the sub-cellular distribution of Cactus, in larval fat body and Drosophila S2 cells in response to Toll signaling. Our results indicate a more differentiated regulation and role of Cactus. Immunostaining and Western blot analysis revealed that Cactus is distributed in both the cytoplasm and nucleus of fat body cells in uninfected larvae. Upon immune induction, the relative nuclear-cytoplasmic distribution of Cactus changes, revealing a primarily nuclear presence of Cactus 30 min post-induction. Similar results were obtained in Drosophila S2 cells.

637A
Blood cell homing and self-renewal in the Drosophila larval hematopoietic system. Kalpana Makhiijani1, Brandy Alexander2, Tsusba Tanaka1, Atsushi Miyawaki1, Katja Brückner1,2,3. 1) Department of Cell and Tissue Biology, University of California San Francisco, 513 Parnassus Ave, San Francisco, CA, USA; 2) Department of Anatomy, University of California San Francisco, 513 Parnassus Ave, San Francisco, CA, USA; 3) Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California San Francisco, 513 Parnassus Ave, San Francisco, CA, USA; 4) RIKEN Brain Science Institute, 2-1 Hirosawa, Wako, Saitama, Japan.

Understanding the mechanisms that govern cell colonization and self-renewal of differentiated cells is central in developmental biology and regenerative medicine. Here, we describe Drosophila larval hematopoiesis as a model to address these questions. In contrast to embryonic and lymph gland hematopoiesis, larval hematopoiesis is based on blood cell homing to resident hematopoietic sites. Surprisingly, larval hematopoiesis solely depends on the expansion of differentiated embryonic hemocytes that leave quiescence and self-renew. We demonstrate that resident hemocyte clusters in segmentally organized lateral patches and in the terminal segment are sites of elevated hemocyte proliferation, implying a role for attractive, inductive and trophic cues from neighbouring tissues, or microenvironments. Here we describe the identification of the attractive microenvironment for larval hemocytes. Using loss- and gain-of-function conditions that disrupt the microenvironment or that lead to supemmary ectopic microenvironment cells, respectively, we demonstrate functional dependence of larval hemocytes regarding their homing pattern and trophic survival. Drosophila larval hematopoiesis parallels blood cell homing in vertebrate hematopoiesis and allows to genetically dissect mechanisms of hematocyte self-renewal in the differentiated state, blood cell homing, and the role of the microenvironment in these processes.
Differential Gene Expression Related to Nora Virus Infection of Drosophila melanogaster. Ethan Cordes, Kimberly Carlson. Biology Department, University of Nebraska at Kearney, Kearney, NE.

Investigating the host relationships of certain viruses is useful for understanding possible threats to human, animal, or plant health. Nora Virus is a recently discovered RNA picorna-like virus that has been shown to produce a persistent infection in Drosophila melanogaster. Unfortunately, the regulation of the virus or the genes that the virus interacts with is unknown. In fact, very little is known about this novel D. melanogaster virus. With this in mind, we performed cDNA microarray analysis comparing the gene expression profiles of Nora Virus infected and uninfected wild-type D. melanogaster. This analysis yielded 57 genes having a 1.5 fold change or greater and p-value less than 0.01. Of these genes, 45 were significantly up-regulated and 12 were down-regulated in response to infection. To validate the microarray results, qRT-PCR was performed with probes for Chorion protein 16 (C16p), which showed a three fold up-regulation on the microarray, and Troponin C isoform 4 (Tpc4), which showed a three fold down-regulation. This genome-wide expression profile of Nora Virus infection of D. melanogaster can pinpoint genes of interest for further investigation of antiviral pathways employed, genetic mechanisms, sites of replication, viral persistence, and developmental effects. The project described was supported by the NIH grant number P20 RR016469 from the INBRE Program of the National Center for Research Resources.

Wolbachia promote stem cell division and display tropism for the germline stem cell niche. Eva M. Fast1, Michelle E. Toomey1,2, Kanchana Panaram1, Barrett Steinberg1, Horacio M. Frydman1,2. 1) Biology, Boston University, Boston, MA; 2) National Emerging Infectious Disease Laboratory, Boston University, Boston, MA.

Wolbachia are obligatory intracellular bacteria that infect up to 70% of insect species of which are vectors for the transmission of devastating infectious diseases. Since Wolbachia’s main mode of transmission is vertical through the female germline the bacteria is under selective pressure to reach the germline and infect the next generation. The molecular and cellular mechanisms of Wolbachia transmission and germline targeting are poorly understood. Our lab has shown that stem cell niches in the Drosophila ovary play an important role in Wolbachia transmission and manipulation of host reproduction. In particular by screening multiple species in the Drosophila genus we have identified two main types of stem cell niche targeting in the Drosophila ovary; targeting of the germline stem cell niche and somatic stem cell niche (1) and targeting of the somatic stem cell niche only (2). We compared the Wolbachia species (wMau) present in Drosophila mauritiana as an example for germline stem cell niche and somatic stem cell niche tropism (1) to the Wolbachia species (wMel) present Drosophila melanogaster as an example for somatic stem cell niche tropism (2). Using quantitative image analysis we established a developmental profile showing that these two Wolbachia species have distinct patterns of accumulation and cellular tropisms in the embryo, larvae and pupae. This leads to infection of the stem cell niche and germline at different time points during development. In the adult germarium, for both Wolbachia species, the escort cells play a role in transmission to the germline. Furthermore our data show that stem cell niche tropism can be a source of Wolbachia into the germline but the reverse is not applicable.

Wolbachia determine differential stem cell niche tropism in the Drosophila ovary and testes. Michelle E Toomey1,2, Kanchana Panaram1, Eva Fast1, Catherine Beatty1, Horacio Frydman1,2. 1) Biology, Boston University, Boston, MA; 2) National Emerging Infectious Disease Laboratory (NEIDL), Boston University, Boston, MA.

The intracellular bacteria Wolbachia infect up to 70% of all insect species. Even though Wolbachia infections are the largest pandemic on this planet, the cellular and molecular mechanisms for bacterial spreading in the Drosophila ovary are still unknown. Typically, Wolbachia are vertically transmitted through the female germline. We have previously reported that Wolbachia target the somatic stem cell niche in the Drosophila melanogaster ovary, facilitating germline infection and contributing to vertical transmission. To assess if niche targeting is an evolutionarily conserved mechanism across the Drosophila genus, we investigated niche targeting in ecologically diverse Wolbachia strain-Drosophila species pairs. Our data revealed different types of niche tropism among naturally infected Drosophila species: 1) somatic stem cell niche tropism; 2) germline stem cell niche tropism; 3) hub tropism in the testes. Each host-bacteria pair displays qualitative and quantitative differences in niche tropisms. Furthermore, phylogenetic analyses suggest that the different patterns of niche tropism are more closely related to the Wolbachia strain than the host species. Using transinfection and hybrid introgression crosses we confirm that bacterial factors play a major role in determining the characteristics of stem cell niche tropism in both the female and male stem cell niches. This work highlights a widespread targeting of stem cell niches in the Drosophila genus, contributing to Wolbachia transmission in nature.
The role of tailless in the adult pars intercerebralis in Drosophila aggression. Krystle J. Nomie\textsuperscript{1}, Herman Dierick\textsuperscript{1,2,3}. 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX; 3) Department of Neuroscience, Baylor College of Medicine, Houston, TX.

Aggressive behavior is pervasive throughout the animal kingdom; however, little is known about the molecular underpinnings or circuitry that regulate this complex behavior. Furthermore, the conservation of aggression and the mechanistic similarities of aggression between distantly related animal species are unknown. We previously developed a novel two-male arena assay that allows the rapid and reproducible quantification of aggressive behavior in different strains of Drosophila. Here, we investigated whether the mechanisms of aggression in Drosophila are conserved with mammalian mechanisms that regulate aggression. To this end, we analyzed the role of the orphan nuclear receptor tailless (tl) in fly aggression. A homozygous deletion of the mammalian ortholog of tl, Nr2e1, causes extreme aggression in mice, and this phenotype is rescued using a human genomic clone covering the orthologous locus. We present evidence that adult specific knock down of embryonic lethal fly tl causes a strong aggression phenotype, similar to the loss-of-function Nr2e1 phenotype observed in mice. We further show that tl is expressed in the adult pars intercerebralis (PI), a brain region with structural, functional and developmental similarity to mammalian hypothalamus. In Drosophila, evidence has accumulated to support a critical role for the hypothalamus in mammalian aggression. We show that PI specific knock down of tl using several driver lines expressed in the PI recapitulates the aggression phenotype. Also, we show that this phenotype is rescued by co-expressing fly tl or human Nr2e1 DNA. Together, our results suggest that aggressive behavior in Drosophila is transcriptionally regulated by tl in the adult pars intercerebralis. To our knowledge, for the first time, this work demonstrates a conserved role of a transcription factor in aggression as well as a conserved role of the neurosecretory neurons in flies and mammals in the regulation of aggressive behavior.

Natural genetic variation in aggressiveness social structure and male reproductive fitness in Drosophila. Julia B. Salt\textsuperscript{1,2}, Sergey Nazhdin\textsuperscript{1}. 1) Dept Population Biol, Univ California, Davis, Davis, CA; 2) Molecular and Computational Biology, University of Southern California, Los Angeles CA.

Aggression is a central facet of animal life and a critical target for human mental health intervention. Drosophila melanogaster has emerged as a leading model system for studying the genetic and neurobiological basis of aggressiveness, but the adaptive and sociological significance of this behavior is still unknown. In a series of independent experiments with natural heterozygous genotypes, we investigated the effects of genetic variation in aggressiveness on group formation and male mating success. Aggressive male genotypes were highly motivated to chase other males away from a food resource, but they were less likely than non-aggressive genotypes to perch on unoccupied patches. Further, aggressive genotypes were more attracted to male odors (cis-vaccenyl acetate, cVA) than non-aggressive genotypes. These results suggest that aggressive genotypes have high social motivation and want to establish territories on food patches only if they have to aggressively displace another male to do so, a phenomenon we call the ‘Fight Club Hypothesis.’ To further understand how male aggression affects social interactions and reproductive fitness, we are collaborating with Simon Tavaré’s group at USC. We have developed a novel camera system that can track multiple individual flies in a three-dimensional ecologically-relevant environment (see Reza Ardekani’s abstract). Using this system, we have characterized how combinations of aggressive and non-aggressive genotypes affect the expression of aggressiveness and male mating success. Our work sheds light on how sexual selection has shaped fly aggressiveness and suggests a potential mechanism for the widespread persistence of genetic variation in this behavior.

kayak modulates circadian behavior in Drosophila melanogaster. Jinli Ling\textsuperscript{1,2}, Raphaëlle Dubruille\textsuperscript{1,2}, Patrick Emery\textsuperscript{1}. 1) Department of Neurobiology, University of Massachusetts Medical School, Worcester, MA; 2) Current: Centre de Génétique Moléculaire et Cellulaire, Université Claude Bernard Lyon I, Mèndel, Villeurbanne Cedex, France.

Circadian rhythms exist in most organisms, from cyanobacteria to mammals. In Drosophila melanogaster, the pacemaker generating circadian rhythms is a negative transcriptional feedback loop. PERIOD and TIMELESS inhibit their own gene transcription by repressing the dimeric transactivator CLOCK/CYCLE which binds to the per and tim promoters. The detailed mechanism underlying this repression is still unclear. In this study, we identify kayak (kay) - the Drosophila homolog of e-cfos - as a transcription factor that helps maintaining accurate circadian rhythms in constant conditions. There are 5 predicted kay splicing variants. RNAi transgenes targeting different kay isoforms were specifically expressed in the ventral Lateral Neurons, the circadian pacemaker neurons. We observed a long period phenotype (26 hours) in constant darkness (DD) when the most 5' exon was targeted. This exon is found in two kay transcripts. The first one codes for the full-length KAY-α isoform, and the second one for a truncated protein called KAY-β. We have obtained antibodies specific to KAY-α (and KAY-β) and are currently examining its pattern of expression in circadian pacemaker neurons.

jim lovell; a neural transcription factor with roles in behavioral responses and gamete development. Kathleen M. Beckham, Sonia Bjorum, Rebecca A. Simonette, William J. Deery, Raul Alansi. Dept Biochem & Cell Biol, Rice Univ, Houston, TX.

The gene jim lovell (lov), CG16778, was identified in a screen for mutations that alter responses to gravity. The gene encodes a transcription factor with a BTB domain. lov is thus related to genes such as fruitless, Broad and tramtrack. We have investigated lov function through three approaches: i) examination of the protein expression pattern, ii) phenotypic analysis of additional mutations made by mobilization of the original gravitactic P(GawB) insertion and iii) under- and over-expression of Lov in the nervous system using the GAL4 system and lov cDNA and RNAi constructs. Lov is expressed in elements of both the CNS and PNS. In the embryonic PNS, it is expressed in subsets of chordotonal and external sense organ neurons, suggesting that Lov acts as a “neural identifier” gene, providing information that differentiates the functions of individual neurons within a given class. Two new mutations, which delete different lov 5' flanking sequences, produce strikingly dissimilar phenotypes.lov47 is associated with enhanced sensitivity to crowding in the larval stage and defects in courtship and climbing in adults. In contrast, mutant lov66 shows no obvious larval phenotype but has severely reduced female fertility, suggested with mild chordin abnormalities and egg retention when genetic expression of Lov throughout the nervous system with elav-GAL4 produces a morphologically normal, but highly uncoordinated larvae that are incapable of hatching from their egg cases. Suppression of Lov expression in the nervous system using RNAi produces normal development but complete male infertility as a result of both reduced courtship and non-functional sperm. This suppression of courtship by lov RNAi involves a role for Lov in FruM-Gal4/UAS lov RNAi males. In summary, lov appears to function in multiple behavioral responses and both male and female gamete development.

Juvenile hormone modulates the maturation of female receptivity in Drosophila melanogaster. Julilde Bilen, Lynn Riddleford. Janelia Farm Research Campus, Howard Hughes Medical Institute Ashburn, VA.

Sexual reproduction is crucial for the survival of most animal species including Drosophila melanogaster. Genes and neurons that specify courtship behavior in males are relatively well known; however, very little is known about the regulation of female mating behavior. Juvenile hormone (JH) is necessary for egg maturation in D. melanogaster, and previous studies suggest that JH may act as a switch to turn on the female mating behavior. JH is secreted by the glands called corpora allata (CA) just before eclosion. In order to test the role of JH in female mating behavior, we genetically ablated the corpora allata by expressing diphertheria toxin in the CA at the late pupal stage. Comparison of the time course of the receptivity of allactomized and control females showed that CA ablation delays the onset of female receptivity significantly, suggesting that JH is one of the factors involved in female receptivity. To determine whether the delay is due to lack of JH rather than nonspecific effects of the GAL4 driver lines or the diphertheria toxin in other tissues,
we topically applied the JH analog methoprene to allatectomized females at eclosion. The treated females showed the normal timing of the onset of female receptivity. Previous studies indicated that Methoprene tolerant (Met) is one of the putative receptors involved in the role of JH in metamorphosis. Therefore, we tested if Met alleles have any effect on maturation of female receptivity. In parallel to CA ablation experiments, we found that two independent Met alleles, one of which is a null, also cause delay in female receptivity, suggesting that JH acts through Met to modulate female receptivity. To examine where the JH acts, we reduced the expression of Met only in the nervous system and found that receptivity was similarly delayed, indicating that JH acts at least partially in the nervous system to regulate female mating behavior.

647B

Functional Role of Flight Muscle in Male Courtship Song of Drosophila melanogaster. Samya Chakravorty, Matthew Wajda, Jim Vigoreaux. Department of Biology, University of Vermont.

As part of the mating ritual, males of Drosophila species produce a species-specific courtship song through wing vibrations. While previous studies have shown that indirect flight muscles (IFM), the major power producing muscles for flight, are neurologically activated during song, the precise role of the flight musculature in courtship has not been investigated. Therefore, we employed wildtype male flies as controls and tested the role of IFM in the generation of the wing beat frequency. We found that the expression of the wildtype protein in control, socially-responsive genes and their role in Drosophila melanogaster male reproductive behaviors. Met, a neuronal receptor that regulates male mating behavior. We propose that flightin fulfills a dual role in enhancing power output for flight and influencing song parameters important for pre-mating isolation.

648C

A novel GPCR is required in fru neurons for male courtship behavior. Brigitte Dauwalder, Yuvali Li, Valbona Hoxha, Chamala Lama. Dept Biol/Biochem, Univ Houston, Houston, TX.

It is well established that fruittes (fru) and doublesex (dos) expressing neurons in the male brain are required for male courtship behavior. However, very little is known about the molecules and pathways that mediate their role or give them their male-specific identity. We have identified a novel G-protein coupled receptor (GPCR) that is required for male courtship behavior. Mutants display reduced male courtship. The GPCR is expressed in numerous neurons, many of which also express fruittes. Feminization of these neurons leads to male courtship defects. We have found that a mutation in the GPCR interacts genetically with a mutation in fruittes, indicating that they contribute to the same overall pathway. Reduction of the GPCR RNA in fru neurons by RNAi reproduces the courtship phenotype of the mutants. Furthermore, the courtship defects of the mutants can be rescued by expression of the wildtype protein in fru neurons. These findings identify this GPCR as one of the first identified molecules that mediate the male specific function of a subset of fru neurons.

649A

Socially-responsive genes and their role in Drosophila melanogaster male reproductive behaviors. Lisa L. Ellis1,2, Ginger E. Carney2. 1) Entomology, Texas A&M University, College Station, TX; 2) Biology, Texas A&M University, College Station, TX.

The perception of sensory information and the integration of those cues into a physiological output are vital to an organism’s survival and reproductive fitness. Drosophila courtship behavior is influenced by genetics and environmental interactions. Therefore, we proposed that examining the courtship- or mating-specific genome-wide expression changes occurring in Drosophila melanogaster would identify behaviorally important loci. Indeed, aside from identifying targets of the somatic sex-determination hierarchy that regulates Drosophila sexually dimorphic development and behavior, for two candidate genes we identified novel functions in male courtship behavior. The socially-responsive egghead (egh) and the mating-responsive Juvenile hormone esterase (Jhe) is required for robust male courtship behavior. We also show that Jhe regulates male mating behavior. We hypothesize that further analysis of these socially-responsive genes will identify novel sex-determination hierarchy targets and other behaviorally important genetic networks.

650B

Chromatin regulators control the formation of neural sexual dimorphism in Drosophila. Hiroki Ito1,2, Masayuki Koganzewa1, Manabu Ote1,2, Ken Matsumoto1, Chihiro Hama1, Daisuke Yamamoto1,2. 1) Graduate School of Life Sciences, Tohoku University, Sendai, Japan; 2) Advanced Institute for Science and Engineering, Waseda University, Tokyo, Japan; 3) Center for Developmental Biology, RIKEN, Kobe, Hyogo, Japan.

fruittes (fru), a Drosophila sex-determination regulatory gene, encodes Zinc-finger-containing transcription factors. Male-specific expression of Fru proteins in the brain and peripheral nervous system underlies the formation of neural sexual dimorphism. In male interneurons of the brain, Fru proteins prevent cell death of some of these neurons that are fated to develop male-specific projection, resulting in the formation of sexually dimorphic neural circuitry (Kimura et al., Nature, 438, 229-233 (2005)). In gustatory receptor neurons (GRNs) of foregut, Fru proteins determine whether or not their axons project across the midline of the ventral nerve cord, assuring male-specific midline crossing (Mellett et al., Development, 137, 323-332 (2010)). Here we report the following: 1) Bon (a transcriptional cofactor), HDAC1 (a histone deacetylase) and HP1 (heterochromatin protein 1) are included in Fru-containing protein complexes, 2) Bon recruits HDAC1 or HP1 to the complexes, 3) knockdown of HDAC1 in males de-masculinizes mAL interneurons in the cell number and projection pattern and suppresses axonal midline crossing of GRNs, 4) knockdown of HP1 counteracts the de-masculinizing effects of Fru hypomorphic mutations on mAL interneurons and GRNs in males, and 5) Fru and HDAC1 mutations synergistically interact and decrease male courtship activities, whereas HP1 mutations counteract the de-masculinizing effects of Fru hypomorphic mutations in males. These data indicate that the sexually dimorphic neurons are masculinized or de-masculinized depending on whether Bon recruits HDAC1 or HP1 to the Fru-containing complexes.

651C

Rival-induced increased mating duration of Drosophila male is dependent on the visual stimulus, circadian neural pathways, and ellipsoid body function. Woo Jae Kim, Yun Nung Jan. HHMI, Physiology, Biochemistry, and Biophysics Dept, UCSF, SF.

Drosophila males invest more in mating duration when they are exposed to males in the 4–5 days prior to mating. This phenotype, RilTic (rival-induced longer time in copula) is evolutionary important because increased mating duration result in higher reproductive success. RilTic is reversible and plastic behavior, which means natural selection favored context-dependent behavior for male’s mating duration strategy. However, physiological mechanism and neural pathways for RilTic are not known. Here we show RilTic can be induced by visual stimuli only. Blind animals and most of vision mutants didn't show RilTic. Olfactory, gustatory, auditory stimuli is not involved with RilTic. We also found that circadian clock genes and per are involved in this phenotype. Besides classical circadian and per mutants, other mutants which show lengthened or lessened daily rhythm such as perS, perL, timS, timUL didn't show RilTic. Another core circadian pacemaker genes Clk and cec is not involved with this phenotype. Constant dark condition during 5 days eliminates RilTic but constant light had no effect. Genes directly involved with the regulation of tim and per such as cry and dlt also eliminate RilTic. Eliminated RilTic can be rescued with pan neuronal overexpression of tim and per transgene. These results suggest that RilTic is not daily rhythmity-based but time-based function of tim and per genes. Small set of pdf neurons were critical for this phenotype. Inactivation of pdf neurons with pdf-GAL4/UAS-Kir2.1 could eliminate RilTic. Memory circuits might be required for
POSTER: Neural Physiology and Behavior
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

652A

Influence of the Male’s Wing Shape for their Success in Drosophila melanogaster Mating, Bianca F Menezes1, Felipe M Vignoder2, Alexandre A Peixoto2, Blanche C Bitter-Matthé1. 1) Genetic Dept, LPGD, UFRJ, RJ; 2) Oswaldo Cruz Institute, LBM, FIOCRUZ, RJ.

Males of Drosophila melanogaster court the females through a series of steps. One of which is when the male vibrates its wings producing a lovesong that is species-specific. Variations in the shape of the Drosophila wings were described among populations and species, but whether these variations influence the courtship process, it is still an open question. In our laboratory, replicate lines were obtained by artificial selection for rounded or elongated wing shapes from a natural population of D. melanogaster. We used those lines to test the influence of male’s wing shape in their mating success. We observed a significant success of males with elongated wings when competing with those with rounded wings regardless of the female type. However, the advantage of the elongated over rounded lines disappeared when wingless males were used. Trials using control males (without selection) competing with rounded lineages did not show any difference in female choice. However, elongated wing males tended to be more successful than control males. No association was detected between the relative body size of competing males and the outcome of crosses. We also recorded the courtship songs produced by males of the different wing shape lines and significant differences were observed in the first principal component of the parameters analyzed. Our results therefore suggest that male wing shape might influence mating success and the lovesong.

653B

Investigation of octopamine’s role in the ovulin-dependent increase in ovulation, C. Dustin Rubinstein, Mariana F. Wolffner. Molecular Biology & Genetics, Cornell University, Ithaca, IL.

Drosophila proteins transferred during mating strongly affect a female’s reproductive capacity, but little is known about the mechanism by which they induce physiological changes. One seminal fluid protein, ovulin, rapidly increases the ovulation rate of females after mating. Ovulation is controlled by neurons that release octopamine (OA) and glutamate on to the oviducts1-3. These neurons project from the ventral ganglia to the oviduct4. By using neurogenetic tools to manipulate the excitability of OA neurons in females and assessing ovulation by these females following mating to normal or ovulin-null males, we are testing whether ovulin acts through OA to increase ovulation. Experiments are also aimed at identifying populations of neurons that may be affected by ovulin. 1Monastirioti (2003) Dev Biol 264: 38-49 2Middleton et al. (2006) BMC Biol 4: 17 3Rodríguez-Valentin et al. (2006) J Cell Physiol 209: 183-189.

654C

An RNAi Screening for Fast Evolving Genes involved in male mate choice in Drosophila melanogaster, Rue S. Sousa-Neves1, Youngmin Chu1, Joseph Schinaman1, Ramon N dos Santos2, Alexandre Rosas2. 1) Biol, Case Western Reserve Univ, Cleveland, OH; 2) Dept of Physics, Universidade Federal da Paraíba.

In nature females quite selectively accept the courtship and mate with some types of males and reject others. This innate behavior is conserved in many species and of great significance for the evolution of the species. Indeed, several lines of evidence suggest that during speciation or right after species separation, females of an emerging new species rapidly develop genetic mechanisms to reject males of the former species. By exerting new preferences for males, females actively select new behaviors and body plans. In addition to its significance in evolution, male mate choice serves as a platform to begin deciphering the molecular mechanisms that govern decision-making. To begin addressing the molecular mechanisms of female mate choice, we selected three very closely related species of Drosophila (D. simulans and D. sechellia) that exhibit markedly different preferences for the males that they prefer to mate. Next, we screened computationally their genomes and by pair wise comparisons identified some fast evolving genes as candidates (Sousa-Neves and Rosas, 2010). Currently we are testing whether these genes are involved in female mating choice by knocking them down using RNA interference in D. melanogaster. To knockdown the genes of interest and avoid lethality, we used the GAL4/UAS system coupled with Flipase/FRT and RNA interference. The results obtained so far suggest that mating is reduced or significantly delayed in 12% of the fast evolving genes tested.

655A

Mapping brain regions required for female acceptance, Rui Sousa-Neves, Joseph Schinaman. Biology, Case Western Reserve Univ, Cleveland, OH.

A central question in neurobiology is how sensory inputs are integrated during the process of decision-making. A promising model system in which this process can be explored is female response to courtship in Drosophila melanogaster. This system is ideal because a female is given an array of sensory inputs by a courting male (in the form of dancing, singing, phonemal cues and touch) which she must translate into a binary output of acceptance or rejection. Recently we identified the neuronal–expressed transcription factor datitilografo (dati), whose downregulation causes constitutive rejection in females. In order to implicate the regions in the brain where dati expression is necessary to allow acceptance in females, we took an unbiased approach in which we clonally knocked down dati expression in the brain using a novel system to generate somatic clones of fourth chromosome genes, the FYT method. Thus, we can generate flies in which dati is knocked down in random patches of brain tissue, test these flies individually for acceptance or rejection, and map the mutant cells using a GFP reporter. By this method we aim to identify brain regions or circuits that integrate the complex sensations of courtship into usable information, which fall under the control of this novel transcription factor.

656B

A Drosophila model for analyzing ion and volume homeostasis in the nervous system, William M. Leiserson1, Biff Forbush2, Haig Keshishian1. 1) MCD Biology Dept., Yale University, New Haven, CT; 2) Cellular and Molecular Physiology Dept., Yale University School of Medicine, New Haven, CT.

Ion and extracellular volume homeostasis is often disrupted following stroke or traumatic brain injury, with generally catastrophic consequences. In both mammals and insects, the homeostatic mechanism depends on the pumps, cotransporters, and exchangers of glia and accessory cells. The larval nerves of Drosophila are well suited for modeling ion and volume homeostasis, as there are homologs to mammalian molecules at the level of morphology, physiology, molecular mechanisms, and mutant phenotypes. We show that the gialt cells that form the Drosophila blood-nerve barrier have a conserved molecular mechanism that regulates extracellular volume. This involves the serine/threonine kinase Fray, an ortholog of mammalian PASKPAK, and the Na-K-Cl cotransporter Ncc69, which we show is an ortholog of human NKCC1. Larvae mutant for Ncc69 or fray develop a peripheral neuropathy consisting of nerve bulges, where fluid accumulates between glia and axons. In vitro flux assays show that Ncc69 is a Na-K-Cl cotransporter, with binding affinities similar to human NKCC1. Furthermore, human NKCC1 can rescue the Ncc69 mutant phenotype. Behavioral, genetic and physiological tests of Ncc69 and fray mutants reveal no loss of nerve function, suggesting that the extracellular potassium ion concentration is fairly normal. We also show that the hyperactive K channel mutation combination, evg Sh, causes an accumulation of extracellular fluid in larval nerves, strikingly similar by TEM to the Ncc69 and fray mutant bulges. We propose a model where excess K left in the wake of action potential firing is removed by the Na/K ATPase, leading to an increase in extracellular solutes and an increase in osmotic pressure. Fray and Ncc69 normally function to remove these solutes, restoring osmotic balance and maintaining the normal extracellular volume.

657C

Long-term Effects of Early Application of Juvenile Hormone, Kathryn J. Argue, Amber Yun, Wendi S. Neckameyer. Pharmacological and Physiological Sciences, Saint Louis University School of Medicine, St. Louis, MO.

Throughout the life span of an individual, the nervous system is constantly changing in response to both endogenous and exogenous factors. During adolescence the nervous system is particularly vulnerable to many different biological and environmental factors. Flies do not engage in reproductive behaviors until they are fully sexually mature,
implying that their brains are still developing (Romeo et al., 2002). Because of this, any changes made to the environment or internal environment of an adolescent animal can have long lasting effects. Normal behavioral competence requires not only proper development of the brain, but also maintenance of the appropriately sexually differentiated state via the actions of hormones. Gonadal development requires (at least in part) juvenile hormone (JH), which controls reproductive maturity and mature sexual signaling (Teal et al., 2000). JH interactions with DA have been established in vitro in various insect species. Past studies have also implied the existence of direct interactions between DA and JH to effect the organization of neural circuitry in Drosophila (Gruntenko et al., 2005 and 2007). Here we show in vivo evidence of interactions between JH and DA in adult Drosophila. Since both JH and DA have been shown to play a role in ovarian development, we used Drosophila ovaries as a model to show evidence of a direct interaction between JH and DA. To assess the effect of early manipulation of JH on adult behaviors, we analyzed courtship and locomotion, two behaviors known to be modulated, at least in part, by DA. Pharmacological application of a JH analog (methoprene) or JH synthesis inhibitor (precocene) immediately after eclosion affects locomotor and reproductive behaviors in the adults in a gender- and age-specific manner. In addition, treatment with these agents in flies with reduced neuronal DA synthesis or ovarian DA transport imply a change in the modulation of DA circuitry through the actions of JH.

658A
Resilin RNAi alters form and function of wing hinge tendon in a transgenic fruit fly. D Neff1, J Galloway2, J Hogan1, N Wang3, S Collier1. 1) Biology Dept, Marshall U, Huntington, WV; 2) Fairfield HS, Lawrence Co, OH.

In this report, we describe our successful attempt to manipulate the formation of an elastic tendon that functions in wing control in the fruit fly. Hovering flight is an extremely energy intensive form of locomotion yet is primary in the numerous and diverse forms of winged insects. The skeleton of insects acts as a rigid yet flexible framework upon which muscles attach. A specialized elastic protein, Resilin, contributes to this function in most described insect flight mechanisms. We have shown that flies expressing interfering RNA directed against the production of resilin RNAi exhibit atypical morphology in a tendon at the base of the fly wing. This tendon attaches to the thorax at or directly adjacent to the insertion of at least 4 well described steering muscles that are important to flight control in flies. Furthermore, we describe seemingly normal tendon form in a mutant fly strain called rickets which are deficient in a receptor for the hormone Bursicon. Tendon structure is also normal in RNAi knockdowns of bursicon, partner of bursicon, and rickets gene products. Bursicon signals wing unfolding and maturation of the fly cuticle, we had hypothesized that Bursicon signal is important in Resilin maturation. Mature, crosslinked Resilin has a distinctive blue autofluorescence while newly produced Pro-resilin does not, this property facilitates our analysis of tendon development. Flies expressing resilin RNAi were generated via UAS/GAL4 expression system with the GAL4 under ubiquitously expressed Tubulin promoter. We discovered that this expression did not prevent apparently normal development in the fly with the notable exception of the aforementioned tendon. Offspring of resilin RNAi crosses with MS1096 (expression of GAL4 only in dorsal wing) were normal, including tendon morphology. Finally, in ongoing experiments, we are exploring the physiological function of Resilin in flight and mating by comparing resilin knockdowns with wild type flies.

659B
Eclosion Gates the Competence for Wing Expansion in Drosophila. Nathan Peabody, Benjamin White. Lab of Molecular Biology, NIMH/NIH, Bethesda, MD.

The adult eclosion sequence is a hormonally-orchestrated behavioral program in insects, which culminates in expansion of the wings. Wing expansion itself is governed by the secretion of bursicon, which occurs after eclosion. We have used "thermogenetic" techniques to stimulate neurons prior to eclosion and have evaluated effects of this manipulation on bursicon secretion and wing expansion behavior. Our results indicate that eclosion, or some process tightly coupled to it, gates the competence of the nervous system to release both bursicon and the motor programs that drive expansion. Interestingly, a pre-eclosion stimulus, although it has little immediate effect, will initiate bursicon release and wing expansion behavior shortly after eclosion in animals that emerge within half an hour of stimulation. We have previously demonstrated that the targeted activation of a small network of central neurons (N_GABA) using the TRPM8 channel can force bursicon release and wing expansion. To investigate the competence of N_GABA to respond to precocious stimulation, we activated TRPM8 in these neurons for 15 min during the "extended ptilinum" (i.e. EP) stage, approximately 0 to 45 minutes before eclosion. We found that excitation at this stage failed to cause bursicon release into the hemolymph, as determined by Western Blot, and also did not initiate behavioral changes. Most animals eclosed normally, but then rapidly released bursicon and expanded their wings after eclosion. Similarly, if animals in the EP stage were prevented from eclosing for 75 minutes, N_GABA> TRPM8 stimulation during the last 15 minutes of confinement was without effect until after emergence from the pupal case. Animals forced to eclose prematurely and then subjected to N_GABA>TRPM8 activation, immediately released bursicon and performed normal wing expansion behavior. We conclude that eclosion gates the competence of the nervous system to release bursicon and wing expansion behavior, and that this mechanism contributes to the ordering of the ecysis sequence.

660C
Non-genomic actions of the steroid hormone ecdysone on behavioral plasticity in adult Drosophila. Hiroshi Ishimoto1, Z Wang2, Chun-Fang Wu2, Toshihiro Kitamoto1,2. 1) Department of Anesthesiology, College of Medicine; 2) Department of Biology, College of Liberal Arts and Sciences; 3) Interdisciplinary Programs in Genetics and Neurosciences, University of Iowa, Iowa City, IA.

Ecdysone is the major steroid hormone in Drosophila and plays vital roles in development as well as adult physiology and behavior. Although ecdysone signaling is mediated mainly by the nuclear ecdysone receptors (EcRs) that serve as steroid-activated transcription factors (genomic actions), several lines of evidence suggest that ecdysone can also execute its functions by modulating intracellular signaling cascades in a nuclear receptor-independent manner (non-genomic actions). A potential mediator of such non-genomic ecdysone actions is DopEcR, the G-protein coupled receptor (GPCR) shown to be directly activated by ecdysteroids in heterologous expression systems. Here we report that DopEcR is indeed functional in adult Drosophila and involved in regulation of both associative and non-associative learning. Interestingly, a defect of courtship memory in mutants for the Ca2+/CaM-dependent adenylyl cyclase gene rutabaga was effectively rescued when DopEcR mediated ecdysone signaling was genetically or pharmacologically enhanced. In addition, feeding 20-hydroxyecdysone, an active metabolite of ecdysone, to adult flies caused a rapid, DopEcR-dependent increase in cAMP levels in the mushroom bodies. Taken together, this study has demonstrated that non-genomic actions of ecdysone are mediated via GPCR-cAMP signaling and play important roles in behavioral plasticity in adult flies. The finding provides a novel insight into the unconventional steroid signaling in insects that is likely conserved among evolutionarily diverse organisms.

661A
Dissecting the role of Drosophila Orb2 in long-term memory. Sebastian Krueßner, Barry J. Dickson, Krystyna Keleman. IMP - Research Institute of Molecular Pathology, Vienna, Austria.

Long-term behavioral memory and synaptic plasticity both require new protein synthesis locally at activated synapses. Members of the CPEB protein family are potential candidates to be involved in translational control at these synapses. They have been shown to regulate translation, localize to synapses and to function in synaptic plasticity and long-term memory. However, the exact mechanism behind these processes is not clear. We previously reported Orb2, a Drosophila homologue of neuronal CPEB proteins, to be required in mushroom body neurons for long-term courtship memory. Here we aim to define the molecular and cellular mechanisms of Orb2 function in memory formation by generation of an orb2(659B) allele, in which most of the reading frame is replaced with an atp target side for the site specific recombinase FLP. Site specific transgenic can now be used to rapidly introduce any desired modification into the endogenous orb2 locus. As a control, we have generated the deleted region with the genomic rescue as well as with a tagged version and confirmed that both can rescue the long-term memory phenotype equally well. We used tagged Orb2-GFP rescue to analyze the expression pattern during development and in adult nervous system. We also conducted structure function analysis of the highly conserved C-terminal RNA binding domain, the N-terminal glutamine enriched region and for the less conserved regions. We analyzed these mutants in respect to its impact on both the long-term memory as well as the expression pattern of the RNA and protein.
The therapeutic role of neurotrophic factor under regulated expression in Drosophila brain. Ekaterina Nikitina, Anna Medvedeva, Elena Savvateneva-Popova. Dept Neurogenetics, Pavlov Inst Physiology, St Petersburg, Russian Federation.

The neurotrophic factor therapy is a promising approach for the treatment of neurodegenerative disorders. The delivery of neurotrophic factor (NTF) stem cells to locus of neurodegeneration is very effective. L.I. Korochkin offered new using heat-shock (HS) Drosophila promotive approach. This promotive responds on mammalian temperature as shock factor that result in constant expression of NTF gene. HS treatment is adjusted for hs-promoter genes induction, for exposure during critical periods of development of the mushroom bodies (main for learning ability, HS1) and the central complex (main for memory formation, HS2), also to imago (HS). We investigated learning ability and memory formation using conditioned courtship suppression paradigm following HS in stocks Canton-S (wild type), GDNF (transgenic flies which carry glia-cell-line derived nerve factor (GDNF) under HS promoter) and I(1)ts403 (stock is characterized by the temporal disturbance of HSP genes expression). Simultaneously we detected GDNF on brain slices by immunofluorescent methods. Canton-S is characterized by improvement of memory formation and constant fluorescence level GDNF in central complex and optical system at HS. Disturbances of 3 hr-memory is revealed in GDNF males in intact control and after HS1, GDNF expression level is identical with wild type. On the contrary, HS and HS2 leads to improvement of 3 hr-memory in GDNF males and intensive GDNF fluorescence in central complex. Since GDNF has neuroprotective effects we used I(1)ts403 mutants in order to imitate defect of stress response intracellular systems. HS1 leads to repression of memory formation in I(1)ts403 mutants. It results in sharp increase GDNF that repeats increase expression of GDNF mRNA under disorders and injuries. So hs-promoter regulated induction of GDNF gene and defect of HSP synthesis in I(1)ts403 mutants leads to increase of Drosophila central complex subdomains staining by GDNF antibodies.

Developmental timing defects caused by an Imp-L2 mediated local decrease of insulin signaling. Ladan Sarral-Zadeh, Hugo Stocker, Katja Koehlner, Ernst Hafen. Institute of molecular systems biology, ETH Zurich, Switzerland.

To examine the mechanisms of neuronal differentiation in the context of a functioning neuronal network, we are focusing on the Drosophila CCAP-neuronal network that generates a behavioural output critical for ecdysis. Larval ecdysis sheds the old exoskeleton at the transition between larval stages, and pupal ecdysis everts the head and appendages to their adult position during metamorphosis. Previous work by the Ewer lab showed that genetic ablation of CCAP-neurons extended the duration of larval ecdysis and blocked pupal ecdysis. We will present our work describing the development of the CCAP-neuron network from embryonic development through metamorphosis. Additionally, we will present work showing that mutants for the BMP receptor, wishful thinking, phenocopy the behavioral defects in ecdysis observed after ablation of CCAP-neurons. Retrograde BMP signaling in neurons plays conserved roles in synaptic efficacy and subtype-specific gene expression. However, no role for BMP signaling in behavior had been established. Further analysis found that signaling via the ligand (Gbb), the receptor (Wit) and the transducer (Mad) regulates the expression of the peptide hormones CCAP, MIP and BurD, in a small subset of the Drosophila CCAP-neurons (the effector subset; CCAP-ENs). Importantly, restoration of BMP activity exclusively in CCAP-ENs rescued neuropeptide levels, and the ecdysis phenotype. Together, our data have begun to uncover the gene regulatory mechanisms underlying the function of select subsets of CCAP-neurons required for ecdysis.

Recruitment of Dopamine Neurons into the Stress Response Circuitry. Kathryn J. Argue, Ariel Hofman, Wendi S. Neckameyer. Pharmacological and Physiological Sciences, University of California, Vancouver, BC, V6T 1Z3 Canada.

To examine the mechanisms of neuronal differentiation in the context of a functioning neuronal network, we are focusing on the Drosophila CCAP-neuronal network that generates a behavioural output critical for ecdysis. Larval ecdysis sheds the old exoskeleton at the transition between larval stages, and pupal ecdysis everts the head and appendages to their adult position during metamorphosis. Previous work by the Ewer lab showed that genetic ablation of CCAP-neurons extended the duration of larval ecdysis and blocked pupal ecdysis. We will present our work describing the development of the CCAP-neuron network from embryonic development through metamorphosis. Additionally, we will present work showing that mutants for the BMP receptor, wishful thinking, phenocopy the behavioral defects in ecdysis observed after ablation of CCAP-neurons. Retrograde BMP signaling in neurons plays conserved roles in synaptic efficacy and subtype-specific gene expression. However, no role for BMP signaling in behavior had been established. Further analysis found that signaling via the ligand (Gbb), the receptor (Wit) and the transducer (Mad) regulates the expression of the peptide hormones CCAP, MIP and BurD, in a small subset of the Drosophila CCAP-neurons (the effector subset; CCAP-ENs). Importantly, restoration of BMP activity exclusively in CCAP-ENs rescued neuropeptide levels, and the ecdysis phenotype. Together, our data have begun to uncover the gene regulatory mechanisms underlying the function of select subsets of CCAP-neurons required for ecdysis.
Relative positioning of histaminergic to serotonergic cells in the larval CNS. Kelsey Crowley¹, Martin Burg², ¹) Cell and Molecular Biology; ²) Biomedical Sciences, Grand Valley State University, Allendale, MI.

The function of histamine in the Drosophila nervous system has been established for photoreceptors and other sensory neurons (1). Histamine is also present in cells of the central nervous system (CNS), although its function in these cells has not been well established. Of the functions ascribed to histamine, only a few may be mediated by central neurons, including modulating temperature preference (2). The enzyme that catalyzes the synthesis of histamine is histidine decarboxylase (Hdc), which is encoded by the Hdc gene. Recently, a fusion between the putative Hdc promoter and eGFP (pHdc-eGFP) has allowed the immunocytochemical identification of Hdc expression in histaminergic cells. The ability to detect histaminergic cells using a GFP antibody enables double labeling studies with antibodies that are specific to other biogenic amines, such as serotonin. Flies bearing the pHdc-eGFP transgene were used to examine the relative position of cells which contain serotonin, as the serotonin pattern appears similar to that of histaminergic cells. Histaminergic and serotonergic cells were identified in double stained preparations using antisera against GFP and serotonin, respectively. Analysis of brains from second instar larvae has indicated that histaminergic and serotonergic cells are located close to each other in the ventral nerve cord, with the histaminergic cells being located more ventrally and medially than the serotonergic cells. As expected, there was a greater number of serotonergic cells, not just in the ventral ganglion, but also in the brain lobes. Results demonstrate that imaging of histaminergic and other amine cells can be accomplished using the pHdc-eGFP transformant flies. This should allow further studies of the spatial relationship between histaminergic and other amine cells in the CNS of Drosophila. Supported by a GSVS SU award to KC. (1) Melzig et al., J. Neurosci. 18: 7160 (1998) (2) Hong et al, J. Neurosci. 26:7245 (2006).


Biogenic amines including dopamine, serotonin and octopamine regulate an extensive array of fly behaviors, but the synaptic mechanisms that mediate these processes remain obscure. The amineergic regulation of larval locomotion provides model behavior to identify pathways that regulate amine signaling. We have used a mutation in the Drosophila vesicular monoamine transporter (dVMAT) to identify pathways that regulate amine signaling. In flies, as well as other animals, VMATs are required to package into synaptic vesicles all amine monoamine neurotransmitters and dVMAT mutants show several behavioral deficiencies including reduced larval locomotion. We find that larval locomotion in the mutant can be rescued either by feeding octopamine or by expression of dVMAT in octopaminergic neurons, consistent with the idea that an octopaminergic circuit activates the central pattern generator (CPG) for larval locomotion. To explore the molecular mechanisms underlying this pathway, and to identify additional regulatory circuits, we screened for drugs that would increase locomotion in dVMAT mutant larvae. We have identified several amineergic and non-amineergic compounds that dramatically increase larval locomotion, suggesting the possibility that alternative pathways can bypass octopaminergic circuit to initiate locomotion. In addition, by comparing the behavior of null dVMAT mutants versus those expressing very low levels of the transporter, we identified drugs that required VMAT function and may act to potentiate monoamine storage or release. Together, these data help determine the neural mechanisms that regulate amine release and also highlight our assay system as a new method to screen for clinically relevant drugs.

A glutamate transporter mutant that causes episodic ataxia and hemiplegic migraine in humans disrupts glial cell morphology in Drosophila. Christine N. Serway¹, Hafsia Mamsa², Christy C. Dower³, Cuwen He¹, Haitao Ji¹, Jasmine J. Yang¹, Robert W. Baloh², David E. Krantz¹, ³) Semel Institute, UCLA School of Medicine, Los Angeles, CA; ²) Department of Neurology, UCLA School of Medicine, Los Angeles, CA; ³) Department of Psychiatry and Biobehavioral Sciences, UCLA School of Medicine, Los Angeles, CA.

Excitatory Amino Acid Transporters (EAATs 1-5) remove glutamate from the synaptic cleft following exocytotic release. In the CNS, Mammalian and Drosophila EAAT1 and EAAT2 reside primarily on the cell surface of gliia rather than in neurons. Dysfunction of EAATs can result in excess extracellular glutamate, thought to cause excitotoxicity to neighboring neurons, yet it is not known if and how changes in EAAT function might affect glia past embryonic development. Moreover, in humans, rare mutations in EAAT1 cause episodic ataxia, hemiplegic migraine, and epilepsy (designated EA6 OMIM600111), but the mechanisms underlying these symptoms and the potential effects on glia are unclear. To address these questions, we have generated a fly model of the mutation P290R in EAAT1 that causes EA6 in human. We find that expressing the dEAAT1 mutant cause episodic ataxia, hemiplegic migraine, and epilepsy (designated EA6 OMIM600111), but the mechanisms underlying these symptoms and the potential effects on glia are unclear. The aminergic regulation of larval locomotion provides model behavior to identify pathways that regulate aminergic signaling. We have used a mutation in the Drosophila vesicular monoamine transporter (dVMAT) to identify pathways that regulate amine signaling. In flies, as well as other animals, VMATs are required to package into synaptic vesicles all amine monoamine neurotransmitters and dVMAT mutants show several behavioral deficiencies including reduced larval locomotion. We find that larval locomotion in the mutant can be rescued either by feeding octopamine or by expression of dVMAT in octopaminergic neurons, consistent with the idea that an octopaminergic circuit activates the central pattern generator (CPG) for larval locomotion. To explore the molecular mechanisms underlying this pathway, and to identify additional regulatory circuits, we screened for drugs that would increase locomotion in dVMAT mutant larvae. We have identified several amineergic and non-amineergic compounds that dramatically increase larval locomotion, suggesting the possibility that alternative pathways can bypass octopaminergic circuit to initiate locomotion. In addition, by comparing the behavior of null dVMAT mutants versus those expressing very low levels of the transporter, we identified drugs that required VMAT function and may act to potentiate monoamine storage or release. Together, these data help determine the neural mechanisms that regulate amine release and also highlight our assay system as a new method to screen for clinically relevant drugs.

Handedness in Drosophila locomotor behavior. Sean M. Buchanan, Benjamin L. de Bivort. The Rowland Institute at Harvard, Cambridge, MA.

Genetically identical individuals can display variability in their gene expression, morphology and behaviors, but the biological significance of this variation has only recently begun to be appreciated. We have investigated whether Drosophila melanogaster display individual variation in locomotor behaviors. We find that, during walking, individual flies exhibit bias in left-right turning, with some flies being strongly ‘left-handed’ or ‘right-handed’. The distribution of turning scores is much broader than would be expected by chance and an individual fly’s handedness is persistent over its lifetime. Furthermore, this idiosyncratic bias is non-heritable and cannot be explained by gross morphological variation. Surprisingly, we find that locomotor handedness is influenced by vision; when walking in darkness, flies exhibit a narrower distribution of turning biases. This suggests that individual handedness may be under neural control and we have examined the roles that neural circuits within the central complex play in left-right turning. The ring neurons of the ellipsoid body have been implicated in the integration of sensory inputs and locomotion. We find that silencing these neurons reduces variation in handedness, similar to the effect of eliminating visual stimuli during walking. We are currently investigating whether handedness is caused by individual asymmetry in these integration neurons.


Drosophila has seven insulin-like peptides (ILP 1-7). These peptides share structural similarity to Insulin and Insulin-like growth factors. A subset of these peptides is expressed in discrete subsets of neurosecretory cells in the Drosophila CNS. ILP7 is expressed in 8 posterior dMP2 neurons during larval stages; these neurons exit the ventral nerve cord to innervate the hindgut, with unknown function. In the adult, ILP7-containing axon termini have been reported in the hindgut (Miguel-Aliaga et al. 2008) and the female reproductive tract (Yang et al. 2008). Electrical inactivation of ILP7 neurons in adult females abrogates egg-laying and ILP7 has been implicated in egg-lay site selection (Yang et al. 2008). Here we report that in the adult Drosophila posterior ventral nerve cord, there are two distinct clusters of ILP7 neurons, a dorsal cluster of 6-8 neurons and a ventral cluster of 4-6 neurons. We found that a subset of dorsal cluster neurons, and all ventral cluster neurons derive from post-embryonic lineages that arise between 90-100 hours post- fertilization. We selectively killed only the embryonic ILP7 cells and show that embryonic ILP7 are not essential for normal egg-laying. We are currently characterizing which subset of post-embryonic ILP7 cells is necessary and sufficient for egg-laying and the mechanisms by which they regulate oviposition. References: Miguel-Aliaga, I et al. PLOS Biology (2008). Yang, C et al. Science 319 (2008).
infection is not clear. We hypothesized that sleep benefits the host during infection. To test this hypothesis, survival as well as sleep behavior was measured in wild-type flies during bacterial infection (early SD) with 
Serratia marcescens
 or mutants. These data indicate that the Drosophila NF-kB539Bs, Dif and Relish, have redundant roles in the protective effect of early SD. To further investigate a role of NF-kB, transgenic flies containing NF-kB-binding elements upstream of a luciferase open reading frame (kB-luc) were used to measure NF-kB activity in living flies. We found that early SD enhanced peak NFkB reporter activity during infection. We next manipulated sleep by genetically altering neuronal excitability in the mushroom body, a region in the central brain that is known to regulate sleep. Increasing sleep using this method strongly promoted survival during infection. Decreasing sleep, on the other hand, did not produce a net change in survival outcome. However, the increased bacterial clearance in these flies suggested that long-term sleep loss reduces tolerance to infection. In summary, these findings indicate a complex interaction between sleep and the immune response in Drosophila and suggest that sleep during an immune response is beneficial for the host during a recovery process.

POSTER: Neural Physiology and Behavior
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.
POSTER: Neural Physiology and Behavior
See page 16 for presentation schedule. Poster number is above title. The first author is the presenter.

677B
3D Digital Atlas Guided Microsurgery of GAL4 Patterns of a Drosophila Brain. Hanchuan Peng, Peng Lab, HHMI, Ashburn, VA.

We present a preliminary system which uses computer driven automatic microsurgery to target neuronal tissues in 3D, labeled using sparse GAL4 patterns of a Drosophila brain. To make this system work, we first built a 3D digital atlas of hundreds of stereotyped neurite tracts of a fruit fly’s brain using our powerful BrainAligner (for 3D brain registration) and V3D-Neuron (for neurite reconstruction) software systems. Then we assembled a laser scanning microscope which is driven by our controlling software to target these neurite tracts in a newly scanned fly’s brain. In the current test we are able to target and cut individual neurite tracts repeatedly, which demonstrates the potential for a wide range of more sophisticated automatic microsurgery of a brain.

678C
A Wild-Derived Advanced Intercross Panel of Chromosome Substitution Lines for High Resolution QTL Mapping of Startle Behavior in Drosophila melanogaster. Yamin L. Serrano1,2, Akihiko H. Yamamoto1,2, Trudy F.C. Mackay2,3, Robert R. H. Anholt1,2,3. 1) Department of Biology, North Carolina State University, Raleigh, NC; 2) W.M. Keck Center for Behavioral Biology; 3) Department of Genetics, North Carolina State University, Raleigh, NC.

Abnormal startle reflexes are a hallmark of many psychiatric disorders. Drosophila presents a tractable model for studies on startle behavior because both the genetic background and environment can be controlled. Startle behavior can be quantified as the amount of time flies move during a 45s period following a mechanical disturbance. Previous studies on wild derived inbred lines have shown significant phenotypic variation in startle behavior (H2 = 0.58). We selected 40 fully sequenced lines of the Drosophila Genetic Reference Panel (DGRP) to construct a synthetic outbred population by a round robin crossing design followed by 10 generations of random mating. At the tenth generation 482 homozygous 2nd chromosomes were extracted from single males and substituted into a common isogenic Canton S B background. We measured startle responses for 10 flies/sex line (9,640 flies and 482 lines total) for the 2nd chromosome substitution lines and the control (Canton S B, 40 replicates per sex). Response scores ranged from 21.8 to 44.7% showing substantial phenotypic variation. The majority of 2nd chromosome substitution lines showed startle responses that were lower than the controls indicating naturally segregating suppressing epistasis. Analysis of variance showed significant variation among the lines as well as a significant effect of sex. Normal distribution of normalized mean startle responses showed that males display greater startle responses than females. The broad-sense heritability for startle behavior was H2 = 0.16 indicating that a significant fraction of the observed variation is due to environmental factors. This panel of wild-derived advanced intercrossed 2nd chromosome substitution lines, together with a similar collection of 3rd chromosome substitution lines, will provide a valuable resource for high resolution QTL mapping.

679A
Regulation of locomotor behavior depends on isoform-specific action of CASK in the central nervous system. Justin Slawson, Elena Kuklin, Kottak Mukherjee, Lilly Ostrovsky, Leslie Griffith. Biology, Brandeis University, Waltham, MA.

The Drosophila ortholog of mammalian CASK (known alternatively as Camgak or Caki) has been shown to play a role in proper locomotor behavior. The nature of this deficit, however, has remained elusive, and has been complicated by several issues, including the existence of multiple CASK gene products. To address this, we generated a new set of CASK mutants using P-element excision that disrupts only the long isoform of CASK (CASKβ), which like mammalian CASK has N-terminal CaMK-like and L27 domains. CASKα, which lacks these domains and more closely resembles the MPP-1 family of proteins, is unaffected in our mutant. We have run these mutants in a series of locomotor assays, and show that flies with decreased levels of CASKβ expression exhibit a complex locomotor phenotype, including defects in motor initiation, motor maintenance, speed, and acceleration. As the most consistent phenotype, we observe a strong reduction in CASKβ action to the cercal neuron circuitry underlying the complex structure of normal locomotion. CASKβ mutants fail to induce slo or acquire tolerance. We employ a 3xCRE-Luciferase reporter construct and CASK mutant stocks to identify the roles played by the two Drosophila CASK genes in drug response and slo regulation. We have shown that CASKα mutants fail to acquire tolerance to benzyl alcohol. Additionally, we have established a time-course for the CRE-mediated signaling following drug exposure, and are currently testing to see how various mutants affect this sequence of events and the acquisition of tolerance.

680B
Differential Roles of Drosophila CREB Isoforms in Drug Tolerance. Benjamin Troutwine, Nigel Atkinson. Institute for Cellular and Molecular Biology, University of Texas at Austin.

We use Drosophila as a model for addiction studies by examining the shared mechanisms of tolerance to ethanol and benzyl alcohol. Tolerance is defined as a reduced response to a drug caused by previous drug exposure, and is a core endophenotype of addiction. Previous work in our lab showed that induction of the slo gene, which encodes a BK-type Ca2+-activated K+ channel, underlies functional tolerance. slo mutants fail to acquire tolerance, and induction of a slo transgene phenocopies tolerance. Analysis of the slo control region identified three conserved CRE sites, implicating the CREB family of transcription factors in slo induction and the response to drug sedation; furthermore, work in numerous systems has shown that CREB genes play an important role in neural plasticity and drug responses. This led us to investigate the two Drosophila CREB genes, CrebA and Creb2 (sometimes referred to as Creb-B17A). After sedation, CrebA expression increases, a CREB repressor isoform (CrebB2b) is downregulated, CREB occupancy at the slo control region increases, and CRE-mediated gene expression increases. Additionally, transgenic expression of the Creb2b repressor blocks tolerance and slo induction, and Creb2 mutants fail to induce slo or acquire tolerance. We employ a 3xCRE-Luciferase reporter construct and CREB mutant stocks to identify the roles played by the two Drosophila CREB genes in drug response and slo regulation. We have shown that CrebA mutants fail to acquire tolerance to benzyl alcohol. Additionally, we have established a time-course for the CRE-mediated signaling following drug exposure, and are currently testing to see how various mutants affect this sequence of events and the acquisition of tolerance.

681C
Frequent Origins of Brain Genes in the Olfactory System Driving the Evolution of Foraging Behavior. Sidi Chen1, Maria Splettzer2, Liqun Lu3, Manyuan Long1. 1) Dept. Ecology & Evolution, Univ Chicago, Chicago, IL; 2) Dept. Biology, Stanford, CA; 3) CSAIL, MIT, Cambridge, CA.

Throughout the animal kingdom, the nervous system plays a fundamental role in processing sensory information and making proper behavioral responses. The brain is the topological and function center of the nervous system controlling the animal behavior. Thus the evolution of behavior is associated with brain evolution. However, the evolution of the brain and how this influences behavior is an interesting puzzle. Specifically, how genetic changes shape the evolution of the brain and behavior is still vaguely understood. Here we show that, under the force of natural selection, novel genes have been frequently recruited into the brain during recent evolution in Drosophila. These nascent brain genes are often expressed in the mushroom body. A recently fixed gene, Xchp1, evolved to acquire expression in the olfactory system and facilitate foraging behavior. Either silencing the Xchp1 gene, or inactivating Xchp1-positive neurons, led to reduced efficiency in foraging, recapitulating an ancestral phenotype revealed by phylogenetic behavioral analysis. Our data suggest that new genes may have extensively integrated into higher order brain functions, driving genetic changes in the brain and phenotypic evolution of animal behavior.

682A
Hedgehog signaling mediates nociceptive sensitization in Drosophila larvae. Michael J. Galko, Yueon Jo, Daniel T. Babcock. Department of Biochemistry and Molecular Biology, MD Anderson Cancer Center, Houston, TX, USA.

Heightened pain sensitivity is an adaptive response to tissue damage that protects the site of injury while it heals. This sensitivity can manifest as allodynia (responsiveness to previously sub-threshold stimuli) or hyperalgesia (exaggerated responsiveness to supra-threshold stimuli). We recently established a new model of pain sensitization in Drosophila...
the expression patterns and interactions of these crucial hearing proteins.

685A
A family of putative taste receptors. Tong-Wey Koh1, Shannon Stewart1, Jeeyun Chung2, John Carlson1,2. 1) Molecular Cellular & Developmental Biology, Yale University, New Haven, CT. 2) Program in Biological and Biomedical Sciences, Yale University, New Haven, CT.

In insects, taste serves important functions in sensing the availability and palatability of food. While many putative taste receptors have been discovered and characterized, there still appear to be many taste neurons in which no receptor is known to be expressed. In previous bioinformatics screens for novel receptors, we discovered a family of 30 putative receptors, which was recently identified by others as a clade within the chemoreceptor superfamily. This clade of thirty IRs includes several members that appear to have undergone recent gene duplications within the Drosophila lineage, suggesting the possibility of recently evolved functions. To determine the expression pattern of these 30 IRs, we constructed IR promoter-Gal4 transgenic flies for each of them and also performed RT-PCR and immunohistochemistry. Our data indicate that most of these 30 IRs are expressed in taste neurons, with some still co-expressed with previously characterized Gustatory Receptors (GRs). Some GRs are found in taste neurons that do not express any known receptors, including neurons in the pharynx, legs, and wings. Interestingly, a subset of sexually dimorphic expressed IRs is expressed in taste sensilla on the legs that do not respond to food-related tastants and do not express Grhs or any other putative taste receptors. Interestingly, we observed sexually dimorphic expression and foreleg-specific expression among a subset of this IR cluster. We hypothesize that this cluster of IRs may also include pheromone receptors.

686B
Variations of larval social behaviors in Drosophila wild-derived inbred lines. Beika Lu, Whitney Pennington, Yehuda Ben-Shahar. Biology, Washington University in St. Louis, St. Louis, MO.

Drosophila larvae exhibit social behavior in different genetic backgrounds. Here, we used the Drosophila Genetic Reference Panel (DGRP, http://www.bgrc.bcm.tmc.edu/) as a tool to explore the role of genetic background on larval social behaviors. Forty wild-derived inbred lines (“core 40”) were analyzed for larval social behaviors. Assayed larvae were classified into three groups, highly social, medium, and low social strains using a “social behavior index”. Analyses of the released sequencing data from the DGRP revealed that there are multiple SNPs and micro-satellites differences between larva high and low social aggregation lines in several gustatory receptor, olfactory receptor gene families, and chemoensory organ development related genes. These findings will be used to identify possible candidate genes for larval social behaviors, as well as the possible signals associated with “high” and “low” social behaviors.

687C
Dual Roles for the Gustatory Receptor 43a in taste perception and nutrient sensing. Tetsuya Miyamoto, Xiangyu Song, Hubert Amrein. Molecular and Cellular Medicine, Texas A&M Health Science Center, College Station, TX.

In Drosophila, Gustatory receptor (Gr) genes are expressed in subsets of taste neurons where they detect soluble chemicals in the environment. Specifically, some Gr genes are necessary for discriminating between palatable, sugar-rich foods and potential food sources that contain non-edible, bitter-tasting and often toxic compounds. Here we show that the highly conserved GR43a protein has a dual role as a taste receptor in taste neurons as an internal nutrient sensor. We generated Gr43aGal4 knock-in flies to determine expression and function of the Gr43a gene. We find that Gr43aGal4 is co-expressed with putative sugar receptor genes in labial and tarsal gustatory neurons. Moreover, Gr43a is also expressed in about 8 neurons in the lateral protocerebrum of the brain and about 6 neurons associated with the proventriculus. To identify the ligand for GR43a, we determine expression and function of the GR43a gene.
POSTER: Neural Physiology and Behavior
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

developed a calcium imaging assay. We show that Gr43aGal4 expressing neurons respond to sugars, but not bitter compounds. Interestingly, response to fructose, but not other sugars, is significantly reduced in neurons homozygous mutant for Gr43a. Proboscis extension reflex assay of wild-type and Gr43a homozygous mutant flies confirmed that Gr43a is necessary for the detection of fructose, but not other sugars. To investigate the function of Gr43a in the brain/proventriculus-associated neurons, we performed calcium imaging experiments and found that Gr43aGal4 expressing neurons also respond to fructose, but not other sugars. Since fructose is a major sugar in Drosophila food and taken up into the hemolymph, we propose that Gr43a acts as an intrinsic nutrient sensor by monitoring ingested fructose levels. This hypothesis is supported by the observation that Gr43a homozygous mutant flies overfeed on fructose and sucrose, the disaccharide composed of fructose and glucose, when compared to wild type flies. Thus, Gr43a represents a precedent for a sugar receptor that functions as in intrinsic nutrient sensor.

Microbial manipulation of animal behavior: investigating the molecular basis of Drosophila- fungi associations. Kelly M Schiabòe1, Michael B Eisen2,3. 1) Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA; 2) Howard Hughes Medical Institute.

Environontal microorganisms play a significant role in the development of the olfactory system. They secrete molecules that affect the growth of the environmental bacteria, and underly affect the animals that live with them. We are interested in understanding the molecular details of the animal-microbe interactions, using the symbiosis between Drosophila and fungi as a model. Fungi are an essential component of the Drosophila larval diet, and both adult and larve drosophilids have evolved sensory mechanisms to detect and locate fungal species within the environment. Fungi also benefit from this physical association, as their interaction with Drosophila help to disperse these non-motive microbes to fresh, sugary substrates. We hypothesize that small molecules produced by fungi help to disperse this symbiosis by acting as both attractants and behavioral cues for drosophilids, and are testing this hypothesis using as our model the interaction between two well-defined model systems: Drosophila melanogaster and the yeast, Saccharomyces cerevisiae.

We began by using gas-chromatography coupled to a mass spectrometer (GC-MS) to characterize the volatiles produced by different yeast strains grown on different fruit substrates at different stages of fermentation. We developed computational methods to identify, assess and compare these compounds. In parallel, we have created a high throughput video-based assay to evaluate differences in fly preference associated with different yeast fermentations. We are currently using these two methods, in concert with the yeast knockout collection, to systematically identify both the volatile compounds responsible for this Drosophila- fungi association, as well as the underlying yeast genes responsible for the production of these molecules.

A specific role for Myb in generating a precise receptor-to-neuron map in the olfactory system. Choon Kiat Sim1, Joe Lipsick1, Anandasankar Ray2. 1) Department of Pathology and Genetics, Stanford University, CA; 2) Entomology, University of California Riverside, CA.

Drosophila detect the important behavioral cue CO2 using an extremely sensitive 7-transmembrane heteromeric receptor encoded by Gr21a and Gr63a [1,2]. These genes are selectively expressed in a specialized population of antennal olfactory neurons that send axonal projections to a single glomerulus, activation of which causes innate avoidance behavior. Very little is known about the mechanism for the selective expression of the two receptors in the appropriate neuron class during development. By using functional analysis such as single-sensillum electrophysiology, together with expression analysis such as promoter-Gal4 and qRT-PCR, we demonstrate that the Drosophila transcription factor Myb is necessary for the normal expression of Gr21a and Gr63a in the endogenous neurons. Bioinformatic analysis of ChIP-chip data reveals that Myb binds to the promoter of the Gr63a gene, suggesting the possibility of direct regulation. This is the first demonstration of a transcription factor playing a role in expression of Gr21a and Gr63a. Moreover, since Myb is one component of a large complex comprised of other transcription factors and chromatin modifying enzymes [3], our results provide a first step to further investigate the role of Myb in generating a precise receptor-to-neuron map in the olfactory system.

Expression and Function of Drosophila sugar receptors. Jesse Slone1, Xiangyu Song1, Joseph Daniels2, Christopher Jaggie1, Tetsuya Miyamoto1, Hubert Amrein1. 1) Molecular and Cellular Medicine, Texas A&M Health Science Center, College Station, TX, 77843; 2) Duke University Medical Center, Durham, NC, 27710.

In Drosophila and other insects, food chemicals are recognized by multicompartmentalized receptors encoded by members of the gustation clones psGr family. However, natural ligands for and the composition of taste receptors are largely unknown. To gain a better understanding of taste discrimination in general and sugar sensing in particular, we investigated expression and function of all eight putative Drosophila sugar receptor (psGr) genes. We constructed numerous psGrGal4 and psGrGal4 knock-in alleles and found that most psGr genes are expressed exclusively in different, but overlapping subsets of sugar-sensing neurons. Surprisingly, Gr5adGal4 was expressed in additional taste neurons in most labellar tastest sensilla, suggesting that Gr5a functions also in perception of non-sugar ligands. To delineate the function of individual psGr genes, we generated a triple mutant fly strain lacking Gr5a, Gr61a and all six Gr genes of the Gr6d locus. While these flies showed virtually no proboscis extension reflex (PER) when stimulated with most sugars, their behavioral response to fructose and sucrose was almost that of wild type flies. To determine the best natural ligands for different receptors, we expressed single or double-combinations of psGr genes in sugar-sensing cells of triple mutant flies using the Gal4 system. The results of this complementation analysis will be discussed. Our major conclusion from our comprehensive studies are i) the eight different psGr genes are expressed in overlapping but not identical subset of taste neurons that mediate sugar perception, ii) the eight psGr genes mediate recognition of most sugars, including glucose, trehalose, maltose and arabinose, iii) the eight psGRs play no major role in sensing the sucrose (fructose and its related disaccharide sucrose), iv) different sugars are detected by multicompartmentalized complexes of psGR proteins.

The larval neuromuscular junction and its presynaptic active zone: Bruchpilot as one organizer of structure and function. Sara Mertel1, Werner Fouquet2, Harald Denes1, David Orwalt2, Carolin Wichmann2, Robert Kittel3, Stefan Haenig3, Jan Schmitt3. 1) Institute of Biologie/Genetics, Free University Berlin, 14195 Berlin, Germany; 2) Cluster of Excellence NeuroCure, Charité, 10117 Berlin, Germany; 3) Institute of Physiology, University of Würzburg, 97070 Würzburg, Germany; 4) Carl-Ludwig-Institute of Physiology, Medical Faculty, University of Leipzig, 04103 Leipzig, Germany.

At synaptic contacts between neurons, the presynaptic active zone organizes Ca2+-mediated release of neurotransmitter to activate neurotransmitter receptors localized at the postsynaptic specialization. How these synaptic compartments assemble and control their function is under intense investigation. Genetic analysis in the fruit fly Drosophila allowed us to identify a master organizer of presynaptic active zones, a protein we called Bruchpilot. At neuromuscular synapses lacking Bruchpilot, clustering of presynaptic Ca2+-channels is defective, and efficiency of neurotransmitter release is dramatically reduced. Thus, this protein might well organize changes of synaptic performance in vivo. We now address the architecture of active zones systematically analyzing synapses in Drosophila larvae. To this end, genetic and biochemical analysis is combined with a recent advance of light microscopy, stimulated emission microscopy (STED). STED drastically increases resolution of fluorescence microscopy, uncovering so far unseen substructures in the molecular architecture of synapses.
The nervous system contains large numbers of neural cell subtypes. While substantial progress has been made with respect to the mechanisms generating neuronal diversity, understanding how matricellular proteins modulate the adhesive and signaling changes that must occur within distinct compartments of complex cells. We observe similar phenotypes in flies lacking /g533PS integrin function suggesting possible physical or genetic interactions. Our results contribute towards these cells. The projections of transmedullary (Tm) neurons in flies without dCCN function do not fasciculate as a layer correctly and instead outgrowth continues into "tree-like" arborizations of optic lobe neurons. We generated a series of single neuron clones to analyze the arborization pattern and dissect the presynaptic and postsynaptic processes of spatiotemporally regulated. amenable to light-mediated acute inactivation. We show that this construct is capable of rescuing both the viability and the synaptic transmission defects in lap mutant flies, and are localization of a subset of SV proteins, including CSP, Synaptobrevin, and Synaptotagmin I. We have also generated a LAP transgene expressed in its full genomic context and sort and retrieve the SV proteins on a rapid timescale, as SV assembly occurs on the order of seconds. Here we test a model in which the Drosophila protein LAP (Like AP180) faithfully reconstitute functional SVs, CME must mediate at least two processes: 1) correct recognition of SV components amid the myriad proteins and lipids in the PM; and, 2) Synaptic Vesicles (SVs) act as principal functional units that store and release neurotransmitters from presynaptic terminals. During synaptic transmission a single SV will go The Role of Drosophila AP180 (LAP) in Reconstitution of Synaptic Vesicles. Phillip Vanlendingham, Hong Bao, Liner Chastain, Chelsea Springer, Bing Zhang. Dept of Zoology, University of Oklahoma, Norman, OK. Synthetic Vesicles (SVs) act as principal functional units that store and release neurotransmitters from presynaptic terminals. During synaptic transmission a single SV will go through repeated rounds of fission and fusion while being refilled with neurotransmitters during each cycle. Therefore, the precise regulation of SV trafficking is fundamental to communication across chemical synapses. Following fusion with the plasma membrane, SVs are endocytosed as their protein and lipid content is simultaneously reconstituted. To faithfully reconstitute functional SVs, CME must mediate at least two processes: 1) correct recognition of SV components amid the myriad proteins and lipids in the PM; and, 2) sort and retrieve the SV proteins on a rapid timescale, as SV assembly occurs on the seconds. Here we test a model in which the Drosophila protein LAP (Like AP180) functions as a core component of the clathrin-mediated endocytic machinery by coupling the recruitment of SV components to the reformation of SVs during endocytosis. To test this model we are using a combination of quantitative biochemical analysis and imaging of SVs in lap mutants. LAP is shown to regulate SV size, synaptic transmission, and the localization of a subset of SV proteins, including CSP, Synaptobrevin, and Synaptotagmin I. We have also generated a LAP transgene expressed in its full genomic context and amenable to light-mediated acute inactivation. We show that this construct is capable of rescuing both the viability and the synaptic transmission defects in lap mutant flies, and are
POSTER: Neurogenetics and Neural Development
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

exploring the specific consequences of the acute inactivation of LAP on SV recycling. The mechanisms underlying SV reconstitution during synaptic transmission are poorly understood. Further, this process may play an important role in disease progression as the human homologue of LAP was recently shown in a wide genome wide association study to be significantly associated with late onset Alzheimer’s disease. Supported by NSF grant #IOS-0822236.

697A Cut mediated transcriptional regulation of the COPII secretory pathway directs class specific dendrite morphogenesis in Drosophila. Strividiya Chandramouli Iyer, Eswar P.R. Iyer, Ramakrishna Meduri, Madhu Karamsetty, Daniel N. Cox. Dept. of Molecular and Microbiology, Krasnow Institute for Advanced Study, George Mason University, Fairfax, VA.

The Drosophila peripheral nervous system (PNS) is a powerful model system in which to investigate the complex processes of neuronal development. Elucidating the molecular mechanisms controlling dendrite development is key to our understanding how neuronal morphologies arise and how they function in achieving synaptic integration and neuronal function. Recent evidence has shown that mutations in select secretory pathway genes preferentially affect dendritic growth. Phenotypic analyses of loss-of-function sec31 mutants, revealed a reduction in dendritic branching indicating a cell autonomous role in mediating da neuron dendritic complexity. Furthermore, gain-of-function analyses indicate sec31 can lead to decreased in dendritic complexity in Class IV da neurons and an increase in complexity in Class I da neurons. Microarray analyses, quantitative RT-PCR and immunohistochemistry experiments reveal that over expression of the homeodomain transcription factor Cut in class I da neurons leads to upregulated expression levels of the components of the COPII-mediated secretory pathway. Microarray analyses and RT-PCR experiments further demonstrate that ectopic over expression of Cut in class I da neurons also leads to an upregulation in the expression of the transcription factor CrebA, previously implicated in secretory activity of the Drosophila salivary gland. Moreover, simultaneous expression of UAS-Cut and CrebA specific UAS-RRNA elements in class I da neurons, suppressed the cut GOF phenotype indicating that CrebA is likely a downstream effector of Cut mediated transcriptional regulation in da neurons. Consistent with this regulatory relationship, overexpression of CrebA in da neurons likewise leads to higher expression levels of components of ER-to-Golgi transport. Collectively, these findings provide novel insight into the role of transcriptional regulation of the COPII-mediated secretory pathway in mediating class specific dendrite morphogenesis.

697B NMNAT protects against hypoxia-induced dendrite degeneration. Yuhui Wen, R. Grace Zhai, Michael D. Kim. Molecular and Cellular Pharmacology, University of Miami, Miller School of Medicine, Miami, FL.

The proper maintenance of dendritic arbors is important for neuronal connectivity and function. Loss of dendrites induced by hypoxia is one of the pathological hallmarks of brain injury after stroke. Dendritic fields of Drosophila class IV dendritic arborization (da) sensory neurons provide a unique system to investigate the mechanisms important for dendrite maintenance. We previously found that the NAD synthase Nicotinamide mononucleotide adenylly transferase (NMNAT) is required for the proper maintenance of class IV dendrites during larval development. Here, we show that NMNAT is important for maintaining dendritic integrity under hypoxia. We found that wild-type flies under anoxic conditions (extreme condition of hypoxia, 0.1% O2) exhibited slightly reduced dendritic branching, but no signs of degeneration. However, nmnat heterozygotes showed reduced dendritic branching along with severe dendrite degeneration phenotypes under the same anoxic conditions. These results suggest that normal levels of NMNAT are important for maintaining dendritic integrity under anoxia. Our preliminary findings suggest that dendrite degeneration in nmnat heterozygotes under anoxia is an autophagy-related process. Our findings show that NMNAT is required for dendrite maintenance and that it can exert protective effects in dendrites under hypoxia.

699A Genetic control of dendrite pruning in Drosophila dendritic arborization neurons. Fengwei Yu1,2,3, Daniel Kiritly1, Ying Gu1,2, Edwin Lim1, Zhihao Wu1, Arash Bashirullah1, Boon Chuan Low2, Alex L. Kolodkin1, Hongyuan Wang1,2. 1) Temasek Life Sciences Laboratory, Singapore, Singapore; 2) Department of Biological Sciences, National University of Singapore, 14 Science Drive, Singapore 117543; 3) Neuroscience and Behavioral Disorder Program, Duke-NUS Graduate Medical School Singapore, 8 College Road, Singapore 169857; 4) Solomon H. Snyder Dept. of Neuroscience, Howard Hughes Medical Institute The Johns Hopkins School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205; 5) Division of Pharmaceutical Sciences, 777 Highland Ave., University of Wisconsin, Madison, WI 53705; 6) Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597.

Pruning that selectively eliminates neuronal processes is crucial for the refinement of neural circuits during development. In Drosophila, the class IV dendritic arborization neuron (ddaC) undergoes pruning to specifically remove its larval dendrites but not axons during metamorphosis. We identified Sox14 as a transcription factor that was necessary and sufficient to mediate dendrite severing during pruning in response to eyeosmotic salting. We found that Sox14 mediated dendrite pruning by directly regulating the expression of the target gene mical. mical encodes a large cytosolic protein with multiple domains that are known to associate with cytoskeletal components. mical mutants had marked severing defects during dendrite pruning that were similar to those of sox14 mutants. Overexpression of Mical could significantly rescue pruning defects in sox14 mutants, suggesting that Mical is a major downstream target of Sox14 during pruning. Thus, our findings indicate that a previously unknown pathway composed of Sox14 and its cytoskeletal target Mical governs dendrite severing. Since Mical is localized in both axon and dendrite, one intriguing question is how Mical mediates dendrite-specific pruning in ddaC neuron. We will present recent findings on this issue as well as a generic screen to identify mutants with dendrite pruning defects.

700A APC/Cmediated CNS neurodegeneration in Drosophila melanogaster. Alexander Braun1,2, Rosa Mino1, Jermaine Lawson1, Armandeep Kaur2, Israel Nnahn1, Elena Kaplan1, Ivan J. Santiago1, Fejzije Balaj1, Tadmiri Venkatesh1. 1) Department of Biology, City College of New York, New York, NY 10031; 2) CUNY Graduate Center, Biology PhD Program, New York, NY 10016.

Cdhn1 is the regulatory subunit of the APC/C (Anaphase promoting complex/cyclosome), a conserved Ubiquitin ligase well known for its role in the regulation of mitosis. Recently, Cdhn1 has been implicated in the regulation of neurogenesis and synaptic differentiation in the CNS (Stegmüller and Bonni, 2010; Yang et al., 2010). We have shown that the Drosophila homolog of Cdhn1 (Rap/Fzr) regulates neuron and glia differentiation in the developing CNS (Kaplow et al., 2008). Our recent studies show that targeted expression of Drosophila Cdhn1 in the CNS results in the loss of a subset of glia in the CNS and leads to progressive age-dependent neurodegeneration, neuronal death and significantly reduced life span. Glia specific overexpression of Cdhn1 also results in temperature sensitive paralysis and disruption of the blood brain barrier in the adult CNS. Our studies involving immunolocalization and confocal microscopy show that neurodegeneration is correlated with increase in ubiquitin accumulation, presence of intracellular aggregates, and altered profiles of autophagy. We have identified specific subtype(s) of glia which are critical for neuro- protection and neurodegeneration in the CNS.

701B Study of Cell Lineage in Drosophila Retinal Basal Glia. Yu Fen Huang1,2, Y. Henry Sun1,2. 1) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan; 2) Department of Life Science and Institute of Genome Science, National Yang-Ming University, Taipei, Taiwan.

Glia cells play important roles in neuronal development and function. The retinal basal glia (RBG) is a subset of glial cell that originates in the optic stalk in second instar of Drosophila. RBG cells start to migrate into the eye disc as photoreceptor cells (R cell) begin to differentiate. The presence of RBG cells in eye discs is essential for R cell axons to enter the optic stalk. Three main classes of RBG cells have been identified including carpet glia, surface glia, and wrapping glia. According to the “sequential differentiation model” proposed by Silses et al. (J. Neurosci. 27:13130-9), surface glia located at the basal side migrates forward along the carpet glia. Once they reach the anterior margin of carpet glia and contact the neuron, these migratory glia starts to differentiate into wrapping glia and wrap around R cell axons. I am trying to follow the temporal sequence of RBG migration and differentiation. My results will be presented.
Identification of new glial cell specific gene functions. Imke Schmidt, Silke Thomas, Christian Klämbt. Institute for neurobiology, University of Münster, Münster, Nordrhein-Westfalen, Germany.

Main functional features of the nervous system have been conserved during evolution. Likewise the biology of neurons and glial cells is remarkable similar in different animal species. The interaction of these two cell types is crucial for the correct formation and subsequent functionality of the neural circuit. Important supportive functions including the insulation and support of neurons, the facilitation and modulation of electrical conductance and synaptic transmission are performed by glial cells. In the embryo and the larval nervous system of Drosophila every segmental unit of the ventral nerve cord contains only 65 glial cells. Due to this lower complexity and a manageable number of glial cells Drosophila represents a well-suited model system to study glial cell biology. To get further insights into Drosophila glial cell biology we performed a glial cell specific RNAi Screen. So far we screened about 5,000 different genes for a cell autonomous requirement in all glia using the panglial driver line repoGal4. The knock down of about 14% of the tested genes leads to lethality. Interestingly, we noted in about 1% of the cases flies with reduced viability and locomotion defects. To determine whether the function of these 800 candidate genes can be attributed to a specific glial cell class we silenced their expression using Gal4 driver lines specific to individual glial cell types (perineurial glia, subperineurial glia, wrapping glia and astrocytic glia). The knock down of kinesin heavy chain (Khc) or tubulin severely affects viability and renders the flies hyperactive. Khc is a well known protein and has been thoroughly analyzed for its role in axonal transport.


Drosophila dendritic arborization (da) neurons have emerged as a powerful model system in which to investigate the molecular mechanisms mediating class-specific dendrite morphogenesis. Recent studies have identified the homeodomain transcription factor Cut as a key regulator of class-specific dendritic architectures of the Drosophila da neurons and mammalian cortical pyramidal neurons. Furthermore, ectopic Cut expression in the morphologically simple class I da neurons has been demonstrated to dramatically increase dendritic branching complexity. However, the molecular mechanism by which Cut exerts differential effects on distinct neuronal classes remains largely unknown. Transcriptional profiling was performed on isolated wild-type and ectopically Cut expressing class I da neurons thereby generating a comprehensive molecular profile of Cut-induced transcriptional changes in Class I da neurons that likely contribute to the substantial changes in dendritic morphology. Microarray analyses and bioinformatic mining of class I da neurons ectopically expressing Cut revealed differential regulation of over 7000 genes among which over 400 genes were significantly upregulated and had conserved Cut consensus binding sites. Functional-enrichment analyses revealed many important biological categories upregulated as a result of ectopic Cut expression, including many transcription factors, cell adhesion/receptor proteins, ion channels, enzymes, secretory pathway genes and cytoskeletal regulators thereby covering a broad range of biological functions. To functionally validate the role of putative downstream Cut transcriptional targets, we performed an in vivo, cell type specific enhancer/suppressor RNAi screen for qualitative suppression of the Cut GOF phenotype in class I da neurons. The results of these studies provide novel mechanistic insight into the molecular programs by which Cut functions in regulating dendritic development.

Drosophila acyl-CoA synthetase long-chain family member 4 regulates axonal transport of synaptic vesicles and is required for synaptic development and transmission. Zhilu Liu, Yan Huang, Yi Zhang, Di Chen, Yong Q. Zhang. Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China.

Acyl-CoA synthetase long-chain family member 4 (ACSL4) converts long-chain fatty acids to acyl-CoAs that are indispensable for lipid metabolism and cell signaling. Mutations in ACSL4 cause non-syndromic X-linked mental retardation. We previously demonstrated that Drosophila dAcsl is functionally homologous to human ACSL4, and is required for axonal targeting in the brain. Here we report that Drosophila dAcsl mutants exhibited distally-biased axonal aggregates of synaptic cargoes. Electron microscopy revealed accumulation of heterogeneous membranous organelles. The profile of these aggregates resembled that of retrograde moving cargos. Live imaging analysis revealed that dAcsl mutations increased the velocity of anterograde transport but reduced the flux, velocity, and processivity of retrograde transport of Syt-eGFP labeled vesicles. Immunohistochemical and electrophysiological analyses showed significantly reduced growth and stability of neuromuscular synapses on the posterior segmental muscles, and impaired neurotransmission in dAcsl mutants. However, the neuromuscular synapses on the anterior muscles were overgrown in dAcsl mutants. The BMP signaling was increased in dAcsl mutant synapses and the synaptic overgrowth was suppressed by the mutations of BMP receptors. The axonal aggregates and synaptic defects in dAcsl mutants were fully rescued by neuronal expression of human ACSL4, supporting a functional conservation of ACSL4 across species in the nervous system. Together our findings demonstrate that dAcsl regulates axonal transport of synaptic vesicles and is required for synaptic development and function. Defects in axonal transport and synaptic function may account, at least in part, for the pathogenesis of ACSL4-related mental retardation.

Identification of novel genes involved in endocytosis and synaptic vesicle recycling. Rui Tian1, Jasmin Podufall2, Carolin Wichmann3, Yong Qing Zhang3, Volker Haucke2, Stephan Sigrist1. 1) Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Da tun Road, Chao Yang District, 100101, Beijing; 2) Institut für Chemie / Biochemie, Freie Universität Berlin, Takustr.6, 14195 Berlin; 3) Charité-Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin.

Drosophila GIT interacts with Stoned B involved in endocytosis and synaptic vesicle recycling. Rui Tian1, Jasmin Podufall2, Carolin Wichmann3, Yong Qing Zhang3, Volker Haucke2, Stephan Sigrist1. 1) Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Da tun Road, Chao Yang District, 100101, Beijing; 2) Institut für Chemie / Biochemie, Freie Universität Berlin, Takustr.6, 14195 Berlin; 3) Charité-Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin.

GIT (G Protein-coupled receptor kinases interacting proteins) is a multimodular scaffolding protein involving in many cellular functions, including focal adhesion remodeling, receptor internalization, membrane traffic and actin cytoskeleton regulation. Communoprecipitation and pull down experiments showed that Drosophila GIT (DGGT) could interact with endocytic molecule Stoned B (StnB) physically. The dgt mutant shows an accumulation of large size vesicles as well as decreased vesicle density at the 3rd instar NMJ by electron microscopy (EM). The accumulation of large size vesicles could be rescued by recruitment of DGGT in dgt mutant background. Large size vesicles are also detected in other endocytic mutants, including stoned, stonpatagom and dynamin mutants (Fergerstad, 1999; Loewen, 2006; Sever, 2000 and Cooney, 2002). These results strongly support the above suggestion that DGGT interacts with StnB involved in the regulation of endocytosis or recycling of synaptic vesicles. However, the biology of neurons and glial cells is remarkable similar in different animal species.
membrane to the cytosol. We have previously shown that phospho-adducin levels are elevated in spinal cord tissue from patients who died with Amyotrophic Lateral Sclerosis, a neurological disease caused by the degeneration of motoneurons. To explore further the roles of adducin in the nervous system, we decided to study Drosophila adducin, encoded by the hu-li tai shuo (hts) locus. We provide evidence that Hts localizes to the postsynaptic membrane of larval neuromuscular junctions (NMJs) in a complex with Discs large (Dlg), a scaffolding protein involved in synaptic plasticity during NMJ development. Hts mutant NMJs contain higher levels of Dlg at the postsynaptic membrane and are severely underdeveloped. In contrast, hts overexpression in the muscle disrupts Dlg localization at the postsynaptic membrane and causes severe NMJ overdevelopment. Previous studies have shown that Dlg postsynaptic targeting is regulated by two kinases: Partitioning-defective 1 (PAR-1) and Calcium/calmodulin-dependent protein kinase II (CaMKII). We show that hts overexpression elevates PAR-1 and CaMKII protein levels in the muscle, causing increased phosphorylation of Dlg. This effect is blocked when the putative target site for PKC in the MARCKS domain of Hts is mutated. We conclude that Hts is a signalling-responsive component of the cytoskeleton that contributes to synaptic plasticity during NMJ development, at least partly by regulating Dlg post-synaptic localization through PAR-1 and CaMKII dependent phosphorylation.

707B


Neuronal differentiation during development is directed by two mechanisms that determine the cell-specific expression profile of terminal differentiation genes: i) The combinatorial action of cell-specific sets of transcription factors (TFs). ii) Retrograde signals secreted from the target cells that the neurons innervate. Although we have a basic understanding of the differentiation of neuronal phenotypes in the developing nervous system, our understanding of the mechanisms that maintain the differentiated state of mature neurons in adults is very rudimentary. Previously, we demonstrated that retrograde bone morphogenetic protein (BMP) signaling in concert with a unique combinatorial code of TFs induces expression of a phenotypic marker, the neuropeptide FMRFamide (FMRFa), in Drosophila Tv neurons (Cell; 2003; 113, 73-86). More recently, we have now turned to an analysis of whether the transcription factors that act in development to differentiate Tv neurons and initiate FMRFa expression, act in mature neurons to maintain differentiation and FMRFa expression. In this presentation, we shall discuss our evidence that while numerous TFs retain a critical role in maintenance, their cross- regulatory interactions and relative importance for FMRFa expression is altered from initiation to maintenance.

708C

Cis-Regulatory Integration of Intrinsic Transcription Factors with Target-Derived Signals in Neuronal Differentiation. Anthony Berndt1, Marc Riedy1, Tianshun Lian1, Jonathan Tang1, Douglas Allan1. 1) Cellular & Physiological Sciences, University of British Columbia, Vancouver, British Columbia, Canada; 2) Department of Molecular and Cellular Biology Harvard University 16 Divinity Ave. Cambridge, MA 02138.

Drosophila Tv neurons extend their axons to the dorsal neurohaemal organs and activate the expression of the neuropeptide, FMRFa, in response to target-accessible source of the BMP ligand, Gbb. Gbb signaling activates canonical BMP signaling via the type II receptor Wishful Thinking and the type I receptors Thickveins and Saxophone, which acts via Mad to induce FMRFa expression in concert with a well-characterized combination of transcription factors. It has not yet been established whether or how any of the FMRFa transcription factor code integrates with BMP signaling to cell-specifically activate FMRFa expression. We investigated how these inputs are integrated in Tv neurons through mutational analysis of a previously identified 446 bp cis-regulatory module (CRM) that faithfully reproduces FMRFa expression selectively in Tv neurons. Mutant analysis of transcription factors and the BMP pathway reveals that most known FMRFa regulators such as Apterous and BMP signaling regulate expression of the Tv CRM. Using the integrase transgenesis system, we assayed the functional relevance of predicted transcription factor binding sites in this CRM. We present data supporting a model by which the Tv CRM integrates BMP signaling and cell-specific transcription factors in the regulation of FMRFa neuron differentiation.

709A

An RNAi-based Screen to Identify Transcription Factors Required for the Development of the Wing Expansion Circuit in Drosophila. Isaac Dripps, Fengqiu Diao, Benjamin White. LMB, NIMH, NIH, Bethesda, MD.

When an adult fly exits the pupal case after metamorphosis, it seeks out a suitable perch and expands its wings. This process, requiring the stereotyped execution of behaviors to drive hemolymph into the wings to unfold them, must behardwired during development since it is executed flawlessly without prior experience. Although the wing expansion circuit has been partially characterized, the genetic determinants that guide its assembly are largely unknown. To identify these determinants, we sought to find transcription factors (TFs) that specify the functional and anatomical properties of neurons in the circuit. To do so, we conducted a TF RNAi-based screen targeting known to be essential components of the circuit. These neurons include a set that expresses the neuromodulator CCAP and a subset that expresses the hormone bursicon. Using CCAP- and Burs-Gal4 drivers, we screened two groups of candidate TFs to identify those that produce wing expansion defects when their expression is knocked down. The first group included 23 TFs in the edysone response pathway and was selected because the wing expansion circuit forms during metamorphosis as part of the generalized nervous system remodeling governed by the steroid hormone edysone. The second group includes all 28 TFs containing a BTB binding domain and was selected because several BTB-containing TFs, such as Fruitless, Lola, and Chimo have been implicated in neural circuit formation. In total, 13 TFs from the edysone response pathway and 12 of the BTB-containing TFs appear to play a role in wing expansion progress. The most robust wing expansion deficits resulted from knock-down of the edysone-inducible nuclear receptors Eip75B and Hr46 and the BTB-containing TFs lola and mamo. To further define the roles of these factors in specifying the properties of CCAP- and bursicon-expressing neurons, we are currently assessing the effects of knock-down on the development, gene expression, and connectivity of these neurons by immunostaining CNS wholemounts from candidate lines.

710B

Subdivision of the neuroectoderm into discrete dorsal-ventral domains: generated by a conserved mechanism? Francisco F Esteves1, Erika Kague2, Shannon Fisher2, Ethan Bier1. 1) Dept Biological Sci, Univ California, San Diego, La Jolla, CA; 2) Department of Cell and Developmental Biology University of Pennsylvania, Philadelphia, PA 19104.

The relative patterns of transcription factor expression that determine the main rows of neurons are similar in Drosophila and vertebrates. The main candidates for conserved pattern determination signal between these animals at this stage are the Bone Morphogenetic Proteins (BMPs). However, the prevailing view is that this signal seems to be affected by different modalities in Drosophila (graded repression) versus Vertebrates (graded activation). In the present work, we explore the possibility that BMPs are an ancestral source of patterning, whether there really is a difference in modality and how Drosophila and Vertebrate cis-regulatory modules integrate BMP signaling during neuroectoderm patterning.

711C

Epigenetic Regulation of Odorant Receptor Expression in the Drosophila Olfactory System. PELIN C. VOLKAN1,2, QINGYUN LI1. 1) DEPARTMENT OF BIOLOGY, DUKE UNIVERSITY, DURHAM, NC; 2) DUKE INSTITUTE FOR BRAIN SCIENCE, DUKE UNIVERSITY, DURHAM, NC.

Inheritance of transcriptional states via regulation of chromatin structure is one of the proposed mechanisms by which cellular fates are restricted during development. In the adult Drosophila olfactory circuitry developmentally related 1200 odorant receptor neurons (ORNs), each expressing generally one out of 60 possible odorant receptor genes (ORs) are specified from olfactory precursor cells. Specific expression of single OR genes in different classes of ORNs provides a unique system with a major regulatory challenge during the development of these neurons. In a genetic screen for genes regulating ORN class-specific organization in the olfactory system, I recovered mutants in alhambra, which encodes an evolutionarily conserved protein with chromatin-mediated gene silencing function. Very little is known about mechanisms by which Alh regulates chromatin. Loss of all function changes the expression pattern of Or47b, Or88a, and Or65a in all ORNs. The phenotype is associated with an increase in the total number of neurons that express
Or7b, and a concomitant decrease in the Or88a neurons. The Or7b expressing ORN synapses innervate both an enlarged Or7b glomerulus (due to increase in the total number of Or7b neurons) and Or88a glomerulus. So both Or88a and Or88a neurons adopt an Or7b sensory identity. Disruption of OR expression pattern in all mutants, suggests a role for epigenetic mechanisms in regulating OR expression among ORs in the same sensilla.

712A

**Robust specification of sensory neuron by dual function of Charlatan, a Drosophila NR5F1/REST-like repressor of extramacrochaetae and hairy.** Yasutoyo Yamasaki, Young-Mi Lim, Leo Tsuda. National Center for Geriatrics and Gerontology, Obu, Aichi, Japan.

Sensory bristle formation of Drosophila is well-characterized system for studying the sensory organ development at the molecular level. The main proneural genes, of *achaete-scute* (*ac-sc*) complex encoding basic-helix-loop-helix (bHLH) transcription factors, have been shown to be necessary and sufficient for the sensory bristle formation. Charlatan (Cln) was originally identified as a transcriptional activator for ac-sc expression through interaction with their enhancer and promotes sensory bristle development. In the previous study, however, Cln was also identified as a transcriptional repressor molecule which shares the characters with mammalian neural restricted silencing factor/repressor element (NRSF/REST), an important transcriptional repressor for vertebrate neurogenesis and stem cell development through epigenetic gene silencing (Tsuda et al., 2006). To assess the transcriptional function of Cln in sensory organ development, we performed *in vivo* and *in vitro* analyses. Immunohistochemistry revealed that in the context of sensory neuron development Cln acts in addition as a repressor of *extramacrochaetae* (*emc*) and *hairy*, inhibitory molecules for ac-sc expression. This double negative mechanism, together with direct activation via *achaete* enhancer, increases expression of *achaete* and ensures robust development of sensory neurons. Using luciferase assay, we show that Cln has transcriptional repression activity. Moreover ectopically expressed mutated Cln, which has a mutation in the C-terminal repressor region of Cln, converted Cln into an activator of *emc* and *hairy* in the imaginal disc, suggesting that Cln is a dual functional regulator of transcription. Since Cln sequences are found among arthropods, the regulation of neuronal development by Cln-like molecules may be widely conserved.

713B

**Pharmacogenetic regulation of acetylcholinesterase activity in Drosophila reveals the regulatory mechanisms of AChE inhibitors in synaptic plasticity.** Wontae Kim1,2, Daeweon Lee2, Jinkyu Choi1, Ayoung Kim2, Sangmi Han1, Kwanho Park1, Jiyoung Choi1, Jonggil Kim1, Youngeheol Choi1, Sibyeock Lee1, Youngho Koh2. 1) National Academy of Agricultural Science, Suwon, Korea; 2) Ilsong Institute of Life Science, Hallym University, Anyang, Korea.; 3) Seoul National University, Seoul, Korea.

We conducted experiments in Drosophila to investigate the consequences of altered acetylcholinesterase (AChE) activity in the nervous system. In ac hypomorphic mutant larvae, the amount of ace mRNA and the activity of AChE both in vivo and in vitro were significantly reduced compared with those of controls. Reduced Ace in Drosophila larvae resulted in significant down-regulation of branch length and the number of boutons in Type 1 glutamatergic neuromuscular junctions (NMJs). These defects in ac hypomorphic mutant larvae were suppressed when Musca domestica AChE was transgenically expressed. Because AChE is decreased in aphids, it is utilized for medications for Alzheimer’s disease, we investigated whether pharmacological inhibition of AChE activity induced any synaptic defects. We found that controls exposed to a sublethal dose of DDVP phenocopied the synaptic structural defects of the ace hypomorphic mutant. These results suggest that down-regulation of AChE activity, regardless of whether it is due to genetic or pharmacological manipulations, results in altered synaptic architecture. Our study suggests that exposure to AChE inhibitors for 6 to 12 months may induce altered synaptic architectures in human brains with Alzheimer’s disease, similar to those reported here. These changes may underlie or contribute to the loss of efficacy of AChE inhibitors after prolonged treatment.

714C

**Trophic actions of neuronal dopamine during the development of a serotonergic feeding circuit in Drosophila melanogaster.** Wendi S. Neckameyer, Parag Bhatt. Dept Pharmac & Physiol Sci, St Louis Univ School Med, St Louis, MO.

The neurotransmitter actions of serotonin (5-HT) depress feeding, and decreased neuronal 5-HT levels increase appetite. However, knockdown of neuronal 5-HT synthesis to reduce 5-HT levels during central nervous system development results in increased branching of the 5-HT axonal fibers projecting to the gut, as well as increased size and number of 5-HT presynaptic varicosities along the neurite length, in the larval foregut. As larvae, these animals display decreased feeding rates relative to controls, and, when given exogenous 5-HT, feeding is significantly enhanced. Late-stage wild-type embryos exposed to 5-HT to augment 5-HT levels during CNS development display, as mature larvae, a significant decrease in gut branching and total varicosity number, as well as increased feeding and a hypoglossic sensitivity to the effects of 5-HT. Dopamine (DA) does not act as a neurotransmitter to modulate larval feeding behavior, and DA fibers do not innervate the foregut. However, both decreased and increased neuronal DA levels during the last 6 hours of embryogenesis result in depressed larval feeding, a hypoglossisensitivity to the neurotransmitter actions of 5-HT, and increased branching of the 5-HT axonal fibers projecting to the gut, as well as increased size and number of 5-HT presynaptic vesicles along the neurite length. The developmental actions of DA and 5-HT are not redundant, and both are required for normal development of the feeding circuit. This trophic action for DA is modulated, at least in part, by the DA D2 receptor expressed during earlier embryogenesis in 5-HT neurons.

715A

**Comparative analyses of the corazonin gene in dipteran insects.** Kai Sha, Craig Conner, Dae Choi, Jae Park. University of tennessee - Knoxville, BCMB, TN.

We describe a gene encoding a neuropeptide Corazonin (Crz) in *Musca domestica* (*M. domestica*), characterized its spatial and developmental expression in the central nervous system (CNS). The Crz-encoding gene of *M. domestica* (*MdCrz*) contains two introns, one within the 5' untranslated region (UTR) and the other in the open reading frame (ORF). The 150-amino-acid precursor deduced from the 738-bp full-length cDNA consists of an N-terminal signal peptide, and mature Crz followed by Crz-associated peptide (CAP). The CAP peptide region is highly diverged from those of other insect precursors, whereas the primary structure of the mature Crz is identical to other dipteran members of the Crz family. In situ hybridization and immunohistochemistry consistently found three pairs of MdCrz-producing neurons in the dorso-lateral protocerebrum, and eight bi-lateral pairs of weakly expressing neurons in the ventral nerve cord (VNC) in the larva. In adults, the expression was found exclusively in the fifth to seventh pairs of abdominal neurons located in the pars-lateralis of the brain. Comparative expression profiles of the MdCrz to those observed in other distantly related dipteran species suggest that the regulatory mechanisms of the Crz expression and Crz functions are conserved during the course of evolution, although the structure of the Crz-encoding gene is rapidly diverged.

716B

**Drosophila FMRP regulates microtubule network formation and axonal transport of mitochondria.** Ai Y. Yao1, Shan Jin1,2, Xin H. Li1, Zhi H. Liu1,2, Xue H. Ma1, Jing Tang1, Yong Q. Zhang. 1) Institute of Genetics and Developmental Biology, Chinese Academy of Sciences , Beijing, China; 2) Hubei University, Wuhan, Hubei, China.

Fragile X syndrome, the most common form of inherited mental retardation, is caused by the absence of the fragile X mental retardation protein FMRP. The RNA-binding FMRP represses translation of the microtubule-associated protein 1B (MAP1B) during synaptogenesis in the brain of the neonatal mouse. However, the effect of FMRP on microtubules remains unclear. Mounting evidence shows that the structure and function of FMRP are well conserved across species from *Drosophila* to human. From a genetic screen, we identified *spastin* as a dominant suppressor of rough eye caused by dfmr1 over-expression. *spastin* encodes a microtubule-severing protein and its mutations cause neurodegenerative hereditary spastic paraplegia. Epistatic and biochemical analysis revealed that dfmr1 acts upstream of or in parallel with *spastin* in multiple processes, including synapse development, locomotive behaviour and microtubule network formation. Immunostaining showed that both loss- and gain-of-function mutations of dfmr1 result in an apparently altered microtubule network. Western analysis revealed that the levels of α-tubulin and acetylated microtubules remained normal in dfmr1 mutants but increased significantly when dfmr1 was over-expressed. To examine the consequence of the aberrant microtubules in dfmr1 mutants, we analysed the microtubule-dependent mitochondrial transport and found that the number of mitochondria and the flux of mitochondrial transport are negatively regulated by dfmr1. These results demonstrate that dfMRP plays a role in regulating the mitochondrial transport via FMRP.
crucial role in controlling microtubule formation and mitochondrial transport. Thus, defective microtubules and abnormal mitochondrial transport might account for, at least partially, the pathogenesis of fragile X mental retardation.

717C

Regulation of neural stem cell fate in Drosophila cell death mutants. RICHA ARYA, YING TAN, MEGUMI YAMADA-MABUCHI, KRISTIN WHITE. CBRC, MGH/HARVARD, CHARLESTOWN, MA.

Cell death is important for normal development and differentiation of an organism. Defects in the normal cell death pathway contribute to degenerative diseases and cancer. In both mammals and non-mammalian models, neural stem cells are eliminated by cell death. To study how the death of neural stem cells is regulated and the consequences of their prolonged survival, we are using a Drosophila model in which neural stem cells survival is prolonged due to defects in the cell death pathway. We have generated mutants that lack key cell death regulators; in various combinations these mutants interrupt the developmental cell death process. In the cell death mutants, the neural stem cells (Neuroblasts) continue to proliferate, and their progeny send out extensive axon bundles throughout the CNS. These ectopic neural progeny are incorporated into the adult CNS, resulting in significant dysmorphology. We found that these inappropriately surviving neuroblasts do not divide randomly in their patterns of proliferation. Both temporal and positional cues regulate the quiescence of these ectopic stem cells. Furthermore, the ectopic stem cells express appropriate identity markers, but their progeny often project incorrectly when compared to their normal counterparts. However, these inappropriate surviving stem cells do not divide indefinitely and are eliminated by an unknown mechanism. The study suggests that the behavior of stem cells is likely governed by both intrinsic and extrinsic factors. Patterns of proliferation obey spatial rules, which may be “remembered” by the neural stem cell, or may reflect cues from the environment. It seems that the identity of a neural stem cell is predefined and the cells can remember, “Who they are” even if they are allowed to survive in a novel background. However, we identified abnormal projection pattern for many neural stem cell lineages indicating that the fate of their progeny may be influenced by extrinsic factors. We expect that further studies will help us to identify the intrinsic and extrinsic factors that govern neuroblast behavior in this model.

718A


Mechanoreceptor organs are typically highly structured, with extracellular and cytoskeletal structures adapted to transmit mechanical stimuli to the sensory endings where transduction occurs. In Drosophila type I mechanosensory organs, the sensory ending is directly contacted by the extracellular matrix of the dendritic cap or sheath, suggesting the cap delivers the mechanical stimulus to the sensory ending. NOMPA, which is a modular extracellular protein with a ZP domain and several Plasminogen N-terminal (PAN) modules, is the only component of the dendritic cap known so far. To investigate the role of NOMPA domains in connecting mechanosensory cilia to the dendritic cap, we generated transgenic flies expressing various versions of modified NOMPA proteins. The interaction of the sensory endings with the dendritic caps containing the modified NOMPA proteins were analyzed by anti-NOMPC antibody that specifically labels the distal most part of the mechanosensory cilia. The results will be presented.

719B

Exploring the basis of neural enhancer sub-pattern specificity: Fine structure analysis of cis-regulatory sequences that control the late temporal network determinant castor. Muktia Kundu, Alexander Kuzin, Jermaine Ross, Thomas Brody, Ward F. Odenwald. Neural Cell Fate Determinants, NINDS, Bethesda, MD.

Our analysis of sequences regulating the late temporal neuroblast (NB) determinant castor (cas) has revealed 6 modular enhancers. Located upstream of the structural gene, each consists of a highly conserved sequence cluster (CSC) and each exhibits temporally delimited patterns of expression in overlapping but non-identical sets of NBs that constitute the wild-type cas expression pattern. In order to discover the functional basis for their sub-pattern specificity we have embarked on the bioinformatics and functional analysis of the conserved sequences within these enhancers. One set of cas late temporal network enhancers contains both bHLH binding sites and POU domain binding sites, targeted by the intermediate temporal network determinants Pdm-1 and Pdm-2. Using cis-decoder alignment algorithms we have discovered a second class of enhancers that lack these identifying characteristics but share other novel conserved sequence elements. Our mutational analysis has revealed that two of these CSCs can be sub-divided into sub-clusters that can function independently to drive expression in more delimited patterns of NBs. We will report on base substitution experiments that identify specific roles for conserved sequence clusters in enhancer function. Together the results reveal an unexpected regulatory complexity in the late temporal network in terms of the DNA targeted by temporal network transcriptional regulators.

720C

Notch signaling regulates neuroepithelial stem cell maintenance and neuroblast formation in Drosophila optic lobe development. Hong Luo, Wei Wang, Wenke Liu, Yue Wang, Liya Zhou, Xiaofang Tang. School of Life Sciences, Tsinghua University, Beijing, China.

Notch signaling mediates multiple developmental decisions in Drosophila. In this study, we have examined the role of Notch signaling in Drosophila larval optic lobe development. Loss of function in Notch or its ligand Delta leads to loss of the lamina and a smaller medulla. The neuroepithelial cells in the optic lobe in Notch or Delta mutant brains do not expand but instead differentiate prematurely into medulla neuroblasts, which lead to premature neurogenesis in the medulla. Clonal analyses of loss-of-function alleles for the pathway components, including N, Dl, Serr(H) and E(spl)-C, indicate that the Delta/Notch/Serr(H) pathway is required for both maintaining the neuroepithelial stem cells and inhibiting medulla neuroblast formation while E(spl)-C is only required for some aspects of the inhibition of medulla neuroblast formation. Conversely, Notch pathway overactivation promotes neuroepithelial cell expansion while suppressing medulla neuroblast formation and neurogenesis; numb loss of function mimics Notch overactivation, suggesting that Numb may inhibit Notch signaling activity in the optic lobe neuroepithelial cells. Thus, our results show that Notch signaling plays a dual role in optic lobe development, by maintaining the neuroepithelial stem cells and promoting their expansion while inhibiting their differentiation into medulla neuroblasts. These roles of Notch signaling are strikingly similar to those of the JAK/STAT pathway in optic lobe development, raising the possibility that these pathways may collaborate to control neuroepithelial stem cell maintenance and expansion, and their differentiation into the progenitor cells.

721A


P21-activated kinases (PAKs) are well established downstream effectors of Rho-GTPases and regulate various cellular processes such as cell motility and survival, transcription and mitosis. Although they have been implicated in neural development, a role in the proliferation of neural progenitors has not been described for PAKs so far. Mushroom bodies (Mbt), the only type II PAK in Drosophila, was identified in a screen for structural brain mutants. Loss of mbt affects neural development and results in a rough eye phenotype and a reduction of the mushroom body, a central brain neuropil. During eye development, Mbt was found to localize to adherens junctions where it controls morphogenetic movements. Overexpression of Mbt in the larval brain leads to strong hyperplasia. We found that these inappropriately surviving neuroblasts do not behave randomly in their patterns of proliferation. Both temporal and positional cues regulate the quiescence of these ectopic stem cells. Furthermore, the ectopic stem cells express appropriate identity markers, but their progeny often project incorrectly when compared to their normal counterparts. However, these inappropriate surviving stem cells do not divide indefinitely and are eliminated by an unknown mechanism. The study suggests that the behavior of stem cells is likely governed by both intrinsic and extrinsic factors. Patterns of proliferation obey spatial rules, which may be “remembered” by the neural stem cell, or may reflect cues from the environment. It seems that the identity of a neural stem cell is predefined and the cells can remember, “Who they are” even if they are allowed to survive in a novel background. However, we identified abnormal projection pattern for many neural stem cell lineages indicating that the fate of their progeny may be influenced by extrinsic factors. We expect that further studies will help us to identify the intrinsic and extrinsic factors that govern neuroblast behavior in this model.

282

During nervous system development, neural progenitor cells divide asymmetrically, renewing themselves and budding off daughter cells with more restricted potential. Daughter cells can either differentiate directly, or divide to expand a certain branch of a lineage tree. However, how the mitotic potential of any given daughter cell is controlled during neural lineage progression is not well understood. In the Drosophila embryo, neural progenitor cells, neuroblasts (NBs), generate the CNS by series of asymmetric cell divisions. Daughter cells from such divisions, ganglion mother cells (GMCs), typically divide once to generate two neurons or glia. However, in certain lineages, such as thoracic NB 5-6, there is an intriguing switch in division mode, such that the four last-born daughter cells never divide, instead differentiate directly into the Apterous (Ap) neurons. The prospero gene controls cell cycle exit of GMCs, and in pros mutants there is an expansion of the early NB5-6 lineage, as a result of extra GMC divisions. However, the directly-generated Ap neurons are not affected, and thus the lineage topology switch is not controlled by pros. In a genetic screen we have identified two loci that fail to execute the switch. Here, Ap cells undergo one ectopic round of division before differentiating, resulting in a doubling of the number of Ap neurons. These two loci map to kuzbanian and neutralized-two components of the Notch signaling pathway. We find that the Notch pathway is not critical for the specification of Ap neurons, but acts exclusively to control the switch in daughter cell division potential. The reason why Ap neurons only divide ectopically one round in Notch mutants is that each Ap neuron is converted into a GMC, and GMCs are prevented from dividing by pros. Strikingly, in pros, Notch double mutants, Ap cells now divide multiple times. To our knowledge, this is the first identified mechanism for a genetic switch in neural lineage topology.

Regulation of neural stem cell self-renewal and differentiation. Hongyan Wang1,2, Kai Chen Chang1, Gisela Garcia-Alvarez1, Gregory Somers2, Rita Sousa-Nunes3, Fabrizio Rossi3, Cayetano Gonzalez2, William Chia1. 1) Duke-NUS Graduate Medical School Singapore, Singapore, Singapore; 2) Temasek Life Sciences Laboratory, 1 Research Link, Singapore, Singapore 117604; 3) Department of Genetics, La Trobe Institute for Molecular Science (LIMS), La Trobe University, Vic 3086 Australia; 4) National Institute for Medical Research, Mill Hill, London NW7 1AA, UK; 5) Cell Division Group, IRB-Barcelona, PCB, c/Baldri Reixac 10-12, Barcelona, Spain; 6) Dept. of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597.

Drosophila larval brain neuroblasts have recently emerged as an excellent model for studying stem cell self-renewal and tumorigenesis. During asymmetric divisions neuroblasts utilize the asymmetric localization/segregation machinery to distribute "proliferation factors" to the neuroblast daughter and "differentiation factors" to the GMC daughter. Failure in asymmetric cell divisions of neuroblasts can result in two daughter cells that both retain proliferative stem/lineal cell-like properties, leading to overproliferation in the brain. Atypical protein kinase (aPKC) promotes self-renewal of Drosophila larval brain neural stem cells, neuroblasts. This study identifies a C2H2 type zinc-finger transcription factor that we have named 'Zif', which regulates aPKC expression and its cortical polarity. Zif is essential for the asymmetric localization of aPKC, and our evidence suggests that it directly represses aPKC transcription by binding to the aPKC promoter region. In addition, we show an intriguing reciprocal activity of aPKC on Zif: that aPKC directly phosphorylates Zif, regulating its activity via control of its subcellular localization. Therefore, our studies show mutual inhibition between Zif and aPKC plays a key role in regulating asymmetric division and self-renewal of neuroblasts.
Axonal transport is essential for proper synapse function and contributes to distal synaptic abnormalities seen in neurodegenerative disease. Shermali D. Gunawardena¹, Min J. Kang¹, Monique Michiewicz¹, Tadeusz J. Kaczynski¹, Samantha Fye¹, Hong Bao², Bing Zhang², Shermali D. Gunawardena¹. 1) Department of Biological Sciences, The State University of New York at Buffalo, NY 14260; 2) Department of Zoology, University of Oklahoma, Norman, OK 73019.

Formation of new synapses or maintenance of existing synapses requires the delivery of synaptic components from the soma to axodendritic sites, and problems in axonal transport could initiate distal synaptic abnormalities. Synaptic alterations observed in neurodegenerative disease could also arise due to perturbations in long distance transport. Individuals with early-stage Alzheimer’s Disease suffer from failure to form new memories and subtle alterations of synaptic efficacy are seen prior to neuronal degeneration. In Huntington’s Disease, neurotransmission problems are observed early in disease. Here we examine how perturbations in axonal transport influence synaptic morphology and function. Using pre and post synaptic markers we find that both kinesin and dynein motor protein mutants show defects in synaptic morphology, in the number of boutons, the ratio of pre and post synaptic bouton size and in synaptic length. Similar defects are observed in larval synapses expressing pathogenic polyQ repeat protein or the human amyloid precursor protein with an FAD mutation. Both these disease mutants contain axonal transport defects. Synaptic transmission defects are observed in dynein mutants and in larvae expressing pathogenic polyQ repeat proteins. However, a synaptic protein mutant that shows severe synaptic defects does not show axonal transport defects. Our results indicate that perturbations in axonal transport can propagate defects in synapse maturation and function; however, synaptic problems have no direct consequence on axonal transport. Further, proper maintenance and function of synapses requires the efficient transport of BMP signaling components. Thus, disruptions in axonal transport by disease proteins can contribute to synaptic abnormalities observed early in neurodegenerative disease.

Mechanical force initiates the neuromuscular synapse. Tiffany Li¹, Wylie Ahmed², Jie Sun¹, Scott Siechen¹, Franklin Carrero-Martínez³, Taher Saijf², Akira Chiba¹. 1) Biology, University of Miami, Coral Gables, FL; 2) Mechanical Science and Engineering, University of Illinois, Urbana, IL; 3) Biology, University of Puerto Rico, Mayaguez, Puerto Rico.

The usage-dependent assembly and enhancement of synapses is central to learning, memory and other forms of behavioral plasticity in animals. We show that mechanical force is applied naturally at nascent neuromuscular synapses and, furthermore, required during presynaptic molecular assembly. Using micromanipulation, genetics, pharmacology and imaging, we uncouple force production from neurotransmission and demonstrate short-term autonomy in synaptogenesis. Our work links mechanical force to neuronal connectivity.


We previously identified a genetic interaction between spastin and p21-activated kinase 3 (Pak3) in the eye, and now show that the two genes cooperate during synapse development at the larval neuromuscular junction (NMJ). Spastin, an AAA ATPase that severs microtubules, is required for proper neuronal function in flies, and underlies the progressive neurodegenerative disease Autosomal Dominant Hereditary Spastic Paraplegia in humans. Pak3 was identified as an effector of Rac/Cdc42 that regulates the actin cytoskeleton in cell culture, but its in vivo role remains unknown. Using a combination of hypomorphic and null alleles, we found that Pak3 loss of function results in larval and pupal lethality demonstrating that Pak3 is an essential gene. Loss of spastin function results in severe synaptic defects at the larval NMJ, including an increased number of smaller synaptic boutons, reduced microtubule expression in distal boutons, and increased bouton clustering. The clusters are a disorganized array of similarly-sized boutons that resemble a bunch of grapes. We thus examined larvae lacking spastin for similar neuronal phenotypes. Pak3 homozygotes did not differ from wildtype in total bouton number or branching, but distal microtubules were slightly reduced. Furthermore, supporting the genetic interaction between spastin and Pak3 observed in the eye, the bunches of small boutons characteristic of spastin nulls were almost completely rescued in a Pak3 loss of function background. Pak3 is therefore functionally relevant to the process of synaptic bouton formation through an antagonist interaction with spastin function. We are now working to identify the Pak3 site of action, and the mechanism underlying its interaction with spastin.
POSTER: Pattern Formation
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

730A
Quantitative Analysis of Dorsal Ventral Patterning in Pre-blastula Crossveinless 2 Drosophila Embryos. Christina Brakken-Thal1,2, David Umulis3, Hans Othmer4, Michael O'Connor1, 1) Chemical Engineering, University of Minnesota, Minneapolis, MN; 2) MD/PhD Program, University of Minnesota, Minneapolis, MN; 3) Agricultural and Biological Engineering, University of Purdue, West Lafayette, IN; 4) Department of Mathematics, University of Minnesota, Minneapolis, MN; 5) Genetics Cell Biology and Development, University of Minnesota, Minneapolis, MN.

The dorsal surface patterning of the Drosophila embryo is determined by the Dpp morphogen gradient. Dpp is produced at uniform levels on the dorsal surface of the embryo, but forms a concentration gradient through interactions with extracellular modulators. The Dpp protein gradient contracts around the dorsal midline, creating a region of high signaling specifying the amnioserosa and a low signaling region specifying the dorsal ectoderm. Current models for explaining how the Dpp gradient contracts and intensifies around the dorsal midline require positive feedback, but the mechanism has not been characterized to date. Initial results indicate that Crossveinless 2 (Cv-2) may be a surface localized Dpp binding protein that is upregulated by Dpp signaling, and may buffer against low temperature fluctuations. We are developing techniques to quantitatively analyze the changes in Dpp signaling on the dorsal surface of the embryo in Cv-2 embryos at 18-28°C using fluorescence microscopy and imaging software written in Matlab.

731B
A novel mutation affecting the patterning of the appendage primordia in the follicular epithelium. Mariana Fregoso Lomas, Biology, McGill University, Montreal, Canada.

We use the follicular epithelium of the Drosophila ovary as a model to study signaling pathways and patterning during development. The cells within this epithelium acquire different fates and are responsible for the secretion of asymmetric eggshell structures, like the two dorsal appendages. We are interested in knowing how the follicle cell primordia producing these appendages are determined. They are located in the dorsal anterior region of the tissue, can be recognized through the high expression of the Broad-Complex (Broad) transcription factor, and are separated by a region of cells at the dorsal midline that do not express Broad. Outside of this region, all follicle cells express basal levels of Broad. Dorsally-localized EGF signaling coming from the underlying oocyte initiates this complex spatial pattern along the dorso-ventral axis, but the signals involved in patterning the anterior-posterior (AP) axis are less well understood. To better understand AP patterning, we are characterizing a new locus called F27 required for this process. F27 mutant follicle cell clones located in a dorsal region posterior to the midline and the two appendage primordia express ectopic high Broad, suggesting that dorsal anterior fates have been determined in posterior follicle cells. Consistent with this change in cell fate, the eggshell produced by such egg chambers exhibits extra appendage material at the midline of the appendage base. We show that the competence of F27 mutant cells in this region to express high Broad can be visualized in earlier stages by looking at the expression of the transcription factor domain. In addition we see ectopic expression of rhomboid and down regulation of Capicua and Echinoid, molecules that normally pattern the dorsal appendages. These observations suggest that the changes in fate induced by the F27 mutation involve features of the endogenous appendage primordia. The shape of this uncovered domain and the fact that it acts upstream of mirror strongly suggest that the F27 mutation could be affecting an early signal, presumably within the EGFr pathway.

732C
The formation of the Bicoid morphogen gradient requires protein movement from anteriorly localized mRNA. Shawn C. Little1, Gasper Tkacik2, Thomas Knessel3, Eric F. Wieschaus1, Thomas Gregor1. 1) Department of Molecular Biology, Princeton University / HHMI, Princeton, NJ; 2) Department of Physics and Astronomy, University of Pennsylvania, Philadelphia, PA; 3) Joseph Henry Laboratories of Physics, Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ.

The Bicoid morphogen gradient directs the patterning of cell fates along the anterior-posterior axis and serves as a paradigm of morphogen-mediated patterning. The simplest model of gradient formation relies on constant protein synthesis and diffusion from anteriorly localized source mRNA, coupled with uniform protein degradation. Recent work has proposed that bicoid mRNA spatial distribution is sufficient to produce the observed protein gradient, minimizing the role of diffusion. Here, we adopt a novel method of fluorescent in situ hybridization to quantify the global spatio-temporal dynamics of bicoid mRNA particles. We determine that greater than 90% of all bicoid mRNA is continuously present within the anterior 20% of the embryo. bicoid mRNA diffusion along the body axis remains nearly unchanged despite dynamic mRNA translocation from the embryo core to the cortex. To evaluate the impact of mRNA distribution on protein gradient dynamics, we provide the first quantitative measurements of nuclear Bicoid levels during the formation of the protein gradient. We find that gradient establishment begins 45 minutes after fertilization, and that the gradient requires about 50 minutes to reach peak levels. In numerical simulations of gradient formation, we find that incorporating the actual bicoid mRNA distribution yields a closer prediction of the observed protein dynamics compared to modeling protein production from a point source at the anterior pole. We conclude that the spatial distribution of bicoid mRNA contributes to, but cannot account for, protein gradient formation, and therefore that protein movement, either active or passive, is required for gradient formation.

733A
Genetic screens of mutants on the second and third chromosomes to identify genes involved in the left-right asymmetric development of the embryonic gut in Drosophila. Mitsutoshi Nakamura1, Naotaka Nakazawa1, Kiichiro Taniguchi1, Reo Maeda1, Takashi Okumura1, Ryo Hatori1, Akira Ishio1, Ayumi Ozaki1, Kenji Matsuno1,2. 1) Department of Biological Science and Technology, Tokyo University of Science, Yamazaki, Noda, Chiba, Japan; 2) Reserch Institute for Science and Technology, Tokyo University of Science.

Although bilateral animals appear left-right (LR) symmetrically on the outside, their internal organs often show directional and stereotypical LR asymmetry. In vertebrates, the mechanisms of LR specification and LR asymmetric development are largely unknown. To address this issue, we have been studies the LR asymmetric development of the Drosophila embryonic gut, which shows stereotypical LR asymmetry. To identify the genes involved in the LR asymmetric development of the embryonic gut, we performed genetic screens to identify mutations affecting LR asymmetry of this organ. About 4,500 mutants on the second and third chromosomes, predicted to cover about 80% of Drosophila genes, were induced by ethyl metanesulfonate, and LR defects in homozygote of each mutant were screened. From these screens, we isolated 31 mutants that showed various LR defects in the embryonic gut. We classified these mutants into the following types on the basis of their LR defects in three parts of the embryonic gut, the foregut, midgut, and hindgut. Type 1 : the laterality of the foregut was randomized. Type 2 : the laterality of the midgut and hindgut was synchronous by inverted. Type 3 : the laterality of the anterior midgut was randomized, or this organ became bilateral. Type 4 : the laterality of the posterior midgut was randomized, or this organ became bilateral. Type 5 : the laterality of the hindgut was randomized, or this organ became bilateral. Further genetic characterizations of these mutants will be presented.

734B
Role of an E3 ubiquitin ligase in ventral eye development. Meghana Tare1, Madhuri Kango-Singh1,2, Amit Singh1,2. 1) Department of Biology, University of Dayton, 300 College Park Drive, Dayton OH; 2) Premedical Program, University of Dayton, 300 College Park Drive, Dayton OH; 3) Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, Dayton, OH.

During early eye development, axial patterning transforms a single sheet of organ primordium cells to a three-dimensional organ by generating Dorsal (D) - Ventral (V), Anterior (A)-Posterior (P) and Proximo (P)-distal (D) axes. Among these, Dorsal-Ventral (D-V) axis generation is the first lineage event, which essentially requires a large number of eye specific proteins. Drosophila eye anlagen initiates with a ventral ground state on which the dorsal eye fate is established. Members of the Notch signaling pathway, Lobe (L) and Serrate (Ser), play an important role in ventral eye growth and development. Loss of function of L/Ser results in loss of ventral half of the eye. In a screen performed for the search of genetic modifiers of L, cul-4 mutant phenotype. cul-4 encodes an E3 ubiquitin ligase - an enzyme that ligates ubiquitin molecules to the protein targeted for degradation. We are trying to study possible role of cul-4 during the event of axial patterning to promote cell survival. We are studying genetic interactions between L and cul-4 to analyze their effect during eye development. We hypothesize that cul-4 possibly promotes cell survival in the ventral region of eye by targeting Wingless (Wg) for degradation. This study will help in discerning the importance of protein degradation and role of E3 Ubiquitin ligase as a possible axial patterning gene.

The Drosophila sex comb is a sexually dimorphic character that is dynamic in both its ontogeny and evolution. In Drosophila melanogaster males, the comb develops and orients itself through a complex process of cell rearrangement in the surrounding tissue. Homologous to the female transverse row, sex combs originated in a relatively small clade within the family Drosophilidae, and show striking variation between species. We show that the leg patterning gene dachshund, which is highly expressed in transverse row bristles but absent from the sex comb, plays a role in controlling the thickness and curvature of sex comb bristles. Ectopic expression of dachshund in the sex comb transforms the bristles to a transverse row phenotype. Furthermore, disruption of the expression and localization of genes involved in the planar polarity pathway and in tissue elongation in the embryo affect sex comb rotation. Our results show how multiple pathways interact in the regulation of this male-specific trait.

736A Drosophila histoblasts: one population many destinies. CARLA PRAT-ROJO, ELENA REBOLLO, ENRIQUE MARTIN-BLANCO. BIBM-CSIC. PARC CIENTIFICO DE BARCELONA, BARCELONA, Barcelona, Spain.

Histoblasts are the founder cells of Drosophila adult abdominal epidermis. They have been shown to remain dormant during larval stages and become invasive during metamorphosis, in response to Dpp signalling. Their proliferation and expansion are coordinated with the apoptosis of the surrounding obsolesce tissue, the larval epithelial cells or LECs. However, we have observed that they are not the only precursors of abdominal epidermis but also from other cell types including bristles, oenocytes and abdominal tendon cells, pointing to their "pluripotency" and stem-cell-like identity. We are trying to elucidate which signalling pathways diversify this population of cells, when and where the different cell types are specified, how their pattern is coordinated and maintained with the expansion of the tissue and up to which extent histoblasts can be defined as stem cells. For this purposes we are using live-imaging techniques and IHC to unravel the activity of different signalling pathways in the nests and the timing of specification of the different histoblasts derivatives by specific markers. Finally, the analysis of GFP labelled histoblasts destiny upon injection in wt larvae will provide clues regarding the "stem-cell-likeness" of these cells.


The number of dorsal appendages (DA) in the follicular egg chamber of Drosophila is a trait that distinguishes its two subgenaera, SOPHOPHORA (1 pair) and DROSOPHILA (2 pairs). The formation of these DAs in D. melanogaster involves the rearrangement and elongation of cells in the anterior dorsal lateral region of the egg chamber, and involves the coordinated activities of several canonical signal transduction pathways including the EGFR/MAP kinase, Dpp, and Notch signaling pathways. These pathways signal via known transcriptional effectors such as Pnt, Mad/Med, and Su(H), respectively. Since these pathways work together to pattern the cells, we hypothesize that their downstream transcription factors will act as combinatorial inputs to regulatory modules of loci involved in DA formation. We are using this working assumption to identify developmental enhancers underlying divergent DA morphology. Using a novel configurable, computational pipeline, we screened the genomes of two SOPHOPHORA (D. melanogaster and D. pseudoobscura) and two DROSOPHILA (D. viridis and D. mohaveensis) species and identified all Su(H) binding sites embedded within modules that are conserved across the entire genus. We then searched within this set of conserved Su(H)-bearing modules to identify 91 sequences containing Pnt binding sites in sequences from one of the subgenera but not from the other. We then identified 18 sequences located near genes that are involved in DA development and/or EGFR signaling, and cloned representative sequences from each subgenus (D. melanogaster and D. viridis). For example, we cloned 4 pairs of putative enhancers located at the Delta, Serrate, lekkon-1, and blistered loci into a lacZ reporter and established transgenic strains of D. melanogaster. We report on differences in expression patterns driven by homologous pairs of enhancers from each subgenus. In conclusion, our approach allows the identification of novel evolving enhancers as well as a quantitative assessment of the number of similar genomic changes for a particular node in the Drosophila phylogeny.

738C The retinal determination gene Dachshund controls the dynamics of cell shape changes during the differentiation of the Drosophila eye. Catarina Bras-Pereira1, Fernando Casares2, Florence Janody1. 1) Actin Dynamics Lab, Instituto Gulbenkian de Ciencia, Oeiras, Portugal; 2) Centro Andaluz de Biología del Desarrollo (CABD), UPO-CSIC-IA, Sevilla, Spain.

The Drosophila dachshund (dac) gene, the founder member of the DACH subfamily of nuclear proteins, forms part of the retinal determination pathway. Different expression and genetic studies suggest that this network is conserved in mammals. In Drosophila, previous work suggested that dac is required for the initiation of retinal differentiation. Accordingly, dac mutant flies are eyeless. However, in Dach2/Dach1 double mutant mice eyes are still present, suggesting that the functions of dac/DACH genes are not conserved. Due to this discrepancy, we have re-examined dac function during Drosophila eye development. We show that dac is not required neither for normal eye specification nor retinal differentiation, since dac mutant cells can still differentiate as photoreceptors. Furthermore, our data uncover a novel role for dac in the propagation of retinal differentiation: dac-mutant cells show abnormal cell shape dynamics that seems to cause a delay in exiting the morphogenetic furrow (MF) state, affecting MF formation and progression. Our results argue that the role of dac is not to promote the specification of the eye field, but to regulate MF dynamics.

739A Role of Centrosomin in Apical Domain Regulation during Drosophila Photoreceptor Morphogenesis. Geng Chen, Sang-Chul Nam. Department of Biology, Baylor University, Waco, TX.

Cell polarity genes including Crumbs (Crb) and Par complexes are essential for controlling photoreceptor morphogenesis. Among the Par complex, Bazooka (Baz) acts as a nodal component for other cell polarity proteins. Therefore, finding other genes interacting Baz will help to understand the cell polarity genes’ role in photoreceptor morphogenesis. Here, we have found a genetic interaction between baz and centrosomin (cmn). Cmn is a core protein for centrosome which is a major microtubule-organizing center. Here we analyzed the effect of the cmn mutation on developing eyes to determine its role in photoreceptor morphogenesis. We found that Cmn is dispensable for retinal differentiation in eye imaginal discs during the larval stage. However, photoreceptors deficient in Cmn display dramatic morphogenesis defects including the mislocalization of Crumbs (Crb) and Bazooka (Baz) during mid-stage pupal eye development, suggesting that Cmn is specifically required for photoreceptor morphogenesis during pupal eye development. This role of Cmn in apical domain modulation was further supported by Cmn’s gain-of-function phenotype. Cmn overexpression in photoreceptors caused the expansion of the apical Crb membrane domain, Baz and adherens junctions. These results strongly suggest that the interaction of Baz and Cmn is essential for apical domain and AJ modulation during photoreceptor morphogenesis, but not for the initial photoreceptor differentiation in the Drosophila photoreceptor.

740B Role of Kinesin-heavy-chain in Crumbs localization along the rhabdomere elongation in Drosophila photoreceptor. Garrett P. League, Sang-Chul Nam. Department of Biology, Baylor University, Waco, TX.

Crumbs (Crb), a cell polarity gene, has been shown to provide a positional cue for the extension of the apical membrane domain, adherens junction (AJ), and rhabdome along the growing proximal-distal axis during Drosophila photoreceptor morphogenesis. Identification of additional players that function with Crb in rhabdome elongation is important in understanding Crb-dependent photoreceptor morphogenesis. Here, we found a genetic interaction between erb and kinesin heavy chain (khc), a component of the kinesin 1 motor
defective proventriculus (dve), a new member of DV patterning in the eye. Oorvashi Roy G. Pulí1, Takeshi Yorimitsu1, Hideki Nakagoshi1, Amit Singh2,3,4. 1) Department of Biology, University of Dayton, 300 College Park Drive, Dayton, OH; 2) Premedical Program, University of Dayton, 300 College Park Drive, Dayton, OH; 3) Graduate School of Natural Science and Technology, Okayama University, 3-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530, Japan; 4) Center for Tissue Regeneration and Engineering at Dayton (TREN D), University of Dayton, Dayton, OH.

Axial patterning is crucial to eye development. During eye development, Dorsal-ventral (DV) axis determination is the first lineage restriction event. The early eye primordium begins with the default ventral fate on which the dorsal eye fate is established by expression of GATA-1 transcription factor, pannier (pn). Loss-of-Function (LOF) of pnr results in dorsal eye enlargements and antennal duplications in adult flies. We found similar phenotypes in LOF of defective proventriculus (dve). dve encodes a homeobox protein which is a target of decapentaplegic (dpp) and wingless (wg) signaling. The Gain-Of-Function (GOF) of dve results in suppression of Retinal Determination (RD) and thereby leads to the loss of eye. Based on our studies we found that Dve plays an important role during eye development. Dve expression domain in the eye isnal function of the Morphogenetic Furrow (MF) on the dorsal eye margin. This expression domain of Dve also overlaps with Wingless (Wg), which is present at the lateral margins of the developing eye disc. Here we present genetic interactions of Dve and Wg and their role during eye development.

742A Extramacrochaete Controls the Morphogenetic Furrow by Regulating Hedgehog Signaling. Carrie M. Spratford, Justin P. Kumar. Biology, Indiana University, Bloomington, IN.

Within the Drosophila retina, uncommitted proliferating cells are separated from differentiating cells by a physical indentation known as the morphogenetic furrow. The forward progression of this mobile compartment boundary is mediated by the Hedgehog (Hh) and Decapentaplegic (Dpp) signaling pathways while the rate at which the furrow moves is thought to be under the joint control of the HHIL protein Hairy (H) and the non-basal HIL protein Extramacrochaetae (Emc). The question of whether there is a connection between factors that regulate the velocity of the furrow and those that propel it forward across the eye field has not been adequately addressed. We report here that removal of emc is sufficient on its own to accelerate the furrow. This increase in velocity is due to a surge in the levels of the full-length protein isoform of Cubitus interruptus (C(lac)) within the furrow, which in turn results in an increase in the transcription of dpp. We set out to determine the mechanism by which Emc regulates the levels of C(lac) within the furrow. Our results indicate that Emc regulates the levels of Suppressor of Fused (Sufu) and we propose that the loss of emc results in increased levels of Sufu which in turn participates in stabilizing and sequestering C(lac) within the cytoplasm. We propose a model in which the mis-regulation of Sufu in emc loss-of-function clones is the mechanism by which the furrow accelerates across the eye. During normal development, the Wingless (Wg) pathway prevents ectopic furrows from initiating at the dorsal and ventral margins. As a result, a single furrow initiates at the posterior margin. We observe that the loss of emc at the margins leads to the initiation of ectopic furrows, a phenotype reminiscent of the removal of wg. We are currently investigating the relationship between Wg signaling and Emc. We propose and will discuss a model in which Emc integrates the activities of the Hh, Dpp and Wg pathways with the cellular biology of furrow initiation, progression and rate of movement.

743B Genes patterning the R8 photoreceptor in the Drosophila retina. Ece Terzioglu Kara, Arzu Öztürk, Arzu Celik. Department of Molecular Biology and Genetics, Böğaziçi University, Bebek, İstanbul, Turkey.

In sensory epihelia, the mutual expression of individual receptor molecules in receptor neurons is a common phenomenon. This ‘one receptor one neuron’ rule is also observed in the Drosophila retina, where only one of the six rhodopsin genes is expressed in a single photoreceptor cell. Based on the selective expression of rhodopsins in the inner photoreceptors R7 and R8, two unmitatinal subtypes are being distinguished. In the pale subtype R7 cells express Rh3 and R8 cells express Rh5, while in the yellow subtype R7 cells express Rh4 and R8 cells express Rh6. Rhodopsins are expressed in the late pupal stage, and their patterning is determined through stochastic developmental mechanisms which are not fully understood. We have identified two previously uncharacterized genes in an piggyBac-based enhancer-trap screen with photoreceptor-specific expression patterns. One of these genes encodes a transporter with 12 transmembrane domains and is specifically expressed in R8 cells in early larval stages, and in 2-4 photoreceptors including R8 in the pupal retina. The other is an integral membrane protein with an expression pattern which is restricted to R8 cells during larval and pupal stages. We will present data on their role in patterning the R8 photoreceptor.

744C Eye and So are Necessary for Maintaining Discrete Expression Patterns of Selector Genes in the Developing Drosophila Eye. Bonnie Weasner, Justin Kumar. Dept Biol, Indiana Univ, Bloomington, IN.

Several fate mapping experiments and selector gene profile studies have established that the developing eye-antenna imaginal disc is comprised of distinct developmental zones that give rise to disparate adult structures including the compound eye, antenna, maxillary palp and surrounding head cuticle. Loss-of-function sire oculis (so) and eyes absent (eya) mutants as well as gain-of-function Arrowhead (Awh) alleles are characterized by a replacement of the eye with head cuticle and a variety of bristle types. Previous studies of so and eya mutants have suggested that all retinal precursor cells are removed by apoptosis and only regions that would normally give rise to the surrounding head cuticle remains, whereas in Awh mutants precursor cells never form. In contrast to these studies our examination of these mutants indicates that a subset of retinal precursor cells is preserved. The fate of these cells is switched from eye to head cuticle and/or bristle. We additionally observe that many of the exclusionary selector gene expression profiles present in wild type eye-antennal discs no longer hold true in either so, eya or Awh mutants. For example, the transcription factor Cut is distributed within the antennal disc and the most anterior portion of the eye while expression of the retinal determination factor Eyeless lies adjacent but does not overlap that of Cut. In both so and eya mutants these two factors, which are thought to mutually repress each other’s expression, are co-expressed in a large proportion of cells. Similarly, in normal development Homothorax and Extradenticle partner to suppress transcription of dachshund. However, in mutant discs all three proteins are co-distributed within a subset of cells that lie at the posterior margin of the eye field. We propose that the loss of primary identity is compensated by the maintenance of a progenitor cell pool followed by a reprogramming of selector gene expression and finally a switch to a secondary tissue fate. This ensures the continued development of the head and the survival of the adult fruit fly.

745A The role of the zinc finger homeodomain-2 (zfh-2) gene in Drosophila leg joint development. Ana Guarné1, Cristina Manjón2, Magali Suzanne3, Ernesto Sánchez-Herrero1. 1) Centro de Biología Molecular Severo Ochoa, Madrid, Madrid, Spain; 2) CMIMA building, Passeig Marítim de la Barceloneta, 37-49 08003 Barcelona; 3) LBCMCP, UMR 5088 CNRS - Université P. Sabatier TOULOUSE III.

Developmental morphogenesis relies on cellular mechanisms that are regulated by transcription factors and signalling pathways. One such mechanism, apoptosis, is necessary to form the joints in the tarsal segments of Drosophila legs. In these joints, the Notch (N) and Decapentaplegic (Dpp) pathways are required to regulate the expression of the pro-apoptotic gene reaper (rpr). We have found that the gene zinc finger homeodomain-2 (zfh-2) is expressed in the cells that will form the joints of the Drosophila leg, but that its
absence prevents the development of only the tarsal joints. zfh-2 expression is regulated by the Notch pathway, and the absence of zfh2, in turn, regulates the expression of Enhancer of split, a target of the N pathway. Moreover, zfh2 is also needed for reaper expression and for the correct epithelial folds that precede joint formation. We have also found that there is a dynamic pattern of accumulation of the regulatory chain of non-muscle myosin II, encoded by the gene spaghetti-squash (sqh), and that sqh is required to form both the fold and the joint. Our results show that zfh-2 has an important role in regulating the tarsal joint formation, and that it may be an intermediate in the process whereby the N and Dpp pathways regulate apoptosis and myosin II dynamics to form the joint.

746B

Bristles as barriers to tissue elongation: the case of the rotating sex comb. Nicolas Malagón1, Joel Atallah2, Ellen Larsen1. 1) University of Toronto, Toronto, Canada; 2) Department of Evolution and Ecology, University of California - Davis, Davis, CA, USA.

The elongation of tissues is a fundamental and widespread developmental process in animal morphogenesis. Recent studies in Drosophila melanogaster have found that the underlying cellular mechanisms in elongation are tissue-specific. Most work has focused on early development of a few systems which are themselves quite different. In this study we describe a model system in which we study the elongation process, the elongation is the rotating sex comb. The sex comb is a group of specialized bristles. During development, the epithelial sheet surrounding the sex comb rearranges, thus changing the orientation of these bristles from a horizontal to a vertical position. Through 4-dimensional live-imaging, we describe and quantify the cellular processes in the area adjacent to the sex comb. We find that although both the proximal and the distal regions close to the sex comb elongate, the cellular processes in each region differ. While, in the distal region the elongation is mainly achieved by means of an increase in the cell apical size, in the proximal region it is achieved by a combination of tissue processes: 1) reduction in apical cell size, 2) cell intercalation and 3) cells dropping from the epithelium. In contrast to other well-studied examples of fly tissue elongation, the region studied has a barrier in the form of the rotating sex comb and the process of cell intercalation is several times slower, can change its orientation and may actually reverse. A model integrating the spatial and temporal dynamics of the cellular processes will be presented suggesting that both active and passive cellular processes are involved in tissue elongation during sex comb rotation. Our work highlights sex comb development as a system for investigating complex cell dynamics during morphogenesis. As sex combs display a great diversity of bristle patterns during evolution, this system will also be useful in comparing changes in cell dynamics between related species.

747C

Identification of the gene responsible for the wings apart phenotype in Drosophila melanogaster. Gimmy Morriss, Carmelita Jaramillo, Richard Cripps. Biol, Univ New Mexico, Albuquerque, NM.

The Drosophila wings apart (wap) locus contains a semi-lethal gene that when mutated leads to the absence of the Tergal Depressor of Trochanter (TDT) muscle. wap has been mapped to the proximal X chromosome but it is currently unclear what gene, when mutated, produces the wap phenotype. It is also unknown what aspect of muscle development is disrupted, resulting in the absence of the TDT. To identify the wap gene, we performed complementation mapping of wap mutant flies crossed with known proximal X chromosome deletions. We also sectioned thoraces of progeny from the complementation crosses to determine if these flies exhibit the TDT phenotype associated with wap. The results of complementation analysis and the phenotypic characterization suggest the most likely candidate for the wap phenotype is DIP1. PCR analysis of DIP1 in wild-type and wap mutant flies is currently underway to detect the mutation in this gene that leads to the observed phenotype. Already, we have found a single alanine to threonine amino acid substitution within the coding region of DIP1 in a wap mutant. Loss- and gain-of-function assays are being performed to determine if targeted loss of DIP1 expression will reproduce the phenotypes observed in wap mutant flies and if over-expression of DIP1 can rescue the wild-type phenotype. The physiological impact of the wap mutation will be analyzed by monitoring muscle formation and by measuring changes in muscle mass. The results of these experiments will then be compared to those observed in mutants of other genes involved in muscle development.

748A

Developmental Stage Annotation of Drosophila Embryos. Lei Yuan1, Shuiwang Ji1, Jun Liu1, Charlotte Konikoff1, Timothy Karr2, Stuart Newfeld1, Sudhir Kumar1,2, Jieping Ye1,2. 1) Department of Computer Science and Engineering, Arizona State University, Tempe, AZ; 2) School of Life Sciences, Arizona State University, Tempe, AZ; 3) Center for Evolutionary Medicine and Informatics, The Biosdesign Institute, Arizona State University, Tempe, AZ.

Today, more than a hundred thousand images of spatial patterns of gene expression have recently become available in a canonical model organism (Drosophila melanogaster) for understanding how a single cell, through gene expression and interaction, transforms into a complex organism. Efficient and accurate analyses of these images will provide the next generation of scientists biological insights into gene functions, interactions, and networks. Currently, many tasks in biological image analysis including the developmental stage annotation are conducted manually by domain experts. This manual practice does not scale with the continuously expanding collection of images, and it proves to be a major impediment in making discoveries. Therefore, we are developing novel computational methods for the automated annotation of the developmental stage. This is in sharp contrast with the existing approaches that assign images to stage ranges. The knowledge of the precise developmental stage is important because it enables the biologically-meaningful mining of genes with similar spatial patterns from different genes, calculating the developmental trajectories of gene expression, facilitating stage-sensitive textual annotation of expressions captured in images, and building genome-wide expression maps (GEMs) at critical junctures in development. In particular, we have obtained a collection of about 5000 images annotated with precise stages by expert biologists. Gabor filters are adopted for feature extraction, and sparse structure of the feature space is exploited using a group sparse structure of the feature space is exploited using a group
sets, generated by the BDTNP, showing where Ftz protein is bound in the genome. This combination produced a testable list of novel candidate Ftz/Ftz-F1 target enhancers near the genes of interest from the microarray. To test whether these regions correspond to Ftz/Ftz-F1-dependent enhancers, reporter genes are being constructed in which these genomic regions are fused upstream of a basal promoter and E. coli lacZ. Reporter gene expression will be analyzed in wild type, ftz and ftz-F1 mutant transgenic Drosophila. Once Ftz-responsive enhancer regions are well defined, this will be used to computationally extract the code for Ftz/Ftz-F1 DNA binding.

751A
Role of regulatory elements in the 3'UTR of the pair-rule gene eve in the robustness of patterning, Valerie Hilgers, Michael Levine. Department of Molecular and Cellular Biology, Division of Genetics, Genomics, and Development, University of California at Berkeley, Berkeley, California 94720, USA.

In the Drosophila embryo, segmentation genes are expressed with remarkable spatial and temporal precision to ensure proper developmental patterning. The reliability of these expression patterns and their robustness against environmental perturbation or intrinsic variability depends on transcriptional and post-transcriptional mechanisms that are not well understood. The 3' untranslated region (UTR) of the pair-rule gene even skipped (eve) is thought to play a role in fine-tuning the transcript’s spatial expression, half-life and translatability. However, the 3'UTR is not required for the establishment of the eve pattern. We propose that post-transcriptional regulation of the eve mRNA suppresses developmental plasticity. We will investigate the role of cytoplasmic localization signals, microRNA binding sites and RNA-binding protein recognition elements located in the eve 3'UTR. We will test whether these sequences contribute to ensuring accurate eve expression in contexts of environmental fluctuation, or when other components of the patterning system fail.

752B
Gene expression noise in spatial patterning: hunchback promoter structure affects noise amplitude and distribution, David M. Holloway1, Francisco JP Lopes2, Alexander V. Spirov1. 1) Dept Mathematics, British Col Inst Tech, Burnaby, BC, Canada; 2) Instituto de Biofisica, Universidade Federal do Rio de Janeiro, Brazil; 3) Computer Science and CFWIT, Stony Brook University, NY, USA.

One of the classic systems for studying positional information is the activation of the hunchback (hb) gap gene in early segmentation by the maternal-derived gradient of Bicoid (Bcd) protein. Within single embryos, this process is subject to noise. We address how hb promoter structure and transcriptional dynamics affect noise in protein output, and what features are critical for wild-type (WT) determinate spatial pattern. We use a stochastic model of the hb promoter, in which the number and strength of Bcd binding sites can be varied, as well as the number and strength of Bcd regulatory sites. Model parameters are fit to data from WT embryos, the self-regulation mutant 14F, and lacZ reporter constructs using portions of the 3' hb promoter. Model noise predictions have been corroborated experimentally. This indicates that WT (self-regulatory) Hb output noise is predominantly dependent on the transcription and translation dynamics of its own expression, rather than on Bcd fluctuations. The constructs and mutant, which lack self- regulation, indicate that the multiple Bcd binding sites in the hb promoter provide a rudimentary level of noise buffering; but WT noise is much lower, due to self-regulation. To the degree that features such as self-regulation and multiple binding sites are shared by other genes, we identify particular ways in which promoter structure and regulatory dynamics reduce expression noise, allowing for the determinate formation of spatial patterns in early development. We are now investigating the effects of gap-gap cross-regulation on noise control, by incorporating Krippel (Kr) expression dynamics and Hb-Kr binding site information into the model; we will present preliminary modeling and experimental results on Kr noise.

753C

We present a quantitative, comparative analysis of the gap gene network between dipteran species. We use a reverse-engineering approach to reconstruct these networks in silico, and verified predicted regulatory interactions by RNAi. Our analysis reveals which interactions are conserved, and which ones diverged during dipteran evolution. We demonstrate how such a comparative analysis can yield novel, quantitative insights into the evolution of gene networks.

754A
New gene encoding histone chaperone-like protein interacts with spineliss in the regulation of limbs morphogenesis in Drosophila melanogaster, Olga Simonova1, Elena Modestova2, Julia Vorontsova1, Olga Zatsepina3, Arsen Mikaeleyan1, Mikhail Slezingr1, Roman Chernev1, Boris Kuzin1. 1) Koltsov Institute of Developmental Biology, RAS, Moscow, 119334 Russia; 2) Institute of Gene Biology, RAS, Moscow, 119334 Russia; 3) Engelgardt Institute of Molecular Biology, RAS, Moscow, 119334 Russia.

One of the genes that determine the specificity of antenna and leg structures during development is spineliss, mutation of which (sxspineliss) disturbs distal structures of the leg, and transformed arista into tarsus. To reveal genes that interact with sx we generated P-element mutagenesis in the sxspineliss line. As a result we isolated sxsp flies that differ from those of the initial one by a more pronounced phenotype. Mapping the P-element’s site integration wising sxsp revealed it’s insertion close to the CG5017 gene (5 kb upstream from the 5'-end of its ORF). Using real time PCR we showed that the level of CG5017 gene transcription in the initial sxspineliss line is twofold higher than that in the sxsp. The revealed difference in the level of CG5017 transcription was the basis for the suggestion that the level of CG5017 transcription influence on the sxsp phenotypic expression. To check this suggestion, we modulated CG5017 expression by introduction into the sxsp genome the polyhomocistronic mutation that encodes the chimeric protein which is capable to suppress any transcription around the area of the P-element insertion. As a result the viability of mutant flies significantly decreased and the sx mutant phenotypic expression strongly increased. The CG5017 ORF encodes 283 amino acids protein containing domain, homologous to the chaperones associated with nucleosomes. The modulation of level expression of both CG5017 and sx genes has high potential influence on the morphogenesis of antenna and leg structures. We consider that the variable levels of these genes expression became an important instrument of evolutionary transformations of limbs morphogenesis in arthropods.

755B
Using variable expressivity in Drosophila segmentation to understand the mechanisms of phenotypic stability. Alexander V. Spirov1, Francisco JP Lopes2, David M Holloway1, 1) CEWIT, State Univ New York, Stony Brook, NY; 2) Instituto de Biofisica, Universidade Federal do Rio de Janeiro, Brazil; 3) Mathematics, British Columbia Institute of Technology, Burnaby; Biology, University of Victoria, BC, Canada.

Irrespective of how redundant a given regulatory network is, weak links are inevitable; indeed they are likely to be crucial in evolution. The early gap gene response to maternal signals is an ideal system for studying this: to what degree does the gap network absorb maternal variability; to what degree does it transmit variability downstream in segmentation? Many mutants lose robust gap patterning, but the mechanisms by which this happens occur. Compared to strong alleles with well-defined phenotypes, alleles with incomplete penetrance can partially disturb regulatory processes and display a range of outcomes. This can be very instructive for piecing together the wild-type (WT) mechanisms for robustness. We have studied maternal control with the bicoid allele bcdK57R, in which its gap gene target hunchback (hb) is expressed at a whole range of anterior shifts, from quite weak (nearly WT) to very strong (Lebrecht et al., 2005; Lopes et al., 2008). We also use a wide range of outcomes in Even-skipped (Eve) pair-rule patterning in the bcdK57R background: from nearly WT; to slight anterior shifts; to a missing 4th stripe; to expression in three broad domains. To understand these shifts in terms of the regulatory mechanism, we use a data-driven mid-grained modeling approach: regulator binding in the hb and eve cis-regulatory elements (CREs) is simulated, with hb and eve expression fit to the mutant data. This provides the regulatory interactions and strengths which account for the range of phenotypic outcomes in bcdK57R. These outcomes can be understood in terms of multiple steady states in the regulatory dynamics, with incomplete penetrance taking the system to different steady states.
POSTER: Pattern Formation
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

756C
Analysis and modeling of segmentation gene expression in Drosophila kruppel mutants. Svetlana Surkova1, Konstantin Kozlov1, Lena Panóč2, Maria Samsonova1, John Reinitz1. 1) Department of Computational Biology, State Polytechnical University, St Petersburgh, Russia; 2) Department of Statistics, University of Chicago, IL, USA.

The segmentation gene network in early Drosophila embryo provides a powerful model system to study the role of genes in pattern formation. Here, we have applied a systems level approach to investigate the regulatory effect of gap gene Krüppel (Kr) on segmentation gene expression. We acquired a quantitative data on expression of other segmentation genes in homozygous Kr mutants and used this data to construct a mathematical model. The main features of gap gene expression in Kr mutants are the greater shift of posterior giant (gt) domain, the coincidence of positions of posterior gt and knirs domains and significant decrease in the level of gap gene expression in the second half of cycle 14A. The variability of zygotic gene expression in mutants is reduced over time, however, this reduction starts later than in wild type embryos. As a modeling framework we applied the gene circuit method which extracts regulatory information from spatial gene expression data. This is achieved by fitting the model to gap gene expression patterns in wild type and mutants simultaneously, in order to estimate parameters for regulatory parameters, which predict a specific network topology in mutants. Our model correctly reproduces the characteristic features of gap gene expression in Kr mutants. To understand the regulatory mechanisms underlying both the shift of gt domain and the decrease of gap gene expression we apply the theory of dynamical systems, with a special focus on movement of attractors in hh-kr-gt-kni space. Our results show that the failure of previous attempts to reproduce mutant phenotypes was rooted in oversimplified representation of transcriptional regulation in the model.

757A
STAT is an essential activator of the zygotic genome in the early Drosophila embryo. Amy Tsurumi1, Fan Xia2, Jinghong Li1, Kimberly Larson1, Russell LaFrance2, Willis X. Li1. 1) Biomedical Genetics, Univ. Rochester Med. Center, Rochester, NY; 2) Department of Molecular Biology, Massachusetts General Hospital, Boston, MA; 3) Department of Medicine, University of California at San Diego, La Jolla, CA.

In many organisms transcription of the zygotic genome begins during the maternal-to-zygotic transition (MZT), which is characterized by a dramatic increase in global transcriptional activities and coincides with embryonic stem cell differentiation. In Drosophila, it has been postulated that maternal morphogen gradients and ubiquitously distributed general transcription factors cooperate to upregulate zygotic genes that are essential for pattern formation in the early embryo. Here, we show that Drosophila STAT (STAT92E) functions as a general transcription factor that, together with the transcription factor Zelida, induces transcription of a large number of early-transcribed zygotic genes during the MZT. STAT92E is present in the early embryo as a maternal product and is active around the MZT. DNA binding motifs for STAT and Zelida are highly enriched in promoters of early zygotic genes but not in housekeeping genes and STAT binding to early zygotic promoter was confirmed using Chromatin IP. Our microarray analysis demonstrates that loss of Stat92E in the early embryo, similarly to loss of Zelida, preferentially down-regulates early zygotic genes important for pattern formation. We further show that STAT92E and Zelida synergistically regulate transcription by RT-PCR. We conclude that STAT92E, in conjunction with Zelida, plays an important role in transcription of the zygotic genome at the onset of embryonic development.

755B
Inwardly Rectifying K+ channel Irk2 contributes to wing development in Drosophila through interactions with the Dpp pathway. Emily A Bates1, Giri Raj Dahal2, Joel Rawson2, Brandon Gassaway3, Ben Kwok4, Abigail Gehret5, John Morrell2, Louis Pátek1, Emily Bates1. 1) Chemistry and Biochemistry, Brigham Young University, Provo, UT; 2) University of Texas Health Science Center, San Antonio, TX; 3) HHMI, University of California, San Francisco, CA.

Mutations that disrupt function of Kir2.1, a human inwardly rectifying K+ channel, are associated with the periodic paralysis, heart arrhythmia, and the morphological defects observed in Andersen-Tawil Syndrome: cleft palate, incomplete dentition, skeletal fusion and abnormal curvature of digits. Although the physiological defects observed are logical consequences of a mutation in an ion channel, the link between morphological phenotypes and K+ conductivity is less intuitive. To determine the role of inwardly rectifying potassium channels in development, we use Drosophila as a genetic model. In Drosophila, the Irk2 inwardly rectifying K+ channel is the closest homolog to Kir2.1. Irk2 deficiency lines, RNAi, and expression of a dominant negative Irk2 subunit in Drosophila disrupt pattern formation in the development of the adult wing. Compromised Irk2 function causes wing-pattern defects similar to those found when Dpp signaling is disrupted: wing venation defects, bristle transformations, bifurcations, and missing wing blades. To determine if Irk2 plays a role in the Dpp pathway, we generated animals that were deficient in both Irk2 and in Dpp signaling. Suppression of the Irk2 loss of function phenotypes by disruption of the Dpp pathway suggests an interaction. Enhancement of the Irk2 gain of function phenotype by reduced Dpp signaling supports an interaction between the pathways. In wild type animals, Dpp signaling can be detected in a stripe along the A/P boundary of the wing imaginal disc. When Irk2 is disrupted, phosphorylated Mad is not detected suggesting loss of Irk2 directly or indirectly hinders Dpp signaling in the larval wing disc to contribute to wing development in Drosophila.

759C
The vein organizing activities of abrupt and knirps in Drosophila wing. Orna Cook, Ethan Bier. Dept. of Biology, UCSD, San Diego, CA.

The Drosophila longitudinal wing veins arise in a stereotypical pattern, making them a good model system to study the role of boundaries during pattern formation. The 2nd and the 5th veins, L2 and L5, are induced in similar but opposite positions in the anterior and posterior compartments of the wing, respectively, in response to the long-range morphogen, Decapentaplegic (Dpp). The fates of both veins are determined by organizing genes; knirps (knirps) for L2 and abrupt (abrupt) for L5. These vein organizing genes are expressed along domains of different Dpp target genes. In previous studies we showed that the L2 organizer gene, kni, is expressed along the anterior border of the Dpp signaling target gene, spalt. In a similar way, the L5 organizer gene, ab, is expressed along the posterior border between two other Dpp signaling target genes, optomotor-blind and brinker. The L2 and L5 vein organizers initiate the formation of their corresponding veins by activating and repressing a specific set of genes that lead to vein differentiation. In order to study the roles of Kni and Ab as vein organizing genes, we swapped their expression patterns and rescued L2 and L5 mutations with the reciprocal vein organizer. The nature of the rescued vein organizers was then analyzed by detecting the expression of the vein organizer target genes.

760A
Body-wall cell fate in the Drosophila thorax is initiated by Dpp and propagated by a positive feedback loop involving the Egfr-ligand Vein. Amanda A. Simcox, Litty Paul, Sathya Manivannan, Shu-Huei Wang, Liana Bonanno, Christina Austin, Sarah Lewis. Mole Gen, Ohio State Univ, Columbus, OH.

Development of the Drosophila dorsal thorax requires the precise localization of two signals at opposite ends of the early imaginal wing disc; wingless (wg) distally and vein (vn), an Epidermal growth factor receptor (Egfr) ligand, proximally. vn-expressing cells become the body wall and wg-expressing cells become the wing. Changes in these gene expression domains cause fundamental defects in the pattern of the adult thorax and complete loss of vn expression at the second instar halts all further wing disc growth. Here we investigated the gene regulatory circuit that controls vn expression. We found vn expression is activated by Decapentaplegic (Dpp) signaling and maintained by a positive feedback loop involving the ETS transcription factor, PntP2, a known mediator of the Egfr pathway. PntP2 binds directly to the vn promoter and forms a positive feedback loop. Secreted Vn activates the loop in neighboring cells to establish a community of vn-expressing cells with body-wall fate. The deployment of a self-reinforcing feedback loop involving a secreted factor is a developmental strategy that produces conformity within in a field of cells destined for the same fate; the so-called ‘community effect’. It is also a subcircuit, which due to its self-propagating nature, needs to be tightly regulated by other inputs. In the case of vn, the domain of expression is restricted by repressive signals from Wg and high levels of Dpp and the spatial localization of transcription factor PntP2. Discovering that an Egfr ligand participates in a gene regulatory subcircuit that produces a community effect, previously attributed only to cases involving TGF-ß and Wnt ligands, underscores the possibility that this may be a widespread mechanism in pattern formation.

290
Analysis of miR-277 targets in Drosophila and its role for metabolism and lifespan. Stephanie Esslinger, Klaus Förstemann. LMU Munich, Gene Center, Munich, Germany. MicroRNAs (miRNAs) are small (21-23 nt) single-stranded noncoding RNAs, that repress the expression of corresponding target mRNAs. The miRNA is incorporated into the RNA-induced Silencing Complex (RISC) with Argonaute proteins, the effector molecules in RNA interference (RNAi). We profiled the expression of Drosophila miRNAs living on three different food compositions (with varying amounts of sugar and protein) for age-related compositions (with using qRT-PCR). MIR-277 expression decreases with age on all food regimens. Transgenic flies with constitutive expression of miR-277 show a drastically reduced life span, in particular on food that has a low sugar but high protein content. By genome-wide measurements of transcription and degradation rates via in vivo labeling of newly synthesized RNA in Drosophila Schneider cells we could validate that miR-277 directly regulates several branched-chain amino acid (BCAA) degradation pathway enzymes. Amino acid levels are sensed by the TOR pathway, in particular elevated levels of the branched-chain amino acid leucine. Overexpression of miR-277 in Schneider cells leads to increased TOR activity, consistent with a role of miR-277 in regulating BCAA levels. Hyperactivation of TOR may be the cause for the life span defect observed in flies with constitutive miR-277 expression.

The Effect of Resveratrol and Diet on Lifespan and Nutrient Storage. Alexis A. Nagengast1,2, Charniece Knight2, Michael Polen1, Neha Sirohi2, Timothy Rudolph3, Hemlata Mistry3, Justin Diangelo4. 1) Dept Biochemistry, Widener Univ, Chester, PA; 2) Dept of Biology, Widener University, Chester, PA; 3) Dept Chemistry, Widener University, Chester, PA; 4) Dept Biology, Hofstra University, Hempstead, NY. Caloric restriction extends lifespan in a variety of organisms including mice, worms, yeast and flies. The polyphenol resveratrol can be found in the skin of grapes and several studies have shown that dietary restriction mimics caloric restriction. This oscillatory behavior is blocked when PTTH or torso function is abolished, resulting in nuclear accumulation of the DHR4 protein. Expressing constitutively active Ras/Raf/ERK pathway through its receptor Torso. Here, we demonstrate that Ras/Raf/ERK pathway activates DHR4, a direct target of EcR, and rescues mutants to adulthood. We examined the DNA binding domains of EcR-GFP during 9 periods of known ecdysone signaling spanning embryogenesis through oogenesis. It is difficult to distinguish between the primary and secondary effects of ecdysone and EcR action. To characterize the direct targets of EcR through development, we identified EcR binding sites in whole animals using whole genome chromatin immunoprecipitation assays with sequencing (ChIP-seq). EcR tagged with eGFP (EcR-GFP) was expressed in the adult stage. EcR-GFP was inserted into the genome of Drosophila melanogaster but additional caloric restriction (CR) does not further extend longevity. Mitochondria play an important role in the response to caloric or dietary restriction (DR), and their function can be altered by sirtuin activity. Few studies to date have explicitly examined the interaction between Sir2 expression, diet and mitochondrial function in the context of aging. Our aim in this experiment is to study whether mitochondrial genotype alters Sir2 dependent life span extension, and whether DR/CR treatments can modify potential epistatic interactions between Sir2 and mitochondria. We used UAS GAL4 system to over express Sir2 in 4 different mitochondrial backgrounds (mitotypes): D. melanogaster OreR, D. melanogaster Yari, D. simulans stiI and D. simulans sill. Compared to native D. melanogaster OreR mitochondria, the three divergent mtDNAs differ by a range of 28 to 756 base pairs. The longevity of these genotypes were scored on 5 different diets with high and low levels of sugar and yeast to explicitly distinguish CR from DR. The result shows that Sir2 over expression tends to suppress differences in longevity across a range of calories, but this leads to great differences in longevity when sugar and yeast are present in different ratios. The life span of these flies is significantly altered by diet.

Drosophila Nuclear Receptor DHR4 determines timing of ecdysone pulses by oscillating between nucleus and cytoplasm in a PTHH-dependent manner. Kirst Ing-Jones, Qiujiang Ou, Adam Magico. Dept Biological Sci, Univ Alberta, Edmonton, AB, Canada. In insects, periodic pulses of the steroid hormone ecdysone are released from the prothoracic glands (PG), thereby directing major developmental transitions such as the molts and metamorphosis. The timing of these steroid pulses is controlled by the prothoracicotopic hormone (PTTH), which acts on its target tissue, the PG, by activating the Ras/Raf/ERK pathway. Here, we demonstrate that Ras/Raf/ERK pathway activates DHR4, a direct target of EcR, and rescues mutants to adulthood. We examined the DNA binding domains of EcR-GFP during 9 periods of known ecdysone signaling spanning embryogenesis through adulthood. EcR binding is highly dynamic throughout development and numerous known direct targets were identified, including the ecdysone dependent puff regions. Depending on developmental stage, EcR binds to 760-3152 genomic locations which map to 553-2395 genes. Up to 45% of these genes are known to be differentially expressed upon ecdysone treatment or loss of EcR function. Analysis of target gene annotations revealed that constitutive EcR target genes were enriched for transcription factors and hormone stimulus response, while temporally restricted target genes were enriched for stage-specific functional categories. We found new targets of EcR in known EcR pathways and new pathways. Interestingly, EcR binds near 15 genes involved in the insulin response across development, half of which require EcR at midprepupal development. Future work to characterize the colocalization of binding partners across development is in progress.

POSTER: Physiology and Aging
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.
Juvenile hormone regulation of lipid and carbohydrate metabolism in adult Drosophila. Hua Bai, Rochele Yamamoto, Stephanie Vasquez, Marc Tatar. Department of Ecology and Evolutionary Biology, Brown University, Providence, RI.

Juvenile hormones (JH) produced in corpora allata (CA) are involved in a variety of biological processes, including insect metamorphosis, reproduction, diapause and lifespan. The effects of JH analog on lipid metabolism has been studied in a wing-pyropharmac cricket and other insects. Here we investigated the regulation of JH on lipid and carbohydrate metabolism using CA ablated (CACKO) Drosophila melanogaster. The CACKO flies have reduced level of total JH and altered level of triglyceride and glycogen. Using microarray analysis, we found that the expression of several lipases and amylases were also altered in CACKO flies. Interestingly, we found the expression of fat body specific insulin-like peptide (dilp6) was down-regulated in CACKO flies. In an ex vivo tissue culture study, we confirmed that the expression of dilp6 can be directly induced by JH analog (methoprene) application. These results suggest that JH plays an important role in lipid and carbohydrate metabolism of adult Drosophila, which might be mediated through insulin signaling.

The nuclear receptor dHNF4 is required for the maintenance of glycogen stores during metamorphosis. William E. Barry, Jason M. Tennesen, Carl S. Thummel. Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT.

Little is known about how energy homeostasis is maintained during Drosophila development, despite the significant changes in availability and energy demands as the animal progresses through each stage in the life cycle. For example, at the onset of metamorphosis the growing larva ceases feeding and enters a period of developmentally-programmed starvation until eclosion as an adult fly. Recently, the nuclear receptor dHNF4 was shown to play a key role in the establishment of a transcriptional program for lipid catabolism during starvation in Drosophila larvae. dHNF4 null mutants die as pharate adults, suggesting that this receptor might also play a vital role during metamorphosis. In support of this possibility, a single pulse of wild-type dHNF4 expression just prior to pupariation rescues the lethality of dHNF4 mutants. Expression of key fatty acid-oxidation genes, which are dependent on dHNF4 in the adaptive response to starvation, appears to be normal in dHNF4 mutant pupae. In addition, the amount of stored fat (in the form of triacylglycerol) is largely unchanged throughout metamorphosis. Glycogen stores, however, are depleted at a much faster rate and to a greater extent in mutant animals. In contrast to the role of dHNF4 in lipid catabolism during starvation, these findings point towards a role for the receptor in glycogen homeostasis during metamorphosis.

The mitochondrial protein CG14290 is required for carbohydrate homeostasis in Drosophila melanogaster. Daniel K. Bricker1, Thomas Orsak2, Yu-Chen Chen2, Jared Rutter3, Carl S. Thummel1. 1) Department of Human Genetics, University of Utah, Salt Lake City, UT; 2) Department of Biochemistry, University of Utah, Salt Lake City, UT.

Mitochondria are complex organelles that have important roles in energy production, intermediary metabolism, signal transduction and apoptosis. Consistent with these activities, mitochondrial dysfunctions are associated with a wide range of human diseases, including myopathies, cancers, and neurodegenerative disorders. Given these critical cellular functions and links to human disease, major efforts have been made to determine the identity of all mitochondrial proteins. The most comprehensive study to date identified approximately 1200 proteins in the mouse mitochondrial proteome. Remarkably, approximately one fifth of these proteins are of unknown function. Moreover, many are represented by only one or a few genes in most species, ranging from yeast to humans, suggesting that they have a critical function that has been conserved through evolution. We are characterizing a subset of these evolutionarily-conserved genes through a collaborative effort in both flies and yeast. Our current effort is focused on the Brain Protein 44 Like (BRP44L) family of proteins. The yeast ortholog of BRP44L, YGL080W, is a transmembrane protein that localizes to the outer mitochondrial membrane and is required for normal growth on glucose medium. Deletion mutations in the Drosophila BRP44L homolog, CG14290, have been generated by imprecise excision of a P-element. These mutants are viable, but cannot survive on a diet consisting solely of sugar. Metabolic analysis revealed that CG14290 mutants accumulate the glycolytic intermediates fructose, glucose and pyruvate. Taken together, our results suggest that CG14290 encodes a mitochondrial membrane protein that plays a critical role in maintaining carbohydrate homeostasis.

A frequency- and density-dependent gene that orchestrates dispersive behavior in Drosophila is a modifier of sensory signaling pathways. Maria Capovilla, Laury Arthaud, Selim Ben Rokia-Mille, Hussein Raad, Aviv Dombrovsky, Nicolas Prevost, Alain Robichon. 1) Dubo Tclphon Institute, Dept. of Biology and Evolution, University of Ferrara, Ferrara, Italy; 2) UMR INRA/CNRS/UNSA 6243, University of Nice- Sophia Antipolis, Sophia-Antipolis, France; 3) Agricultural Research Organization, the Volcani Center, Israel.

The foraging (for) gene of Drosophila, which encodes a cGMP-dependent protein kinase (PKG), has been extensively described as a frequency- and density-dependent gene and its transcripts are differentially expressed between individuals, reflecting the population-density context. Alternative for transcripts confer a binary behavioral mode in Drosophila known as Rover or Sitter, i.e., exploratory or sedentary. Some for transcripts, when expressed in a population at high-density for many generations, concomitantly trigger strong dispersive behavior associated with foraging activity. Moreover, genotype-by-environment interaction (GEE) analysis has highlighted a dormant role of for in energetic metabolism in a food deprivation context. In this report, we investigated whether expression of transcripts of for provoking an exploratory behavior might operate in a gene network creating pleiotropic effects at molecular and physiological levels in order to facilitate the dispersion task. Through trajectory analysis of different genetic backgrounds, we found that the for gene influences and regulates the metabolism of odorants emitted by plants, through the Aldehyde dehydrogenase III (aldh III) pathway and through a phosphorylatable adaptor, in order to shorten their action and decrease their toxicity. The rapid modification of aldehyde into carboxyl groups lessens their action and toxicity, which should facilitate exploration and guidance in a complex odor environment. Our present data provide evidence that optimal foraging performance requires the fast metabolism of volatile compounds emitted by plants to avoid neurosensory saturation and that the density-dependent genes that trigger dispersion influence these processes.
Lipids are constantly shuttled through the body to redistribute energy and metabolites between sites of absorption, storage, and catabolism in a complex homeostatic equilibrium. In Drosophila, lipids are transported through the hemolymph in the form of lipoprotein particles, known as lipophorins. The mechanisms by which cells interact with circulating lipophorins and acquire their lipid cargo are poorly understood. We have found that lipophorin receptor 1 and 2 (prr1 and prr2), two partially redundant genes belonging to the Low Density Lipoprotein Receptor (LDLR) family, are essential for the efficient uptake and accumulation of neutral lipids by oocytes and cells of the imaginal discs. Females lacking the prr2 gene lay eggs with low lipid content and have reduced fertility, revealing a central role for lipid uptake in mediating Drosophila vitellogenesis. prr1 and prr2 are transcribed into multiple isoforms. Interestingly, only a subset of these isoforms containing a particular LDLR type A module mediate neutral lipid uptake. Expression of these isoforms induces the extracellular stabilization of lipophorins. Furthermore, our data indicates that endocytosis is not required to mediate the uptake of neutral lipids. These findings suggest a model where lipophorin receptors promote the extracellular lipolysis of lipophorins. This model is reminiscent of the lipolytic processing of triglyceride-rich lipoproteins that occurs at the mammalian capillary endothelium, suggesting an ancient role for LDLR-like proteins in this process.

772A

Activation of the innate immune system in the adult fat body promotes triglyceride storage.

Melody Esmaeili, Michelle Bland, Morris Birnbaum. Department of Medicine, University of Pennsylvania, Philadelphia, PA.

In Drosophila, insulin signaling in the larval fat body promotes growth and nutrient storage; these effects are blocked when the fly’s innate immune system is activated in this organ by expression of the constitutively active Toll receptor. We asked whether insulin’s control of nutrient storage was also impaired in adult flies with activated immune systems. Unexpectedly, yolk-Toll flies exhibited 2-fold higher levels of triglycerides compared with controls (yolk-GFP). Dietary carbohydrates promote lipid storage, and we found that flies raised on a diet with 1M sucrose exhibited 3-fold increases in triglycerides compared with flies fed the normal, 0.15M sucrose diet, regardless of genotype. We again observed a 2-fold increase in triglycerides in yolk-Toll flies compared with yolk-GFP controls on the 1M sucrose diet. Insulin signaling through Akt activates the nutrient-sensing kinase TOR, a protein that has been implicated in lipid metabolism. Overexpression of TSC1 and TSC2 in fat body inhibits TOR and phenocopies the Toll effect on triglycerides. In contrast, activating TOR by co-expressing Rheb with Toll rescues the increased triglyceride phenotype to control levels with no effect on the Rheb transgene on its own. Downstream of TOR, we found that 4E-BP/Thor was neither necessary to increase triglycerides in yolk-Toll flies nor sufficient to increase triglycerides on its own. Furthermore, a constitutively active version of the TOR target S6 kinase did not rescue increased triglycerides when co-expressed with Toll. We are now testing the role of TOR targets such as autophagy and HIF-1α in triglyceride accumulation in adult flies with activated immune systems. In conclusion, we found that Toll activation leads to increased triglyceride accumulation in adult flies, and our data suggest that this occurs through inhibition of TOR signaling.

773B

Rapid effects of dietary restriction in Drosophila through gustatory perception and nitric oxide synthase.

Nancy J. Linford, Tammy P. Chan, Scott D. Pletcher. 1 Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI; 2 Developmental Biology Program, Baylor College of Medicine, Houston, TX.

Dietary restriction (DR) can rapidly confer stress resistance and longevity in multiple organisms, but the mechanism is not well understood. We have identified sleep fragmentation as a rapid behavioral response to DR in Drosophila melanogaster without a change in activity or total sleep. This effect is mediated by a sugar-dependent gustatory circuit and nitric oxide synthase (Nos). Nos is also required for DR-induced longevity, indicating shared molecular regulation of sleep and lifespan. Perception of sugar through Gr5a and Gr64-containing neurons is necessary and sufficient for DR-induced sleep fragmentation, and this response is supressible by activation of Gr64a-containing bitter-sensing neurons. These results demonstrate that a gustatory neuronal circuit mediates the link between diet and sleep behavior and that Nos is a shared molecular regulator of sleep and lifespan in response to dietary cues. This is the first demonstration of a connection between gustatory sensation and sleep response that may impact physiology, diseases, and aging.

774C

Temporal synchronization of O-GlcNAc post-translational modification in Drosophila melanogaster as a function of circadian rhythm.

Amanda C. Zirzow, Dona C. Love, John A. Hanover. Laboratory of Cell Biochemistry and Biology, NIDDK/NIH, Bethesda, MD.

Transcriptional feedback loops are central to the generation and maintenance of circadian rhythms. In animal systems transcriptional repression is believed to occur by catalytic post-translational events. The aim of this study is to determine if post-translational O-GlcNAc modification influences circadian rhythm. O-GlcNAc transferase (OGT) is a transmembrane enzyme that mediates the transfer of N-acetylglucosamine (GlcNAc) from GlcNAc from a-linked UDP-GlcNAc to serine and threonine residues. Conversely, O-GlcNAcase (OGA) removes the O-GlcNAc protein modification. OGT is the terminal step in the hexosome signaling pathway (HSP), implicated as a cellular regulator modulating numerous signaling cascades influencing growth, metabolism, cellular stress, host-pathogen interactions and circadian rhythm. This study focuses on the effect of OGT on circadian rhythm. In plants, previous research implicates two OGTs as essential regulators of circadian rhythm. In a mammalian system, the temporal synchronization of Ogt transcripts in the Rhesus Macaque adrenal gland is demonstrated. In this investigation, Drosophila melanogaster O-GlcNAc mutants are compared to wild-type and period (per) flies which have an arrhythmic circadian clock in constant darkness. The levels of O-GlcNAc protein modifications at different circadian times were determined by quantitative western blot and real-time PCR was used to quantify the levels of Ogt and Oga transcripts and transcripts known to cycle in a circadian fashion. The preliminary results of this study suggest that under normal conditions O-GlcNAc modifications of certain proteins cycle in a circadian manner.

775A

Reliability and precision of Drosophila feeding assays.

Ariadna Amador, Angela Phillips, William Ja. The Scripps Research Institute, Department of Metabolism and Aging, 30 Scripps Way 3B3, Jupiter, FL.

Despite the importance of determining the effects of dietary, genetic, and other manipulations on Drosophila feeding behavior, the precise measurement of food intake in flies remains technically challenging. Existing assays for measuring nutrient consumption are argued to be imprecise, indirect, or inaccurate. In many instances, feeding assays fail to reflect the actual feeding history of the animals; experimental setups or environmental conditions. Poor resolution and precision of food intake measurements can result in erroneous conclusions regarding the effects of dietary modifications on metabolism, nutrition, and behavior. Here, we compare several of the most popular assays used to measure Drosophila food consumption including the Capillary Feeder (CAFE), behavioral measurements that reflect feeding, and medium labeling with radioactive tracers or calorimetric dyes. We measure the resolving power of each technique under typical experimental conditions. Understanding the strengths and limitations of the various available feeding assays will facilitate studies where feeding plays a role, including investigations involving metabolism, growth, aging, drug screens, and feeding behavior and disorders thereof.

776B

Investigating the Role of Nutrition and Insulin Signaling on Autophagy and Neuronal Aging.

Rosanne Kotzabue, Bryan Bartlett, Aysa Gonzalez, Aubrey Sever, Katia Suarez, Kim Finley. 1) BioScience Center, San Diego State University, San Diego, CA; 2) Department of Biology, San Diego State University, San Diego, CA.

Macroautophagy (autophagy) is involved with the recycling of a wide range of intracellular components and regulates cellular homeostasis and stress responses. We have shown that defects in the autophagy pathway result in reduced lifespan, premature aging, the buildup of protein aggregates and neural degeneration. Insulin signaling regulates a complex series of downstream pathways and is known to have a profound effect on aging as well as suppressing autophagy. We have previously shown that autophagy gene expression declines with age, at a time when ubiquitin-positive aggregates are accumulating. We are examining the effects of nutrition on the aging processes including the regulation of neuronal autophagy. Nutritional study includes normal, low calorie, high calorie, low sugar/high yeast and high sugar/low yeast diets. We are also examining genetic loss-of-function (LOF) defects in various components of the insulin-signaling pathway. Preliminary results show that LOF insulin mutants (i.e. chico) and wild type flies exposed to
POSTER: Physiology and Aging
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

reduce caloric conditions have an increased mean lifespan and reduction in age-dependent aggregate accumulation. Drosophila lifespan profiles, health span and aggregate assays show that reduced nutrition and insulin signaling promotes basal rates of autophagy in the aging Drosophila CNS. In addition, we find improved motor function at a time when complex behaviors typically decline. Our work begins to characterize complex molecular pathways that sense nutrition and have a profound effect on cellular aging. Studies involving the role of nutrition, insulin signaling and aging in Drosophila have implications for human health, including risk factors like Type 2 diabetes and metabolic syndrome.

777C
Food protein-carbohydrate manipulation and its effect on fruit fly growth and development. Amber Rico1,2, Spencer T. Behnmer2, Aaron Tarone3, 1) Department of Biology, Ripon College, Ripon, WI; 2) Department of Entomology, Texas A&M University, College Station, TX.
Changes to the nutritional content of an organism’s diet can significantly alter their survival, development rates, fecundity, and lifespan. Here we use the fruit fly, Drosophila melanogaster, to explore how the protein-carbohydrate content of a diet influences larval performance (survival, development time, mass gain, lipid content). In our first experiment we examined the effects of nutrient dilution on larval performance by putting three different larval strains on a standard culture diet (13.7% protein and 61.0% digestible carbohydrate) that contained one of three nutrient densities (100%, 66%, and 33%). In our second experiment we examined the effects of larval fat body composition on larval performance. Here we reared larvae to the pupal stage on one of four different diets (the standard diet - p13.7:c61.0, two carbohydrate-biased diets - p8.2:c67.3 and p11.0:c64.1, and one protein-biased diet - p16.5:c57.8). We discuss how changes in the protein-carbohydrate content of the food influenced performance, and the implications this might have with respect to human nutrition.

778A
The conserved insulin target-of-rapamycin (TOR) pathway couples nutrient availability with tissue and organismal growth in metazoans. Although many inputs to the TOR pathway are known, the downstream effectors used by insulin/TOR to regulate cell growth and metabolism remain largely unknown. We show that in Drosophila these effects are mediated in part by stimulation of rRNA synthesis. We find that nutrient availability controls rRNA synthesis via inhibition of the conserved RNA polymerase III repressor Maf1. Genetic inhibition of Maf1 accelerates larval growth rate and increases final body size. These phenotypes are largely due to regulation of dMaf1 function in the larval fat body, a critical nutrient-sensing tissue equivalent to vertebrate liver or adipose tissue. We show that loss of dMaf1 in the fat body stimulates an increase in peripheral insulin levels and promotes systemic insulin signaling and growth. Significantly, these effects were reproduced in transgenic flies carrying only an extra copy of a single rRNA(iMet). We propose that the regulation of rRNA synthesis represents a mechanism by which nutrient availability controls tissue and organismal growth.

779B
Larval diet manipulation affects transition into the adult stage in Drosophila melanogaster. Madelyn Weeks1,2, Aaron Tarone3, Spencer T. Behnmer2, 1) Department of Biochemistry & Genetics, Clemson University, Clemson, SC; 2) Department of Entomology, Texas A&M University, College Station, TX.
Changes to the nutritional content of an organism’s diet can significantly alter their survival, development rates, fecundity, and lifespan. Here we use the fruit fly, Drosophila melanogaster, to explore how adult performance (survival and development rate) is influenced by the protein-carbohydrate content of the larval diet. In our first experiment we examined the effects of nutrient dilution on adult performance by putting three different larval strains on a standard culture diet (13.7% protein and 61.0% digestible carbohydrate) at one of three nutrient densities (100%, 66%, and 33%). In our second experiment we examined the effects of protein-carbohydrate ratio on larval performance. Here we reared larvae to the pupal stage on one of four different diets (the standard diet - p13.7:c61.0, two carbohydrate-biased diets - p8.2:c67.3 and p11.0:c64.1, and one protein-biased diet - p16.5:c57.8). We discuss how changes in the protein-carbohydrate content of the fly’s larval food influenced their adult performance, and the implications this might have with respect to human nutrition.

780C
Juvenile hormone (JH) plays key roles in controlling insect growth and metamorphosis. However, relatively little is known about the molecular mechanisms of JH action. Recent studies revealed that a 20-hydroxyecdysone (20E)-induced early gene, broad (br), acts as a key regulator in mediating the crosstalk between 20E and JH signaling pathways. The temporal pattern of br expression is the result of 20E and JH interaction. 20E directly induces br expression, but this induction can be prevented by JH in young larvae. Therefore, expression of br is specifically restricted to the larval-pupal transition when 20E is high but JH is low or absent. In order to identify genes involved in JH action, we designed a novel genetic screen based on precocious br expression at early larval stages and identified that Wnt Signaling were involved in JH action.

781A
In the fly brain vacuolization occurs progressively as a function of age but is not associated with brain cell loss. Christopher Beckwith1, Eva Polot1, Atanu Duttaroy2, 1) Dept Human Genetics, Howard Univ, Washington, DC; 2) Dept. of Physiology and Biophysics, Howard University College of Medicine, Washington, D.C; 3) Howard University Department of Biology, Washington, D.C. It has been known for a long time that vacuoles appear in the form of clear spaces in Drosophila brain as it ages. This progressive vacuolization event can significantly alter their survival, development rates, fecundity, and lifespan. Here we use the fruit fly, Drosophila melanogaster, to explore how adult performance (survival and development rate) is influenced by the protein-carbohydrate content of the larval diet. In our first experiment we examined the effects of nutrient dilution on adult performance by putting three different larval strains on a standard culture diet (13.7% protein and 61.0% digestible carbohydrate) at one of three nutrient densities (100%, 66%, and 33%). In our second experiment we examined the effects of protein-carbohydrate ratio on larval performance. Here we reared larvae to the pupal stage on one of four different diets (the standard diet - p13.7:c61.0, two carbohydrate-biased diets - p8.2:c67.3 and p11.0:c64.1, and one protein-biased diet - p16.5:c57.8). We discuss how changes in the protein-carbohydrate content of the fly’s larval food influenced their adult performance, and the implications this might have with respect to human nutrition.

782B
Teaching Old Brains New Tricks: Insulin Signaling, Autophagy and Neural Regeneration. Kim Finley1,2, Bryan Bartlett1,2, Roxanne Kotzebue1,2, Heriberto Sanchez1, Katia Suarez1, Aysa Gonzalez1, Aubrey Sever1, Anne Simonsen3, 1) BioScience Center, San Diego State University, San Diego, CA; 2) Department of Biology, San Diego State University, San Diego, CA; 3) Department of Biochemistry, University of Oslo, 0317 Oslo, Norway.
Insulin signaling regulates complex pathways that regulate both early and adult cellular processes. Macroautophagy (autophagy) is a conserved pathway that is suppressed by enhanced insulin signaling. Autophagy is involved with cellular homeostasis and the turnover and recycling of intracellular components. Our work has shown that autophagic defects lead to the premature accumulation of protein aggregates, degeneration and neuronal cell death. In addition, we find that autophagy gene expression declines with age and...
POSTER: Physiology and Aging
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

at a time that is concomitant with the formation of ubiquitin-containing neural aggregates (IUP). Using Drosophila genetic and transgenic techniques we find that altering key autophagy components has a profound effect on neuronal aging and lifespans. Atg8a mutations reduce lifespans, accelerated IUP accumulation and increase sensitivity to oxidative stress. In contrast, enhanced Atg8a expression in old brains extends adult lifespans over 50 percent and enhances oxidative stress resistance. These animals also show a significant reduction in aging markers such as IUP and Ref(2)P. We have begun examining the role of insulin signaling on the regulation of autophagy in the mature nervous system. We find insulin defects promotes basal rates of autophagy in older animals by preventing the accumulation of neuronal protein aggregate. Depending on the genetic background, expression of key autophagy genes is also significantly altered. This work begins to clarify the genetic and molecular underpinning of neuronal aging and the cellular processes required to maintain a robust and healthy nervous system. In addition, the conservation of both the insulin and autophagy pathways and their role in aging has significant implications for human health and common progressive neuronal disorders such as Parkinson’s and Alzheimer’s disease.

785B
The Drosophila homologue of the human multidrug resistance-associated protein 4 (dMRP4) protects animals against oxidative stress by modulating ROS accumulation.

He Huang1, Ying Lu-Bo1, Gabrielle Haddad1,2. 1) Department of Pediatrics, School of Medicine, UCSD, La Jolla, CA; 2) the Rady Children’s Hospital of San Diego, 9500 Gilman Drive 0735, La Jolla, CA 92039-0735, USA.

MRP4 is a member of the MRP/ABCC subfamily of ATP-binding cassette (ABC) transporters that are essential for many cellular processes requiring the transport of substrates across cell membranes. Despite extensive studies in the past, the physiological and biological functions of MRP4 remain unknown. In this work, we investigate biological functions of a Drosophila ortholog of human MRP4, dMRP4, which was originally identified through an overexpression screen against anoxic stress. We show that dMRP4 expression is significantly upregulated in response to paraquat-induced oxidative stress. Animals lacking dMRP4 are more susceptible to paraquat treatment. dMRP4 mutant flies are viable but have increased ROS (Reactive oxygen species) levels and shortened lifespan. We show that overexpression of antioxidant enzymes, either sod1 or sod2, profoundly increases resistance of dMRP4 mutant flies to oxidative stress, indicating that the primary dMRP4 function is to modulate ROS accumulation. However, overexpression of sod genes had a negative impact on lifespan of dMRP4 mutant flies, suggesting that factors other ROS may have been disrupted by loss of dMRP4 in lifespan determination.

786C
A myeloperoxidase like protein in Drosophila melanogaster.


Myeloperoxidase is the most abundant protein in human neutrophil. It provides immunity by generating hypochlorous acid from hydrogen peroxide, for destroying external pathogens. Elevated myeloperoxidase expression level signifies the risk for cardiovascular disease in humans and therefore used as a biomarker for the disease. Drosophila melanogaster genome carries a homolog of human myeloperoxidase protein known as CG5873 which shares 77% overall homology with the human gene with a highly conserved animal peroxidase domain. Elevated expression of CG5873 was first noted in the SOD2 loss of function mutant Sod2n283 that suffers from acute oxidative stress. To characterize the role of myeloperoxidase homolog in drosophila, we have studied an insertion mutant in the coding sequence of CG5873. Homozygous mutant shows deformed wing phenotype. A myeloperoxidase like protein in Drosophila melanogaster was identified in a Genetic Screen to Identify the Mechanism of General Anesthetics.

Paniz Heidar1, Seth M. Judd1, Adam G. Dawson1, Krista Pearman1, Michael J. Murray2, Gerald B. Cowen. 1) Arizona College of Osteopathic Medicine (AZCOM), Midwestern University, Glendale, AZ; 2) Department of Pharmacology, AZCOM, Midwestern University, Glendale, AZ.

Although more than 150 years have passed since nitrous oxide and ether were first used to induce anesthesia, we still do not understand the mechanism of action of general anesthetics. Much research has been done with no conclusive evidence identifying one pathway or mechanism of action for general anesthetics. Our goal is to determine what the mechanism of general anesthetics is. Interestingly, Drosophila responds similarly to general anesthesia when compared to humans, demonstrating all anesthetic stages at similar doses. With the fly genome being fully mapped and many mutant fly stocks available, we have constructed a system which we are using to perform both forward and reverse genetic screens through the fly genome in search of genes involved in the anesthetic response pathway. Central to this process is a laboratory apparatus known as an inebrimeter, which provides a means for quantitatively analyzing the response of flies to inhaled anesthetics. The initial screen is utilizing the LA insertion stocks from the BDGP Gene Disruption Project. The LA vector is similar to P{EP} in that it has a GAL4-inducible promoter for misexpression of flanking genes. Therefore, these stocks are being tested both as loss-of-function mutants as well as misexpression mutants by crossing with ELAV-GAL4 to induce neuronal expression. Initial data from the screen has identified more than 16 genes that have altered responses to isoflurane (the anesthetic used in the inebrimeter). Included in this list of genes are ones involved in apoptosis, ATP-synthase activity, and neuronal development, all of which have a physiological basis for anesthetic action. Detailed analysis of the screen and its results will be presented.

784A
Pheromone production and perception modulate lifespan and sexual attractiveness in Drosophila through mechanisms involving insulin signaling.

Tsung-Han Kuo1, Jeannie Yew1,2, Tatyana Fedina1, Klaus Dreisewerd1, Herman Dierick1, Edward Kravitz2, Scott Fletcher1,4. 1) Molecular & Human Genetics, Baylor College Medicine, Houston, TX, USA; 2) Department of Medical Physics and Biophysics, University of Muenster, Muenster, Germany; 3) Department of Neurobiology, Harvard Medical School, Boston, MA, USA; 4) Geriatrics Center, University of Michigan, Ann Arbor, MI, USA.

Sensory perception is a potent modulator of aging in multiple species. Flies with significantly reduced olfactory capabilities, for example, are long-lived and resistant to a range of stresses. However, the particular sensory cues, receptors, and neurons that are responsible for these effects remain unclear. We speculated that systems involved in pheromone production and perception play an important role in modulating longevity because of the strong influence of these molecules on animal behavior and physiology. The key pheromones in Drosophila are produced as cuticular hydrocarbons (HCs) and are involved in mating and other behaviors. Previous studies have shown that fly HCs are affected by developmental temperature and regulated by circadian rhythm. Using Gas Chromatography (GC) and Laser Desorption Ionization-Mass Spectrometry (LDI-MS), we show that the composition of HCs are significantly affected by aging in both sexes and that these changes are robust across different genetic backgrounds. Furthermore, we establish that the insulin signaling pathway, which is a conserved mechanism that modulates aging and stress resistance, also modulates HC profiles and regulates the expressions of genes involved in HC synthesis. Interestingly, the changes in HC profiles by aging and by the insulin signaling pathway significantly impact fly attractiveness. Lastly, we show that genetic manipulations designed to alter HC profiles and/or the perception of these pheromones on conspecifics impacts aging and longevity. These studies establish direct links among pheromone production, sensory perception, aging, and attractiveness, and they provide evidence for their regulation by a common mechanism, specifically insulin signaling.
Neuronal synaptobrevin functions in neuronal maintenance independent of its role in neurotransmitter release. Adam S. Haberman, Daniel Epstein, W. Ryan Williamson, Ian A. Meinertzhagen, Robin Flesinger. Dept. of Physiology, UT Southwestern Med Ctr, Dallas, TX; Dept. of Psychology, Dalhousie University, Halifax, Nova Scotia.

The neuron-specific SNARE neuronal synaptobrevin (n-syb) has a well-characterized role in synaptic vesicle fusion. Here we report the discovery that loss of n-syb leads to adult-onset degeneration because of an intracellular degradation defect, but independent of its role in synaptic vesicle fusion. Adult n-syb mutant photoreceptor neurons degenerate in a light-dependent manner as assayed by both electrophysiological and morphological analyses. Electron microscopy of n-syb photoreceptor synapses reveals large accumulations of endocytic endosomes and autophagic organelles. These accumulations are marked by endosomal and autophagic markers and contain heterogeneous undegraded cargo. In order to map the intracellular membrane fusion reaction blocked by loss of n-syb, we assayed the trafficking of the degradative enzyme Capeathisin L, which is activated by strongly acidic pH in degradative compartments. Indeed, loss of n-syb leads to dramatic accumulation of inactive Capeathisin, indicating a failure of ‘degradation machinery’ vesicles to fuse with endosomes and autophagosomes. This phenotype is very similar to loss of the vesicle ATPase protein V100, another neuron-specific protein required for neurotransmission and neuronal maintenance. We have recently shown that V100 functions in both endosomal fusion as well as acidification. Strikingly, we find that over-expressing either the wild type or an acidification-defective variant of V100 partially rescues n-syb-dependent degeneration, but not neurotransmission. Correspondingly, the Capeathisin assay reveals re-routing of ‘degradation machinery’ vesicles by V100 in the rescued neurons. We conclude that n-Syb and V100 function in the same neuron-specific degradation pathway. We propose that this novel mechanism increases neuronal degradative capacity and is required for neuronal maintenance.

Identification and characterization of genes encoding peritrrophic matrix proteins. Lacey Harbour, Nona Amiri, Gae Kovalick. Biology, Univ Texas Permian Basin, Odessa, TX.

In Drosophila the peritrrophic matrix is produced by the cardia, located at the foregut/midgut junction. The matrix is extruded from the cardia into the midgut, where it participates in digestive processes and acts as a barrier between ingested food and the midgut epithelium. In adults, the matrix is absent at eclosion, but appears within the cardia within a hour after eclosion. By 12 hours after eclosion, the matrix has extended the length of the midgut. Matrix synthesis is continuous in the adult and is not affected by feeding or starvation. These characteristics, combined with the wide variety of molecular techniques available for Drosophila, make the Drosophila cardia a good model in which to study the function of individual matrix component specifically, the structure, synthesis, and assembly of extracellular matrices in general. The first goal in establishing the cardia as a model system was to identify genes encoding peritrrophic matrix components. A strategy was developed to identify these genes. Eight potential candidate genes were identified from publicly accessible databases. These genes were then screened by in situ hybridization to verify that they were expressed within the cardia. Transcription of these genes was then assessed at eclosion and 12 hours after eclosion, to verify that the genes were expressed in manner consistent with peritrrophic matrix components.

Changes in the immune system are a hallmark of aging in many living organisms including mammals; as we age, the ability to fight off invaders decreases, yet components of the inflammatory pathway are upregulated with age, leading to low-grade chronic inflammation. The Drosophila immune system mimics molecular and functional aspects of the human innate immune system, and so it is an ideal model to study aging-related changes in immunity. The Drosophila Malpighian tubule provides an isolated system in which to dissect the different pathways plying a role in immunosenescence. Our studies suggest that, while the aging Malpighian tubule is hyper-responsive to immune challenges, its capacity to respond effectively to these challenges is diminished. Possible underlying mechanisms include alterations in protein translation, stability, or secretion, and studies are underway to elucidate between these possibilities.

ILK/Integrin function in organismal and cardiac aging in Drosophila. Mayuko Nishimura, Malene Hansen, Karen Ocorr, Rolf Bodmer. Sanford-Burnham Medical Research Institute, La Jolla, CA.

With age, cardiac function declines in Drosophila and a similar decline is seen in humans. It is not well understood, however, how aging correlates with cardiac functional decline and what are the molecular mechanisms underlying cardiac aging. In order to understand the mechanisms involved in organismal and cardiac aging, we are focusing on Integrin-Linked Kinase (ILK), a putative kinase that reportedly binds to beta1-Integrin. Reduced ILK function extends lifespan in C. elegans and ILK is highly expressed in human hearts (Hansen et al. 2005; Haminian et al. 1996). Therefore, we hypothesize that ILK affects aging and cardiac function in Drosophila. To test whether ILK is involved in organismal aging, we examined lifespan of ilk heterozygous mutants. The lifespan was extended in both male and female ilk heterozygous mutants compared to the genetic background introgression strains, suggesting that moderately reducing ILK signaling retards organismal aging. We also tested ILK and beta1-Integrin expression by immunostaining and found that GFP-tagged ILK and beta1-Integrin were prominently expressed in adult hearts and localized to Z-lines, which implies that ILK and beta1-Integrin act also in the adult heart. To investigate whether heart function is affected by reducing ILK/Integrin signaling, we monitored cardiac function in ilk and beta1-integrin heterozygous mutants using optical heartbeat analysis (Fink et al. 2009) and found that aged hearts exhibited significantly reduced arrhythmia compared to the level of young mutant or wildtype control hearts. On the other hand, reducing ILK signaling (ilk mutant flies) in the heart more strongly compromised heart function at young age, causing increased arrhythmias and dilation. In sum, the data so far suggest that ILK signaling is a fine tunable system used to modulate heart function that is altered during aging. ILK signaling may be critical in modulating longevity as well as vital organ physiology, including the appropriate maintenance of heart function with age.
POSTER: Physiology and Aging
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

792C
Changes in cuticular lipids, water loss and desiccation resistance in a tropical drosophilid: Analysis of within population variation. Bhawna Kalra1, Ravi Parkash2, Dau Dayal2. 1) Department of biology, University of Haifa, Tivon 36006, Israel; 2) Department of genetics, MD University, Rohtak 124001, India.

We investigated within as well as between population variability in desiccation resistance, cuticular lipid mass per fly and cuticular water loss in nine geographical populations of a tropical drosophilid, Zaprionus indianus. Interestingly, the amount of cuticular lipids and desiccation resistance in this non-melanic species are significantly higher as compared with melanic Drosophila melanogaster. On the basis of isolate family line analysis, within population trait variability in cuticular lipid mass per fly is positively correlated with desiccation resistance and negatively correlated with cuticular water loss but show lack of correlation with body size. We observed geographical variation in the amount of cuticular lipid mass per fly in Zaprionus indianus but no such divergence was found in D.melanogaster. In both the species, geographical variations in desiccation resistance are negatively correlated with cuticular water loss but the underlying mechanisms for changes in cuticular permeability are quite different. Thus, we may suggest that body melanisation and cuticular lipids may represent alternative strategies for coping with dehydration stress in melanic versus non-melanic drosophilids. For both the species, desiccation resistance and cuticular water loss are correlated with regular increase in aridity in the northern subtropical localities as compared with southern peninsular humid tropical localities. The role of climatic selection is evident from multiple regression analysis with seasonal changes in temperature and humidity (Tcv and RHcv) of the sites of origin of populations of Zaprionus indianus along latitude.

793A
P-element screen to characterize the multiple dimensions of cold tolerance in Drosophila melanogaster. Robert L. Kobe, Rob Gassert, Kristi L. Montooth. Department of Biology, Indiana University, Bloomington, IN.

Cold tolerance is likely to be an important trait in the evolutionary history of Drosophila melanogaster as its range has expanded from tropical Africa to temperate regions across the world. Cosmopolitan populations in temperate regions are exposed to lower and potentially more variable temperatures than those experienced by the ancestral population. We have measured cold survival across a range of temperatures (-6 °C to 6 °C) for five genotypes (Canton-S, Berlin-K, Hikone-A, Oregon-R, and Raleigh-208). There is a highly significant effect of genotype on cold survival (p<0.001) that is dependent on the temperature of exposure. Median survival times range from only 1-2 hours at -6 °C to 3-4 days at 6 °C. There are two inflection points in the relationship between survival time and exposure temperature. Going from -4 °C to 2 °C or from 4 °C to 6 °C decreases survival time by 5.9-fold and 2.5-fold, respectively, while survival times do not change dramatically across intermediate temperatures. The most cold-tolerant genotype at one temperature is not the most cold-tolerant genotype at all temperatures. One possible explanation for these results is that the physiological cause of cold injury differs across exposure temperatures. For example, at 6 °C we have documented that some genotypes are desiccated when they die from cold exposure, while other genotypes are clearly not dying from desiccation at this temperature. If this is the case, alleles which mediate cold tolerance for one temperature may not mediate cold tolerance at other temperatures. We will test this hypothesis using P-element mutagenesis to identify mutants for cold survival at three different temperatures. This mutant screen augments the few mutations affecting cold tolerance that have been previously identified. Comparing multiple dimensions of cold tolerance for genotypes that differ at a single locus should allow us to better understand both the physiological and genetic factors that mediate cold tolerance across temperatures.

794B
Reduced expression of ribose-5-phosphate isomerase in neurons tolerates oxidative stress, enhances lifespan, and attenuates polyglutamine toxicity in Drosophila. Horng-Dar Wang1,2, Ching-Tzu Wang1, Yi-Yun Wang1, Ming-Hao Huang1, Tzu-Kang Sang1, Yi-Chun Chen1, Si-Chih Cho1, Chao-Yung Wang1, Theodore Brummel2.

1) Institute of Biotechnology, Natl Tsing Hua Univ, Hsinchu, Taiwan; 2) Department of Life Science, Natl Tsing Hua Univ, Hsinchu, Taiwan; 3) Division of Molecular and Genomic Medicine, National Health Research Institutes, Zhunan Town, Miaoli County, Taiwan; 4) Second Section of Cardiology, Department of Internal Medicine, Chang Gung Memorial Hospital at Linkou, Chang Gung University College of Medicine, Taoyuan, Taiwan; 5) Department of Biology, Long Island University, Brookville, NY 11548, U.S.A.

Aging is a stress and associated with many age-related diseases. The identification of mutants and genes which are responsive to stress and postpone aging provides new therapeutic targets for age-related diseases. Here, we reported that an isolated mutant fly EP2456 with a reduced expression of ribose-5-phosphate isomerase (rpi) contains a higher level of NADPH and exhibits increased resistance to oxidative stress and enhanced lifespan. The knockdown of rpi in neurons by double-stranded RNA interference recapitulated the lifespan extension and oxidative stress resistance in Drosophila. It also rescued the abnormal eye morphology and the damaged phototaxis function by the polyglutamine toxicity. The information provides a new avenue of postponed aging and a possible treatment target of polyglutamine-related neurodegenerative diseases.

Very little is known about the regulation of genes encoding splicing factor proteins. Drosophila RNP-4F is a highly conserved protein from yeast to human, and functions as a spliceosome assembly factor during pre-mRNA splicing. We have characterized two major RNP-4F mRNA isoforms during fly development, designated "long" and "short," differing by a 177-nt tract located in the 5′-UTR. MFold analysis predicts that the 177-nt long raf-4 isoform-specific sequence can form an evolutionarily conserved stable stem-loop by pairing of an intron with a highly conserved adjacent exon. Since the coding potential for the two isoforms is identical, the interesting question arises as to the functional significance of this 5′-UTR structure in terms of raf-4 gene expression control. To clarify this question, we evaluated the effects of the wild-type and mutated 177-nt region on modulation of gene expression during fly embryogenesis using GFP reporters. The UAS-GAL4 system was utilized to trigger GFP expression in different tissues.

Fluorescence microscopy, Western blotting and qRT-PCR were used to study and quantify GFP reporter protein and mRNA levels in control (UAS-eGFP/Gal4) and experimental (UAS-Stem WT-eGFP/Gal4) lines. A significant increase in GFP protein expression due to presence of the wild-type 5′-UTR structure was observed with no concomitant increase in GFP mRNA levels, suggesting that the 177-nt region enhances translation at the post-transcriptional level. Functional elements within the 5′-UTR are being identified using a mutational approach, combining the GFP reporter and RNA electrophoretic mobility shift assay. A positive correlation is seen between raf-4 gene expression and two RNA-binding proteins using wild-type and mutated stem-loops. MALDI-TOF mass spectrometry is being used to identify the two trans-acting factors. A model is proposed for evolutionarily conserved regulation of raf-4 gene expression during development.

Psi is required for Histone H3 Acetylation and control of dmyc transcription. Nicola J. Cranna1, Amanda Lee Jue Er1, Naomi Mitchell1, Zuquin Nie2, Hye Jung Chung2, David Levens2, Ross Hannah1, Leone Quinn1. 1) University of Melbourne, Parkville, VIC Australia; 2) National Cancer Institute, Bethesda, Maryland, United States; 3) Peter MacCallum Cancer Centre, Melbourne, VIC, Australia.

The KI domain protein P-element somatic inhibitor (Psi) is the sole Drosophila ortholog of the mammalian Fusion Binding Protein (FBP) family. FBPI and 2 and Psi have previously been implicated in RNA splicing. In addition, in vitro mammalian studies have suggested a role for the FBP proteins in controlling c-myc transcription. In support of these in vitro studies we have evidence that Psi is required for regulation of Drosophila myc (dmyc) transcription. RNA interference of Psi results in dysregulation of dmyc enhancer trap activity, which suggests Psi is necessary for correct regulation of dmyc transcription. ChiP experiments have revealed that Psi is enriched within the dmyc promoter, consistent with a direct role in controlling dmyc transcription. Further support for a transcriptional role for Psi is that the protein is predominantly localized to the nucleus of Drosophila larval imaginal tissues. More specifically, within the nucleus, the Psi protein is predominantly localized to euchromatin. In addition, RNAi for Psi in wing imaginal discs did not result in any detectable defects (H3K9/K14) were strongly reduced in Psi knockdown clones. Furthermore, mammalian studies also demonstrate that FBPI knockout MEFs have reduced levels of active histone marks. Psi and FBP are, therefore, normally required for the maintenance of active chromatin, which suggests this function has been conserved between the mammalian and Drosophila orthologs. In summary, our findings suggest that, like FBPI, Psi is required for control of dmyc transcription and that Psi and FBP are essential for maintaining active chromatin. Our future studies will be directed towards understanding how Psi regulates dmyc and determining the mechanism by which Psi and mammalian FBP regulate active chromatin.

Genome-wide Transcription Factor Binding Varies with Gene Dosage in vivo. Matthew Davis1, Michael Eisen2. 1) UC Berkeley, Berkeley, CA; 2) Howard Hughes Medical Institute.

Statement of Purpose: Transcription factors recognize signals specified by nucleotide sequences to bind and regulate the expression of genes. The recognition of these sequences is understood biochemically as the binding of a DNA binding domain to its nucleic acid ligand. Simple kinetic models suggest that binding of transcription factors should be “switch-like” in behavior, with the concentration of the factor either exceeding the threshold for stable binding, or not. These models, however, are at odds with observations of some transcription factors that act as morphogens, such as Bicoid in the embryo of Drosophila melanogaster. Thus, the complexity of motif recognition and binding in vivo is poorly understood, especially in metazoans. While several in vitro biochemical methods have identified recognition motifs, and some of these methods allow for titration of the affinity of the DNA binding domain for the nucleic acid ligand, none allow the study of concentration dependence in vivo. Using the embryo of D. melanogaster as a model, we have developed a system for measuring concentration-dependent binding of transcription factors genome-wide. Methods used: ChiP sequencing was performed on small numbers of embryos to quantify genome-wide binding in several derived lines of D. melanogaster. Visualization of was performed with in situ hybridization of RNA and immunohistochemistry. Data were analyzed to assess both changes in binding and sequence recognition. Summery of results: We observe that at least some transcription factors known to regulate developmental patterning morphogenetically are not tightly regulated by negative feedback as has been described for many transcriptional regulators in fungi, and that there are changes in binding, sequence recognition context, and gene regulation under conditions of varying transcription factor concentration.

ZLD binds to broad spectrum of zygotic genes prior to their transcription. Xiao-Yong Li1, Milissa Harrison2, Tommy Kaplan2, Michael Botchan2, Michael Eisen2. 1) Howard Hughes Medical Institute, University of California, Berkeley, CA; 2) Department of Molecular and Cell Biology, California Institute of Quantitative Biosciences, University of California, Berkeley, CA.

During the rapid nuclear cycles of early development, the D. melanogaster embryo is transcriptionally silent. Zygotic transcription begins around cycle 8 or 9, and becomes widespread by the blastoderm stage about an hour later. Previous studies have shown that the Zn-finger protein Zelda (Zld) plays an important role in these earliest waves of embryonic transcription. To better understand the range of genes and functions regulated by ZLD, and to gain insights into its mechanism, we used ChiP-seq to examine the genome-wide binding of ZLD in early embryos. We find that ZLD binds more than ten thousand sites genome-wide, including both the promoters of genes transcribed in the early embryo as well as non-promoter regions that regulate their expression. ZLD binding often overlaps with regions bound by maternal and early transcription factors important early embryo pattern formation, but with unique distribution patterns around the genes. To determine whether this strong relationship between ZLD binding and blastoderm function is causal or incidental, we examined ZLD binding at two earlier stages: mitotic cycles 8-10, and mitotic cycles 11-13. To avoid contamination from older embryos, we performed our ChiP-Seq experiments on hand-sorted embryos. The binding at cycles 8-10 was very similar to cycles 11-13 and 14. Crucially, binding to promoters at cycles 8-10, prior to the onset of zygotic transcription, is highly predictive of blastoderm expression, strongly suggesting that ZLD is directly involved in regulating early embryonic transcription. Given that ZLD is ubiquitously expressed, and binds to a wide range of different factors, we suggest that early ZLD binding is necessary for the subsequent binding of other proteins that generate gene specific patterns of expression. X.Li, M.Harrison, and T.Kaplan contributed equally to this work.

Zelda coordinates gene regulatory networks in the early embryos. Hsiao-Lan Liang1, Chung-Yi Nien1, Sheng-Bo Fu1, Steve Butcher2, Nikolai Kirov1, John Manak2, Chris Rushlow1. 1) Biology, New York Univ, New York, NY; 2) Dept of Biology and the Roy J Carver Center for Genomics, 459 Biology Building, Univ of Iowa, Iowa City, IA 52242.

In past years, much attention has focused on the gene networks that regulate early developmental processes, but less attention has been paid to how these processes are coordinated. Recently the discovery of the transcriptional activator Zelda, which binds to CAGGTAG and related sequences present in the enhancers of many early-activated genes in Drosophila, revealed a mechanism for how batteries of early genes are coordinately activated (Liang et al., 2008). To address the extent to which Zelda binds and regulates pre-blastoderm genes, we performed genome-wide binding analysis of early embryos. The results combined with our expression profiling studies reinforce and extend our previous understanding of gene regulation during early development.
Sexually dimorphic expression of Flavin-containing Monooxygenase-2 in Drosophila melanogaster is directly regulated by Doublesex. Dion Luo. HHMI: Janelia Farm Research Campus, Ashburn, VA.

The doublesex (dsx) gene encodes sex-specific transcription factors (DSXF and DSXM) that regulate almost all somatic sexually dimorphic features of Drosophila melanogaster. Yet only a few genes have been identified as the direct targets of DSX. The Flavin-containing Monooxygenase-2 gene (Fmo-2) was identified as a candidate for being directly regulated by DSX using a genome-wide approach. The work presented is to validate Fmo-2’s regulation by DSX and further analyze this gene’s regulation. We found that Fmo-2 was expressed at a higher level at the anterior ventriculus in female than in male adult flies. A 2kb promoter fragment, which contains an optimal DSX binding site, 5’ of the Fmo-2 gene was sufficient to direct the female-biased expression of Fmo-2. Mutation of either the dsx gene or the optimal DSX binding site at the Fmo-2 promoter both diminished the sexual dimorphism in Fmo-2 expression. The evolution of Fmo-2 regulation by DSX was also studied.

Characterization of Zelda binding sites. Chung-Yi Nien1, Hsiao-Lan Liang1, Tenzin Gocha1, Sheng-Bo Fu1, Steve Butcher2, Hsiao-Yun Liu1, John Manak1, Nikolai Kirov1, Chris Rushlow1. 1) New York University, 100 Washington Sq. East, New York, NY, 10003; 2) Dept of Biology and the Roy J Carver Center for Genomics, 459 Biology Building, University of Iowa, Iowa City, IA 52242.

The recent discovery of the transcription factor Zelda hinted at a mechanism for how genes involved in the early developmental processes might be coordinately activated (Liang et al., 2008). Zelda binds to CAGGTAG and related sequences, which are found upstream of many of the early zygotic genes. Genome-wide profiling studies of 1-2 hour embryo lacking maternal expression of Zelda revealed that Zelda activates at least 70% of the early expressed genes, including genes required for cellularization, sex determination and patterning (Liang et al., 2008). However, some genes are downregulated in zelda mutant embryos, but do not have any TAGteam sites within 2 kb of the transcription start site. In order to understand how Zelda regulates target genes, we used genomically approaches to identify Zelda bound regions in early embryos. We found that additional TAGteam sites and other types of DNA sequences are enriched in Zelda bound regions. Whether the newly discovered DNA sequences are directly bound by Zelda will be further investigated. Liang HL, Nien CY, Liu HY, Metzstein MM, Kirov N, Rushlow C. The zinc-finger protein Zelda is a key activator of the early zygotic genome in Drosophila. Nature 456(7220):400-3, 2008.


The eyeless (ey) and type I bHLH gene daughterless express overlapping patterns during eye development. Recent studies show that Ey directly activates the atonal (ato) early eye enhancer ato-3', which is required for the initiation of photoreceptor differentiation in the developing retina. We show that Da enhances the activation of the ato-3' enhancer by Ey in non-eye tissues and increased sizes of ectopic compound eye size induced. In addition, da mutant clones spanning the MF exhibited delayed onset of the ato-3' enhancer activation while clones over-expressing Da surrounding the MF induces precocious activation. These effects of Da appear to be mediated by Da-Da homodimer since tethered Da-Da dimer increased these activities. Interestingly, a non-canonical E-box motif was observed near an Ey binding site in the ato-3' enhancer. DNA sequence substitutions of either the Ey or the E-box motif decreased reporter expression levels, indicating both Ey and Da-Da activate the ato-3' enhancer directly. In conclusion, our data indicate that Ey and Da-Da synergistically activates the ato-3' enhancer and control proper timing of photoreceptor cell differentiation during eye development.

Genetic analyses of CDK8-CycC functions in vivo. Qun Wang, Lauren Bridges, Larry Jiang, Jun-Yuan Ju. Department of Molecular and Cellular Medicine, Texas A&M Health Science Center, College Station, TX.

Precisely regulated gene expression is essential for normal development of all organisms. Biochemical studies have suggested that the transcription cofactor Mediator complexes are required for most of the RNA polymerase II-dependent transcription. Nevertheless, accumulating evidence suggests that the Mediator complexes can have specific functions in different biological contexts. Previously, we identified Cyclin-dependent kinase 8 (CDK8) and its regulatory partner Cyclin C (CycC), two subunits of Mediator complexes, as potent inhibitors of E2F1-mediated transcription. Genes encoding homologs of both CDK8 and CycC are either amplified or deleted in diverse types of human cancer. However, little is known about the functions and regulation of CDK8-CycC in vivo. To address this important problem, we have generated transgenic Drosophila lines that allow us to either over-express or knockdown CDK8 and CycC. When combined with Gal4 lines that are specifically expressed in the developing Drosophila wings, these lines generated stable and multiable phenotypes. We thus performed a dominant modifier genetic screen using the wing phenotype caused by knocking down of CDK8. We have screened 515 deficiency enhancers that we have retested about half of these modifiers (43 lines) using the wing phenotypes caused by CycC knockdown, and we observed similar modification with the majority (72%) of the suppressors and enhancers. Currently, we are screening through the deficiency lines on the X chromosome, and we are mapping the specific modifier genes by using partial overlapping deficiency lines. Further analyses of these novel genetic interactions will provide a better understanding about the functions of CDK8-CycC in vivo. Because CDK8 and CycC are highly conserved during eukaryotic evolution, our findings about the roles of CDK8-CycC in Drosophila will illuminate how their deregulation contributes to tumorigenesis in humans.


Using a bacterial one-hybrid system, we obtained DNA binding motifs for over 250 of 700 predicted Drosophila transcription factors (TFs). Within most TF families, we observe many members with overlapping specificities combined with examples of smaller subsets with distinct specificities. For the third largest class of TFs, the basic-helix-loop-helix (bHLH) proteins, previous studies indicated that they can bind palindromic “E-box” sequences, CANNTG. We determined DNA binding motifs for 46 of the 56 bHLHs and observe a wider range of specificities. 28 of the bHLHs bind E-box motifs with high specificity for the outer CA and TG bases of the motif. These proteins bind at least 11 different types of E-box motifs, with variation at positions 3 and 4 and in positions that flank the E-box. Proteins with only a bHLH domain bind E-boxes with central CC, TA, NA or NN preferences, while proteins with orange or leucine zipper domains have CG or GC preferences. The remaining bHLHs bind to E-box related motifs in which the outer positions have reduced or altered specificities. 9 orange domain bHLHs related to Hairy and E(spl) have reduced specificity at positions 2 and 5 in the E-box. 6 pas domain bHLHs form heterodimers that bind motifs with CAC at positions 1 to 3 followed by specificities that are different for each complex at positions 4 to 6, with reduced specificities at 6. Two bHLH proteins exhibit unique binding behavior. HLH106 binds 2 distinct motifs, one a classic E-box with a central CG, the other a non-palindromic sequence distinct from all other Drosophila bHLH binding motifs. Her appears to bind a monomorphic motif resembling a bHLH half site with additional flanking specificity. Overall, our results indicate that while many bHLH proteins have specificities that conform to the general E-box motif, the full set displays a wide range of variation on this motif suggesting a greater potential
for binding modes and specificities than previously recognized.

805A
Characterization of the Function of cropped in Drosophila Eye Development. Yiyan Chen, Bryce Daines, Yumei Li, Rui Chen. Human Genome Sequencing Center, Department of Human and Molecular Genetics, Baylor College of Medicine, Houston, TX.

cropped (crp), or DAP-4, is the Drosophila homolog of transcription factor AP-4. crp encodes a helix-loop-helix protein and has been found to be partially responsible for the heterogeneous DNA binding activity of SEBP3 (secretion enhancer binding protein). We identified crp as a direct target of eyeless (ey) based on ey ChIP-sequencing, suggesting that it may play a role in Drosophila eye development. To elucidate crp function during Drosophila eye development, we have generated a crp null allele by P-element imprecise excision. Preliminary clonal analysis using ey-FLP and hs-FLP yields only small clones in the adult fly eyes. Clonal analysis in eye discs of third instar larvae, however, led to delay in morphogenetic furrow progression and disruption of normal organization of ommatidia in mutant clones. Further characterization of crp mutant phenotype and its underlying molecular mechanism will be conducted and reported.

806B
Identification of nuclear receptor DHR96 target genes and DNA binding sites in Drosophila. Niloufar Farbedoobi, Emma Nally, Quixiang Ou, Kirst King-Jones. Department of Biomedical Sciences, University of Alberta, Edmonton, AB, Canada.

We recently showed that DHR96 mutants fail to survive on a low cholesterol diet, while controls are normal. DHR96 encodes a nuclear receptor that binds cholesterol in vivo and acts as a cellular cholesterol sensor, presumably by protecting cellular cholesterol levels from dropping below a critical threshold. We also showed that genes with roles in cholesterol metabolism are dependent on DHR96 function. It is unclear; however, which target genes of DHR96 are critical for maintaining cholesterol homeostasis in response to fluctuating cholesterol levels. Thus, it is important to identify DHR96 target genes to better understand the signaling network regulated by this nuclear receptor. To this end, we generated transgenic lines expressing DHR96 fused to the VP16 protein. We predicted that target genes of DHR96 would be significantly induced as a result of VP16-mediated activation. Based on gain-of-function microarrays, we selected 7 genes that are likely candidates for direct regulation by DHR96. Two cholesterol metabolism genes, NPC2c and ACAT, were highly upregulated by VP16-DHR96. To map and identify functional DHR96 response elements, we generated a series of lacZ transgenic reporter lines harboring different fragments from the ~5000 bp upstream of the NPC2c gene. We are currently analyzing these transgenic lines. Besides, we are conducting chromatin immunoprecipitation to detect VP16-DHR96 bound to transgenic reporter lines harboring different fragments from the ~5000 bp upstream of the NPC2c gene, were highly upregulated by VP16-DHR96 bound to NPC2c and ACAT. Preliminary data shows that the VP16 antibody is capable of immunoprecipitating VP16-DHR96 from native nuclear extracts. We are also examining the expression levels of NPC2c and ACAT in VP16-DHR96 lines in combination with RNAi transgenes that interfere with the expression of other nuclear receptor genes. The reason for this is to identify nuclear receptors that form a heterodimer with DHR96. Identification of target genes and recognition sites of DHR96 advances our understanding of the mechanisms underlying cholesterol homeostasis and allows us to discover new genes that are important for maintaining proper sterol balance in vertebrate cells.

807C
fliH, a novel cis-regulatory mutation in Drosophila wup4 gene leads to indirect flight muscle hypercontraction. Hena Firdaus, Mohan J., Arathi B.P., Upendra Nongthomba. MRDG Department, Indian Institute of Science, Bangalore, India.

Vast arrays of muscular dystrophies and cardiomyopathies harbor mutations in genes coding for structural proteins of the muscle. Etiology and genotype-phenotype correlation of many of these diseases have not been very clear. Indirect flight muscles (IFM) of Drosophila serves as an excellent system to address these questions, besides, giving useful insights into myofibrillogenesis process. Present study involves in-detail characterization of the fliH allele which exhibits IFM muscle thinning and tearing after fibres are formed normally. This interesting phenomenon is observed on the mechanisms leading to hypercontraction phenotype. Genetics played a pivotal role in identifying the mutant locus and it showed that this mutation falls in the regulatory region of the wup4 gene which codes for Troponin I (TnI). Our study elucidates that mutation abrogates the proper binding of myocyte enhancer factor 2 (mef2) transcription factor. This in turn leads to reduced level of Tnl transcript and hence reduced amount of protein; as a consequence, troponin complex formation is impeded leading to uncontrolled acto-myosin interactions, thus causing muscle fibre breakdown. This is the first mutation found in the regulatory region of any structural gene which is temperature dependent and leads to muscle hypercontraction. We also report coordinated downregulation of the transcript levels of other myofilament proteins. Overall, this study emphasizes that stoichiometry of structural proteins is important for proper functioning of the muscle. It even makes clear distinction between already known mutations in coding sequence of structural proteins (hdp1 and hdp1α) versus stoichiometric defects (fliH and hdp1β), both of which can lead to muscle hypercontraction.

808A
Identification of cis-regulatory elements in the dmyc gene of Drosophila melanogaster. Jasmine Kharazmi1, Cameron Moslehi2. 1) Molec Biol Lab, Biotech Ctr Zurich, Zurich, Switzerland; 2) Material Sciences Dept, ETHZ Zurich, Switzerland.

Myc is a crucial regulator of development during early stages of normal growth. Many signals and transcription factors tightly regulate the expression of myc yet no clear model exists to explain the complexity of its positive and negative regulators. In this study we use well-established Drosophila transgenesis tools to track the dmyc cis-regulatory elements by using random P-element insertion as well as the site-specific phage ΦC31 integrase-mediated system. Bioinformatic analyses identified conserved sequence blocks in the non-coding regions of the dmyc gene. Investigation of lacZ reporter activity driven by upstream, downstream and intronic sequences in larval imaginal discs and adult female tissues reveals that dmyc is transcribed from multiple independent transcription initiation units, a distal far upstream regulatory region, a TATA-box containing proximal complex and a TATA-less downstream promoter element (DPE) in conjunction with an initiator within the intron 2 region. Our data provide evidence for a modular organization of dmyc regulatory modules that generate different populations of transcripts. The far upstream region is responsible for late embryogenesis, while the other cis-elements control embryogenesis and organogenesis. Modularly structured cis-regulators confirm a finely-tuned control of the dmyc gene expression in a spatial and temporal manner during different stages of development.

809B
A model for the regulation of tailless by three maternal signals in the early embryo. Yoosik Kim1, Kate M. Fitzgerald1, Gerardo Jiménez2, Stanislav Y. Shvartsman1. 1) Department of Chemical Engineering and Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ 08544, USA; 2) Institut de Biologia Molecular de Barcelona-CSIC and Institut Català de Recerca i Estudis Avançats, Parc Científic de Barcelona, Barcelona 08028, Spain.

During the development of the Drosophila embryo, the terminal gap gene tailless (tl) is required to pattern the future acron andelson. In the posterior region, expression of tl requires only the terminal signaling system. However, the anterior tl expression requires inputs from three maternal signaling systems: Bicoid, MAPK, and dorsal. The interaction of these three morphogens leads to a 2-dimensional expression pattern wheretl is repressed at the anterior most and ventral regions, resulting in a dorsal stripe pattern. However, the exact mechanism of the regulation of tl by the three maternal morphogens is still unclear. Using genetic experiments and quantitative imaging approaches, we found that tl is regulated through an incoherent feed-forward loop where Bicoid and MAPK act together to activate both tl and its repressor at the anterior-most region. Our subsequent experiments suggest that anterior repression of tl is mediated by another terminal gap gene, huckebein (hkb). We found that Hkb can repress tl only in the anterior region although the protein is expressed at nearly equal levels at both poles. Indeed, only the enhancer that drives anterior pattern of tl contained Hkb binding site. Our results support the model where multiple enhancers with different inputs are required for spatial regulation of 2-dimensional gene expression patterns.
POSTER: Regulation of Gene Expression
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

810C

Importance of Pol II pausing for transcriptional precision during development. Mounia Lagha, Jacques Bothma, Emilia Esposito, Michael Perry, Vivek Chopra, Chia Hao Tsai, Michael Levine. Molecular and Cellular Biology, UC Berkeley, Berkeley, CA.

How a progenitor cell adopts a specific cell fate is a central question in developmental biology. The stochastic nature of transcription in addition to fluctuating surrounding signals implies the existence of developmental noise that must be filtered in order to ensure precision in cell specification. We would like to understand if “poising” genes for activation, within a progenitor/stem cell population, contributes to the buffering of this noise. One of the big surprises that have emerged from genome-wide studies of RNA Polymerase II (Pol II) binding is that many critical developmental patterning genes contain paused Pol II at their promoter prior to their activation during embryogenesis. Using a combination of BAC recombinaseering and high-resolution imaging methods we examined the consequences of converting paused genes into nonpaused genes, and vice versa. In particular, we swapped the promoters of two major regulators of mesoderm formation in the early Drosophila embryo, snail and twist. ChiP-Seq and Gro-Seq data clearly show that snail is paused in the early embryos, whereas twist is not. We also explored the possibility that pausing works in concert with “shadow” enhancers to suppress transcriptional noise, and examined the activation profiles of chimeric snail BAC transgenes containing the nonpaused twist promoter and just a single enhancer.

811A

Elucidating Regulatory Variation in cis, trans, and more within a population of D. simulans. Bradley J. Main1, Rita M. Graze2, Lauren M. McIntyre2, Marta L. Wayne2, Hysik Jang2, Sergey V. Nuzhdin2. 1) MCB, Univ Southern California, Los Angeles, CA; 2) University of Florida,Gainesville.

Gene regulatory variation has been shown to be common within populations of Drosophila. Illuminating the genetic basis of these differences is important for understanding fundamental evolutionary processes. To do this, we partition gene expression differences into two major sources: cis-regulatory (differences at the gene of interest ) and trans-regulatory (differences elsewhere in the genome). Other sources of variation may also exist due to the non-additive interactions between different loci (e.g. cis-by-trans). How can we assign variation to each of these sources? In hybrids, the parental alleles share the same cellular environment (including trans-factors), resulting in no trans differences between alleles. Thus, we assign all allelic bias in hybrids to cis, while trans is estimated from the difference between homozygous parents (cis + trans) and the hybrid (cis). In this study, we also want to identify genes that are involved in cis-by-trans interactions. To accomplish this aim, we estimated the allelic bias in 5 pairs of F1 hybrids and 3rd chromosome introgression (3Ci) hybrids from a sample population of D. simulans. As only the trans-genetic background is changed, a difference in allelic bias would indicate a cis-by-trans interaction. In summary, we elucidate intrinsic regulatory variation due to cis, trans, and cis-by-trans across a large portion of chromosome 3.

812B

Genome-wide analysis reveals that the FoxA protein Fkh plays a major role in maintaining salivary gland fate and function. Rika Maruyama, Elizabeth Grevengood, Peter Stempniewicz, Deborah Andrew. Dept Cell Biol, Johns Hopkins Sch Med, Baltimore, MD.

The Drosophila FoxA homologue Fork head (Fkh) is expressed in the salivary gland (SG) from the time the primordia first appear until metamorphosis when larval tissues are replaced with their adult counterparts. Fkh plays many roles in the developing salivary gland. It is required to prevent salivary gland cell death, to prevent expression of duct genes in the secretory portion of the SG, and to maintain SG expression as well as expression of at least two additional SG transcription factor genes, CrebA and Sage. Despite this broad range of activities, relatively few transcriptional targets of Fkh have been discovered and characterized. To identify such targets, we carried out whole mount in situ hybridization screens using a set of SG genes identified by the Berkeley Drosophila Genome Project ( BDGP) and did microarrays comparing the transcription profiles of WT to fkh mutant embryos. The in situ screens revealed that Fkh affects expression of 57% (73/128) of SG genes at some stage in embryogenesis. Far more late expressed SG genes (71%) were affected by loss of fkh than early expressed SG genes (36%), indicating that Fkh might play a major role in maintaining the expression of SG genes. For many SG genes, this regulation is likely to be indirect through its role in maintaining expression of the SG transcription factors CrebA and Sage. The microarray analysis of fkh mutants also identified a large number of affected genes, and revealed some unexpected patterns in both the genes that went up and went down in fkh mutants. For example, genes normally expressed in fully differentiated cells (chinup, synapse, cuticle and muscle related genes) were significantly enriched in the genes whose expression was upregulated in early fkh mutants. We are currently exploring the functional implications of these gene expression changes.

813C


Transvection is the modulation of gene activity due to interaction between alleles on homologous chromosomes. Generally, loci on sister chromosomes are not thought to interact, and gene expression is a linear combination of the activities from each chromosome. Transvectional influences on particular loci can, however, lead to gene activities either greater or lesser than the sum of the activities from each contributing locus. At the Malic enzyme locus in Drosophila melanogaster, we have found non-additive levels of malic enzyme (MEN) protein activity in flies that are heterozygous for synthetic, small-lesion, knockout alleles and a wild-type allele. Preliminary results suggest that this increase in MEN activity is not a response to a physiological cue, but an interaction between the homologues. Furthermore, we have found that the size and/or location of the lesion influence the amount of transvection observed in these heterozygous flies. These transvectional effects open the door for more complicated regulation of gene activity than classical models predict and have interesting implications for the evolution of regulatory regions and gene regulation.

814A

Regulation of gene expression in primary spermatocytes by meiotic arrest genes by positive and negative factors. Helen White-Cooper1, Jianqiao Jiang1, Karen Doggett2. 1) Sch Biosci, Cardiff Univ, Cardiff, United Kingdom; 2) Peter MacCallum Cancer Centre St. Andrews Place East Melbourne 3121.

In Drosophila spermatogenesis, meiotic cell cycle progression is linked to spermatid differentiation by the function of the “meiotic arrest” genes. aly-class genes arrest spermatids at the pachytene stage of meiosis and fail to enter meiotic divisions and spermatid differentiation. The proteins encoded by aly-class genes form a complex termed TMAC-cancer arrest proteins, the tests TAFs (tTAF) form a distinct complex. Using microarray analysis we found that aly-class mutants fail to express many tests specific genes. The can-class mutants also show significant defects in transcriptional activation in tests; they regulate a large subset of the aly-class target genes, but typically the mutant tests show basal expression of target genes. Protein purification and yeast-2-hybrid strategies have revealed additional proteins that physically interact with the aly-class gene complex. We have focussed on two genes, mip40 and wake-up-call (wuc, CG12442). mip40 mutants are viable, but male sterile, with a meiotic arrest phenotype. Similarly, RNAi against wuc in testes reveals a meiotic arrest phenotype. Unexpectedly, neither mutant falls into the aly-class, in terms of the gene expression defects seen in the mutant testes. Instead these two genes appear to define a novel meiotic arrest class. Mip40 and Wuc are both required for full expression of many tests specific genes, however their major function is to repress the basal activity tests-specific promoters, this repression is counteracted by aly.

815B

Computational discovery of cis-regulatory elements in Drosophila embryogenesis. Manonmmani Arunachalami1,2, Karthik Jayasurya1, Pavel Tomancak1, Uwe Ohler1. 1) Institute for Genome Sciences & Policy, Duke University, Durham, NC; 2) Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany.

In higher eukaryotes transcription factors control gene expression by binding regulatory DNA segments called cis-regulatory modules (CRMs). We here present an approach that utilizes gene expression data to identify cis-regulatory elements and modules. In our approach, we take advantage of the large database of spatio-temporal patterns of gene expression in D. melanogaster embryogenesis to identify sets of developmentally co-expressed genes (Tomancak et al. 2007). We developed a computational method that identifies
DNA binding sites for transcription factors from families of co-regulated genes that are expressed during Drosophila embryo development. Our method discovers over-represented motifs in a set of co-regulated genes using the exhaustive motif enumeration technique. The predicted motifs often correspond to known TFBSs and many other motifs, which to the best our knowledge are novel. Clustering the predicted motifs identifies the CRMs, which assist in translating a combinatorial code of TF inputs into a specific gene expression output. We detected CRMs that share some degree of similarity in their binding site content. Applying a linear regression classifier the false positive rate on CRM prediction is significantly reduced. It is well known that the gene expression is substantially controlled through CRMs and those key regulatory sequences are conserved in related species. We searched the whole genome for the predicted CRMs and establishing expression pattern of the genes that are associated with these CRMs. We applied our alignment-free method (M. Arunachalam et al. 2010) to test the conservation level of predicted CRMs. We observed that 40% to 55% of candidate CRMs shows clear conservation in all 6 distantly related non-melanogaster species (D. ananassae, D. pseudoobscura, D. mojavensis, D. virilis and D. grimshawi). We will test our predictions by considering the genes flanking the predicted conserved CRM candidates by RNA in-situ experiments.

816C
Regulatory DNA of the engrailed and invected genes. Yuzhong Cheng, Alyne Brown, Stefanie Kremer, Sarah Devido, Catherine Stefanisk, Judith Kassis. Program in Genomics of Differentiation, NICHD, Bethesda, MD.

engrailed (en) and invected (inv) form a gene complex that extends over 100kb. These two genes encode highly related homeodomain proteins that are co-regulated, form a chromatin domain, and are expressed in a complex manner throughout development. Dissection of inv/en regulatory DNA shows that enhancers are spread throughout at least a 70kb region. We used two types of constructs to analyze the function of the regulatory DNA: reporter constructs with small pieces of en/inv DNA fused to the en promoter driving the expression of lacZ and large constructs using the phiC31 system and HA-tagged En and Inv. In addition, we generated some in situ deletions of en and inv DNA; these were also very informative. We report two interesting findings. First, the sum of the parts is not equal to the whole. Some of the enhancer activities we see in the small constructs are not recapitulated in the large constructs. Second, we have not yet located the DNA fragment that causes expression of en/inv in the posterior compartment of imaginal discs. The explanation for this may be trivial, i.e., we may have disrupted the activity of the imaginal disk enhancer in the small constructs by chopping it in two, or it may turn out that a combination of elements is required for imaginal disk expression. Experiments are in progress to distinguish between these two possibilities.

817A
Dissecting the Functionality of an Enhancer Family, Kurt M. Dahlstrom, Niranjana Nataraja, Albert Erives. Dept. Biological Sciences. Dartmouth College, Hanover, NH.

During embryonic development in Drosophila, a nuclear gradient of the protein Dorsal patterns the dorsal-ventral axis. Our lab has recently discovered that four genes controlled by Dorsal have enhancers sharing a pattern of binding sites for Dorsal, Twi, and Su(H). These neuroectoderm enhancers (NEEs) regulate rhomboid (rho), brinker (brk), ventral nervous system defective (vnd) and vein (vn). These genes are expressed as lateral stripes of varying widths and define successive regions of the ectoderm. How each NEE responds differently to the dorsal morphogen gradient has remained an outstanding question. One model previously put forth to explain how these genes are differentially expressed postulates that the enhancers for these genes contain many Dorsal binding sites, and that gene expression is determined by the density and affiliations of these sites in a given enhancer. Our lab has discovered, however, that a DNA spacer which exists between a pair of adjacent Dorsal and Twi sites in each NEE encodes that enhancer’s ability to read its position with respect to the Dorsal nuclear gradient, while loosely-adjacent Dorsal and Twi variant sequences represent deprecated relic elements. Here we discuss new experiments to dissect the molecular mechanisms that readout precise threshold responses. First, we are selectively removing combinations of TF binding sites from the rhb NEE in vivo using tissue specific FLP expression and FRT-containing NEEs. Excising the Dorsal and the Twi binding sites may render the enhancer non-functional due to the disruption of the critical spacer between the two sites. Our lab has observed the level of transcriptional activation or decrease in expression for a number of Dorsal target genes that is gained or lost by eliminating Dorsal gradient sensitivity. Second, we are using chromatin conformation capture (3C) techniques to determine if different NEEs at distant loci physically interact in vivo and whether such interactions play a functional role in precise threshold responses. Third, we are constructing synthetic enhancers to test the sufficiency of the identified elements and architecture of NEE modules.

818B
From morphogens to morphogenesis: cis-regulatory logic in epithelial patterning. Alisa Fuchs1, Enrica Charbonnier1,2, Giorgos Pyrowolakis1,2. 1) Developmental Biology, University of Freiburg, Institute of Biology I, Freiburg, Germany; 2) Centre for Biological Signalling Studies (BIOSS), University of Freiburg, Freiburg, Germany.

The process of pattern formation is a prerequisite for morphogenesis and its understanding remains a major challenge in developmental biology. The follicular epithelium of Drosophila offers this may be trivial, i.e., we may have disrupted the activity of the imaginal disk enhancer in the small constructs by chopping it in two, or it may turn out that a combination of elements is required for imaginal disk expression. Experiments are in progress to distinguish between these two possibilities.

819C
Transcriptional control and evolution of the patterning gene knirps in the second wing vein (L2) of Drosophila melanogaster. Valentina M Gantz, Ethan Bier. Biological Sciences, University of California San Diego, La Jolla, CA.

Wing pattern is one of the best understood developmental systems making it an excellent model for studying the link between patterning and morphogenesis. To understand the mechanisms for developing specific structures such as distinct wing veins, it is crucial to obtain a deep knowledge of the transcriptional control of the genes that play role in it. Here we propose a method for a functional analysis of cis-regulatory elements (CREs), and to study of the effect of variation in their transcription factor binding sites. The gene knirps (kni) is well suited for this kind of study since it has an already characterized enhancer element and is responsible alone for the differentiation a specific structure (second vein - L2) in the wing. Furthermore by analysis of CREs of other Diptera we hope to understand the link between difference in gene expression and difference in shape.

820A

Abd-B expression is regulated by a large cis-regulatory region that is subdivided into 4 segment-specific chromosomal domains (ab-5 through ab-8). Critical to the activity of the regulatory domains are the initiator elements that read the early segmental address set by the gap and pair-rule gene products and coordinate the activity of the nearby cell type-specific enhancers located within the domain. To further analyze Abd-B expression and regulation, we created a 110 kb-long Abd-B-GAL4 BAC that carries the whole Abd-B regulatory domains. When tested the BAC faithfully recapitulates Abd-B expression as it is known in embryos. This BAC allowed us to follow Abd-B expression at development stages that are difficult to probe with antibody staining such as larvae, pupae and adults. Among other tissue we discovered Abd-B expression in the secondary cells of the male
accessory gland. We have narrowed down the enhancer responsible for accessory gland expression to a discrete 2.8 kb region within iab-6. Following the domain model a deletion of the iab-6 initiator was expected to result in the inactivation of the whole domain and hence inactivate the accessory gland-specific enhancer. Surprisingly we found that the iab-6 initiator deletion does not eliminate Abd-B expression in the accessory gland. Accessory gland derives from the mesodermal tissue surrounding the gonad in 3rd instar larvae. Because the domain model derives from the analysis of Abd-B expression in epidermal and nervous system derivatives, our results suggest that the Abd-B regulatory landscape may differ in different germ layers. Male accessory glands are the site of synthesis of numerous peptides that elicit post-mating behaviors in females. There are about 40 secondary cells per gland, characterized by large vacuoles. The lack of Abd-B leads to a morphological and physiological phenotype. Morphological: the cells loose their vacuoles and diminish in size. Physiological: there is a defect in the female post mating response which has been explored in detail by the Wolfr"ernab (see poster by Jessica Sitiuk).

821B
REDfly: The Regulatory Element Database for Drosophila, v3.0. Marc S. Halfon1,2, Steven M. Gallo1,2, Dave T. Gerrard3, David Miner2, Michael Simich2, Benjamin Des Soy3, Casey M. Bergman1. 1) Department of Biochemistry, SUNY at Buffalo, Buffalo, NY; 2) NYS Center of Excellence in Bioinformatics & Life Sciences, Buffalo, NY; 3) Developmental and Biological Sciences, SUNY at Buffalo, Buffalo, NY. 4) Faculty of Life Sciences, University of Manchester, Manchester, UK.

The REDfly database is a highly-curated portal for Drosophila cis-regulatory data containing records for over 900 cis-regulatory modules (CRMs, “enhancers”) and over 1425 transcription factor binding sites (TFBSs), all empirically validated and curated from the published literature. The recent release of version 3.0 of the database extends the utility of REDfly as an important tool for the fly community. REDfly v3.0 now includes all sequences reported as functionally tested in a transgenic reporter gene assay regardless of whether they showed regulatory activity or have activity redundant with other, shorter regulatory sequences. Regions of overlap between CRMs with similar regulatory activity are automatically identified and displayed as “inferred CRMs.” Graphical views show the position of each CRM within its genomic locus, the location of each CRM with respect to its associated gene is provided, and conservation of local synteny between CRMs and their target genes across nine species of Drosophila is assessed. Curation of TFBSs has been expanded to include sites identified by electrophoretic mobility shift assay (EMSA, “gel shift”) in addition to DNase I footprinting. A completely redesigned interface improves access to REDfly for casual and power users alike with greatly improved capabilities for database searching and results filtering. These new features and enhancements make REDfly, which is freely accessible at http://redfly.ccr.buffalo.edu, a comprehensive source of Drosophila cis-regulatory data and a powerful platform to facilitate high-throughput experimental and computational studies of gene regulation.

822C

In Drosophila and other insects, cytochrome P450 enzymes (CYPs) play a major role in conferring resistance to various insecticides including DDT. Generally, DDT-resistant strains show increased expression of multiple P450 genes. However, the mechanism of overexpression is not known. To understand the molecular mechanism of CYP gene regulation, we have been using Cyp6a8 gene of Drosophila as a prototype gene and various xenobiotics, such as caffeine and phenobarbital as chemical tools. We have shown that these chemicals significantly induce Cyp6a8 gene expression in adult flies and S2 cells. We also demonstrated that -11/-199 (0.2-kb) upstream DNA of Cyp6a8 contains cis-regulatory elements for caffeine and phenobarbital induction. In the present investigation the putative TATA box and AP-1 and CREB binding sites present in the 0.2-kb upstream DNA were mutated. The mutated 0.2-kb DNAs were individually cloned in front of the luciferase reporter gene and transiently expressed in S2 cells in presence of caffeine, PB, or both chemicals. Dual luciferase activity assay showed that all three deletions decreased the basal and caffeine-induced activities. However, deletion of the CREB binding site showed the maximum negative effect and decreased the basal and caffeine-induced promoter activity by 6- and 8-fold, respectively. In this deletion construct inducibility with phenobarbital alone or caffeine plus phenobarbital also decreased dramatically by 3.6- and 7.8-fold, respectively. Experiments are in progress to identify the proteins that bind to the Cyp6a8 0.2-kb upstream DNA. [Supported by USDA-CREES # 002-35302-12281].

823A

Notch signalling is involved in many cell fate decisions throughout Drosophila development and, despite a simple and highly conserved transduction pathway, there are multiple possible outcomes to signalling, determined partly by context. We recently identified argos as a Notch target in the adult muscle progenitors (AMPs) of the wing disc and isolated the enhancer mediating this regulation. Previously argos was shown to be repressed by Notch in the wing pouch suggesting that the response to Notch activation is context dependent. We have mapped the regions responsible and shown that these responses are mediated by separable enhancers. One enhancer results in direct activation by Notch in the AMPs, where Twist is an essential co-factor. The second is susceptible to indirect inhibition by Notch in the wing pouch, where its regulation depends on a combination of general activators and restricted repressors that together result in expression in vein primordia.

824B

The integration of distinct developmental pathways to yield tissue- and cell-specific gene expression is fundamental for proper morphogenesis, yet the molecular interactions between pathways remain largely uncharacterized. In this study, we use a combination of transgenic reporter assays, genes, cell culture and biochemistry to show that an Abdominal-A (Abd-A) Hox complex interacts with the thoracic Hox factor Antennapedia (rho) to control EGF signaling from a subset of sensory cells during development. This cell-specific transcriptional complex requires the Extradenticle (Exd) and Homothorax (Hth) Hox co-factor transcription factor binding sites (TFBSs), all empirically validated and curated from the published literature. The recent release of version 3.0 of the database extends the utility of REDfly as an important tool for the fly community. REDfly v3.0 now includes all sequences reported as functionally tested in a transgenic reporter gene assay regardless of whether they showed regulatory activity or have activity redundant with other, shorter regulatory sequences. Regions of overlap between CRMs with similar regulatory activity are automatically identified and displayed as “inferred CRMs.” Graphical views show the position of each CRM within its genomic locus, the location of each CRM with respect to its associated gene is provided, and conservation of local synteny between CRMs and their target genes across nine species of Drosophila is assessed. Curation of TFBSs has been expanded to include sites identified by electrophoretic mobility shift assay (EMSA, “gel shift”) in addition to DNase I footprinting. A completely redesigned interface improves access to REDfly for casual and power users alike with greatly improved capabilities for database searching and results filtering. These new features and enhancements make REDfly, which is freely accessible at http://redfly.ccr.buffalo.edu, a comprehensive source of Drosophila cis-regulatory data and a powerful platform to facilitate high-throughput experimental and computational studies of gene regulation.

825C
Functional Diversification of Su(H) Binding Sites in the Regulation of Su(H) Autoactivation During Drosophila External Sensory Organ Development. Feng Liu, James Posakony. Dept Cell & Dev Biol, Univ California, San Diego, La Jolla, CA.

In Drosophila, activation of the transmembrane Notch receptor leads to the cleavage of its intracellular domain, which enters the nucleus and converts the DNA-binding protein Su(H) from a transcriptional repressor to an activator. As a result, many Su(H) binding motifs act as repressor sites in Notch sending cells, but function as activator sites in Notch responding cells. During the development of external mechanosensory organs, Su(H) autoactivates its expression specifically in the socket cell through an autoregulatory enhancer, the ASE, that contains eight high-affinity Su(H) sites organized in two separate clusters. Here we show that these two clusters of Su(H) sites are functionally diversified. One cluster of three sites (S7-S9) is responsible for the early activity of the ASE in the socket cell, and for repressing inappropriate activity of the ASE in the shaft cell. By contrast, the

303
other cluster of five Su(H) sites (S2-S6) are dedicated activator sites for the function of the ASE in the socket cell, and do not repress ectopic ASE activity in the shaft cell. Furthermore, we find that some local activator sites collaborate only with S7-S9 to establish the early high levels of Su(H) expression required for its autoactivation in the nascent socket cell. As the socket cell differentiates, maintaining the Su(H) autoactivator loop relies on input through S2-S6. Together, our data suggest distinct roles of two clusters of Su(H) sites in respectively establishing and maintaining Su(H) autoactivation.

826A

Functional and evolutionary implications of modularity in gene regulatory sequences. Tara L. Martin, Meghan Bragdon, Kelly Eckenrode, Angela DePace. Department of Systems Biology, Harvard Medical School, Boston, MA.

The cis-regulatory sequences controlling the precise spatial and temporal patterns of gene expression in developing multicellular organisms are often organized into modules (ie. enhancers). This modularity has important implications for both the function and evolution of regulatory sequences. Many biological systems that integrate information make use of modular components to reduce pleiotropy and allow the rapid rearrangement of an existing system to perform a new function. We are investigating the modularity of cis-regulatory sequences, including constraints on module spacing, issues of redundancy within the locus, and contributions of transcription factor binding sites flanking enhancers to the regulatory function. We use the even-skipped (eve) locus as a model system and make transgenic flies containing synthetic regulatory sequences driving expression of a lacZ reporter. We use fluorescent in situ hybridization and 2-photon imaging to measure gene expression patterns at six time points during stage 5 of Drosophila melanogaster development. Using an automated image analysis pipeline, we collect quantitative cellular resolution data from hundreds of embryos. Our large data set provides sufficient statistical power to detect subtle spatial and temporal differences in expression patterns between regulatory sequence constructs. We present results from constructs that explore functional constraints on spacing between enhancers and the contributions of sequences flanking enhancers to gene expression patterns. We also present preliminary results of an experiment to determine whether known enhancers are necessary as well as sufficient to produce the eve stripe expression pattern. The quantitative nature of our measurements allow us to detect more subtle differences in gene expression than has previously been possible, and to relate these differences to the modular organization of cis-regulatory sequences. Our results will inform evolutionary models of binding site turnover and regulatory sequence evolution.

827B

Identifying the oenocyte enhancer of seven-up in Drosophila melanogaster. Grace A. Mason, Kathryn M. Ryan, Richard M. Cripps. Biology, University of New Mexico, Albuquerque, NM.

In Drosophila melanogaster, seven-up is an invertebrate ortholog of the chick ovalalbumin upstream promoter transcription factors (COUP-TFs), and expression ofsvp is required in many cell types for normal development. In the mammalian liver, COUP-TFII is implicated in controlling several metabolic phenotypes, yet the transcriptional regulation ofCOUP-TFII in hepatocytes has also not yet been elucidated. Since regulatory cascades are often conserved between Drosophila and vertebrates, the use of the Drosophila model system to characterize the regulation ofsvp will hopefully give us insight into regulation of the COUP-TFs in vertebrates. Oenocytes are cells in Drosophila that are hepatocyte-like and express svp in addition to other factors. The previously identified embryonicsvp enhancer is located in the first svp intron and is active in: the oenocytes, the ventral nerve cord, the dorsal somatic muscles, the ventral somatic muscles, and the dorsal vessel. We are now investigating which regions within the svp enhancer are crucial for svp expression specifically in oenocytes. We created transgenic animals containing various fragments of genomic DNA tied to areporter gene; by doing so we have identified a ~500-bp fragment, 8-EV3, with strong enhancer activity in the oenocytes. Using 8-EV3 as a basis, we are mutating conserved enhancer regions that were identified through a bioinformatic approach to find putative regulatory elements. Once we locate the minimal oenocyte svp enhancer, we shall attempt to identify direct regulatory factors. Some factors that have been shown to be important in oenocytes development arespalt major (salm) andmirror (mir). By understanding svp expression and regulation in the simpler Drosophila system, we can lay the groundwork for understanding COUP-TFII & III's regulation and interactions in higher animals.

828C

Functional Validation of Genome-wide Enhancer Predictions in Drosophila. Steven Miller1, Nicholas Négre2, Chris Bristow2, Jia Chen2, Rachel Sealfon1, Liija Ma2, Manolis Kellis3, Kevin White2, James Posakony1. 1) Div. of Bio. Sci./CDB, UCSD, La Jolla, CA; 2) Inst. for Genomics & Sys. Bio., Dept. of Human Genetics, The Univ. of Chicago, Chicago, IL; 3) Comp. Sci. and A. I. Lab., Broad Institute of MIT and Harvard, Cambridge, MA.

Though the sequence of the Drosophila genome was completed over ten years ago, vast stretches of nucleotides have yet to be functionally annotated, particularly non-coding sequences. As participants in the modENCODE consortium, we have used ChIP-chip and -seq platforms to map certain histone modifications and transcription factor occupancies genome-wide to infer the locations of different classes of cis-regulatory elements. A key component of this project is the functional validation of predictions derived from these data; here we present the validation of enhancer predictions. To maximize utility to the research community, we have sought to assess the effectiveness of different prediction criteria. One factor that has proven valuable for identifying enhancers in other systems has been CBP. For a subset of our predictions, we examined the binding of CBP across 12 developmental stages. This data allowed us to compare the predictive success of different CBP temporal profiles, absent any other factor binding data. A set of regions with CBP binding restricted to embryonic stages proved particularly interesting, with over 80% of tested sequences exhibiting enhancer activity in embryos with a variety of tissue specificities. However, regions with temporally stable CBP binding do not exhibit activity in embryos nearly as often, indicating the striking utility of the temporal profile we have identified through a bioinformatic approach to find putative regulatory elements. Once we locate the minimal oenocyte svp enhancer, we shall attempt to identify direct regulatory factors. Some factors that have been shown to be important in oenocytes development arespalt major (salm) andmirror (mir). By understanding svp expression and regulation in the simpler Drosophila system, we can lay the groundwork for understanding COUP-TFII & III's regulation and interactions in higher animals.

829A

Decoding the transcriptional program of epidermal differentiation. Francois PAYRE1,2, Delphine MENORET1,2, Marc SANTOLINI2, Isabelle FERNANDES1, Jennifer ZANET1,2, Yvan LATAPIE1,2, Pierre FERRER1,2, Hereve ROAULT3, Ignacio GONZALEZ3, Philippe BESSE3, Vincent HAKIM3, Stein AERTS2, Serge PLAZA1,2. 1) Centre de Biologie du Développement, University of Toulouse, UPS, Toulouse, France; 2) Laboratory of Computational Biology, KU Leuven, Belgium; 3) Laboratoire de Physique Statistique, ENS, Paris, France; 4) Institut de Mathematique de Toulouse, France; 5) CNRS UMR5547, Toulouse, France.

Developmental programs are implemented by regulatory interactions between Transcription Factors and their target genes. How the Cis Regulatory Modules (CRM) that mediate these interactions are build and function during the late stages of development remains poorly understood. We address these questions during the morphological differentiation of embryonic epidermal cells. Epidermal morphogenesis relies on regulatory cascades that ultimately set up the expression of the Shavenbaby transcription factor. Shavenbaby in turn directly triggers the transcription of a battery of cellular effectors, collectively responsible for localized cell shape changes leading to trichome formation. Combining experimental and novel computational approaches, we deciphered the nature and logic of the CRMs regulated by Svb. Our results bring new aspects to the current models of gene regulation since a weak Svb binding site enrichment and several distinct combinatorial codes define functional CRMs.

References:
Chanut & al, Plos Biol 2006
Fernandes & al, Dev Cell 2010
Dissecting the cis-regulatory elements of doublesex. A number of critical developmental control genes contain “shadow” enhancers, which produce patterns of gene expression that are the same or similar to those generated by more proximal “primary” enhancers. A combination of BAC recombineering and quantitative confocal imaging provides evidence that shadow enhancers help ensure robustness of gene expression within the dorsal-ventral (DV) and segmentation-patterning networks of the early Drosophila embryo. In DV patterning, the shadow enhancer of the gene snail fosters robustness of de novo transcription in response to different temperatures and genetic backgrounds. It also appears to be critical for the mesoderm involution (e.g., gastrulation) at elevated temperatures, but is dispensable at optimal culturing conditions. Thus, shadow enhancers represent a novel mechanism for the canalization of gene expression in development.

Most or all of the gap genes controlling segmentation also contain shadow enhancers, including Hunchback, which responds to the Bicoid morphogen gradient. We present evidence that the primary or shadow enhancer is dispensable for the early activation of hunchback expression in the anterodorsal hinge of the early embryo (Eve in Bicoid mutants) or in anterior neuroblasts. Enhancers in Eve and hunchback that are active early are typically activated by the expression of a shadow enhancer. If the primary or shadow enhancer is deleted, the Eve enhancers result in more uniform and reliable transcriptional activation patterns, especially in regions of diminishing Bicoid concentration. We suggest that shadow enhancers, in addition to increasing the reliability of expression at varying temperatures, also ensure homogenous patterns of gene activation in response to graded signals prone to variability and noise.

Comparative and functional analysis of doublesex cis-regulatory elements. Gavin Rice, Michelle Arribas, Jerry Huang, Steve Small. Biology, New York University, New York, NY. Compared to the spatial regulation of gene expression, the mechanism of temporal control is poorly understood. We are investigating the patterning activities of the maternal factor Bicoid, which forms a protein gradient along anterior-posterior axes of the embryo, and activates target genes in different positions of the embryo. In our lab, we conducted a genome-wide search of Bicoid dependent CRMs (cis regulatory elements) by looking for Bicoid binding sites clusters. Some predicted CRMs directly anterior expression patterns in the early embryo, but others are activated only later. By looking at the expression timing of all the Bicoid dependent CRMs, we found a correlation between the numbers of Zebrafish binding sites and the expression times of these CRMs. CRMs without Zebrafish binding sites tend to be activated later or not expressed in early embryo compared to those that have Zebrafish binding sites. This indicates that Zebrafish might cooperate with Bicoid to activate early target gene expression. Currently we are testing whether binding of Zebrafish is required to activate these Bicoid dependent CRMs in the early embryo by testing CRMs activity in Zebrafish mutants. We also generated constructs containing CRMs with manipulated numbers of Zebrafish and Bicoid sites. These experiments will tell us whether Zebrafish is required to temporally regulate Bicoid target genes and help us understand how the binding of Zebrafish could control the timing of gene expression.

Temporal regulation of Bicoid-dependent cis-regulatory elements by Zelda. Zhe Xu, Hongtao Chen, Jerry Huang, Steve Small. Biology, New York University, New York, NY. Compared to the spatial control of gene expression, the mechanism of temporal control is poorly understood. We are investigating the patterning activities of the maternal factor Bicoid, which forms a protein gradient along anterior-posterior axes of the embryo, and activates target genes in different positions of the embryo. In our lab, we conducted a genome-wide search of Bicoid dependent CRMs (cis regulatory elements) by looking for Bicoid binding sites clusters. Some predicted CRMs directly anterior expression patterns in the early embryo, but others are activated only later. By looking at the expression timing of all the Bicoid dependent CRMs, we found a correlation between the numbers of Zebrafish binding sites and the expression times of these CRMs. CRMs without Zebrafish binding sites tend to be activated later or not expressed in early embryo compared to those that have Zebrafish binding sites. This indicates that Zebrafish might cooperate with Bicoid to activate early target gene expression. Currently we are testing whether binding of Zebrafish is required to activate these Bicoid dependent CRMs in the early embryo by testing CRMs activity in Zebrafish mutants. We also generated constructs containing CRMs with manipulated numbers of Zebrafish and Bicoid sites. These experiments will tell us whether Zebrafish is required to temporally regulate Bicoid target genes and help us understand how the binding of Zebrafish could control the timing of gene expression.

A major experimental hurdle in flies has been removing endogenous expression patterns; we present a quantitative comparison of RNAi knock downs to classic null alleles in fly reporters. Together these complementary yeast and fly systems explore how mutations influence gene expression phenotypes over evolutionary time scales.
Forkhead (Fkh) proteins comprise a large family of conserved transcription factors (TFs) with diverse developmental functions. The role of this family in regulating expression of mesodermal genes in Drosophila has been largely unexplored, although the expression of Fkh TFs in various subsets of the larval mesoderm suggests that they may control cell fate specification and/or differentiation. To investigate the potential involvement of these TFs in the regulation of mesodermal gene expression, we first mutated the evolutionarily conserved Fkh binding sites in an enhancer from the Nidogen (Ndg) gene that is active in subsets of cardiac cells, in somatic muscle founder cells and in the gut musculature. In transgenic reporter assays, these mutations resulted in de-repression in myocardial and pericardial cells of the heart, de-repression in fusion competent somatic myoblasts (FCMs) that do not normally express Ndg, and a loss of Ndg enhancer activity in visceral muscle. We then determined the candidate Fkh TFs accounting for the effects seen with the mutant enhancers in each mesodermal cell type. Loss-of-function of jumeau (jum), a Fkh TF-encoding gene expressed in the cardiac mesoderm (CM), caused a de-repression of the ndg reporter in the heart, while jumu over-expression repressed cardiac expression of ndg. A null mutation in mumbler observed in both the CM and FCMs generated no effect on Fkh binding site mutations in both of these mesodermal cell types. These results suggest that different tissue-specific Fkh TFs mediate distinct gene expression responses through the same binding sites in a single enhancer, and support a role for these factors in determining the unique genetic programs that characterize different subtypes of mesodermal cells.


Despite the recent advances in both the understanding of the pathways that control splicing and the ability to perform comprehensive RNA-seq analysis, a systematic analysis of the overall effects of changes in gene dose on alternative splicing is still lacking. We describe a novel strategy for identifying changes in alternative splicing in the context of changes in gene dose using RNA-seq data from the Drosophila genome project. We have performed single- and paired-end and stranded and non-stranded RNA-Seq (Illumina) of poly(A)+ RNA isolated from over 30 distinct time points throughout Drosophila development, in over 25 dissected tissues, 25 cell lines and in and animals treated with a variety of perturbagens, and in 22 cell types and in nearly 60 different RNA binding proteins. In total, we have generated over 10 billion individual sequence reads from these samples that map uniquely to the Drosophila genome or splice junctions. From this data, we have identified thousands of new alternative splicing events and identified alternatively spliced genes regulated by RNA binding proteins. We will present a global analysis of our data including the alternative splicing regulatory network we have constructed and an integrated analysis with RNA-seq data from the 30 development time points and the dissected tissues. Together, these studies have allowed us to identify exons that are regulated by individual RNA binding proteins at an unprecedented level of resolution and are aiding our elucidation of the splicing code of Drosophila melanogaster.

Juvenile hormone modulates ecdysteroid-inducible transcription in the larval salivary gland via the bHLH-PAS protein, Methoprene tolerant (MET). Vincent C. Henrich, Jenna L. Callender, Jesse Plotkin, Joshua Beatty. CBGHR, Univ North Carolina at Greensboro, Greensboro, NC.

The wandering period of the larval third instar of Drosophila melanogaster is marked by an increase in titers of both 20-hydroxyecdysone (20E) and juvenile hormone (JH). The effects of 20E on transcriptional activity have been thoroughly examined in the larval salivary gland (SG), and the timing of 20E-inducible gene transcription during the wandering period has been precisely characterized. For this study, transcript levels for three 20E-inducible genes (E74A, E74B, and Broad-Complex (Bc)) were examined in dissected SGs challenged for two hours with low and high titers of 20E, with and without additional JH. By itself, JH had no effect on transcript levels, but when incubated together with low 20E doses that evoked submaximal transcript levels, the additional presence of JH potentiated 20E response to a maximal level. Conversely, JH repressed maximal activity evoked by the higher dose of 20E. In SGs that were mutant for Methoprene-tolerant (METw3), which substitutes a conserved residue in the bHLH domain, the effects of JH on transcript levels were completely eliminated with no effect on 20E inducibility. An RNAi knockdown of the MET paralogue, germ cell expressed (gce), reduced the maximal level of 20E induction for E74A and Bc in the SG. In Met/gce doubly mutant SGs, the timing of 20E induction was accelerated relative to the larva's development through the wandering phase of the third instar. Together, the results indicate that JH is capable of modulating 20E-inducible transcription in the larval SG during the wandering period of the third instar via the action of the two bHLH-PAS proteins, MET and GCE. These activities apparently involve both inductive and repressive mechanisms. Current efforts are directed toward better understanding the relationship between these two factors and the promoters of 20E-inducible genes.
To examine this question on the global level, RNAseq experiments were performed on effect on the acetylation such that an increase in expression of the reporter only occurs in the absence of the whole MSL complex. Previous studies using selected endogenous expression levels of regulated genes. This analysis unravels the evolutionary links between various levels of transcriptional regulation, from DNA sequence to gene expression through protein binding.

840C
Evolution of Transcriptional Regulation in Early Embryos of the Drosophila Genus. Mathilde Paris1, Tommy Kaplan1, Xiao-Yong Li2, Robert K. Bradley1, Mark D. Biggin1, Michael Eisen1,2. 1) Molecular and Cellular Biology, QB3 Institute, BERKELEY, CA; 2) Howard Hughes Medical Institute, University of California Berkeley, Berkeley, CA; 3) Genomics Division, Lawrence Berkeley National Lab, Berkeley, CA.

To better characterize how variation in regulatory sequences drives divergence in gene expression, we undertook a systematic study of transcription factor binding and gene expression in four species that sample much of the diversity in the 60 million-year old genus Drosophila. D. melanogaster, D. yakuba, D. pseudoobscura, and D. virilis. We used ChIP-Seq to examine the genome-wide binding of four transcription factors (BCD, HB, GT and KR) that play a major role in regulating patterning along the anterior-posterior axis. While the global architecture of the transcriptional islands is conserved (i.e. the same proteins tend to regulate the same genes), only 20-50% of bound loci are shared between species. We analyzed these findings in light of the fully-sequenced genomes of the four species. Most binding events in each species could be associated with nearby underlying binding sites. However, these binding sites were typically not homologous, even in cases of overlapping binding events, demonstrating extensive binding site turnover. To examine the consequences of these changes, we used mRNA-Seq to measure gene expression in blastoderm embryos of each species. Surprisingly, we found relatively few changes in gene expression, suggesting that differences in sequence and binding have limited effect on gene expression or act in a compensatory manner to maintain the overall expression levels of regulated genes. This analysis unravels the evolutionary links between various levels of transcriptional regulation, from DNA sequence to gene expression through protein binding.

841A
Global Patterns of Gene Expression in the maleless mutant. Abhijit Sanyal, Lin Sun, Weiwu Xie, James Birchler. Division of Biological Sciences, University of Missouri, Columbia, MO.

The Male Specific Lethal (MSL) complex associates with the male X chromosome and produces an increased acetylation of Lys16 on histone H4. Using GAL4 binding domain fusions, targeting of the responsible acetylase, MOF, to reporter constructs has been performed in multiple laboratories and revealed that the complex contains a "counteractive" effect on the acetylation such that an increase in expression of the reporter only occurs in the absence of the whole MSL complex. Previous studies using selected endogenous genes and transgenes on the X chromosome or the autosomes revealed that in male larvae homozygous for the maleless (mle) mutation, there was a generalized retention of X chromosome dosage compensation with a generalized increase in expression of the autosomes. To examine this question on the global level, RNAseq experiments were performed on maleless homozygous males and females and the normal controls. In comparing the reads on the X chromosome in the mle mutant to normal males, dosage compensation is maintained for most genes or there is a slight increase in expression. A small number of genes lose dosage compensation. For autosomal genes, a substantial fraction is unchanged but the majority of genes are increased in expression to varying degrees up to about two fold at which point the curve drops off. These results in combination with the targeting experiments suggest that the MSL complex sequesters MOF from the autosomes to mute the increased expression on the autosomes that might otherwise occur due to the genomic imbalance in males and then counteracts the high level of acetylation on the X to allow the proper two-fold upregulation.

842B
Distinct mechanisms of short- and long-range repressors in the Drosophila embryo. David N. Arnosti1,2, Kurtulus Kok3, Sandhya Payankaulam1, Li M. Li3. 1) Dept Biochemistry and Molecular Biology, Michigan State Univ, East Lansing, MI; 2) Program in Genetics, Michigan State University; 3) Dept Microbiology and Molecular Genetics, Michigan State University.

The Drosophila blastoderm embryo has served as a premier platform for understanding mechanisms that regulate complex interactions of multiple transcription factors and enhancers. One of the key findings to emerge from this area is that transcriptional repressors employ functionally distinct pathways, working to inhibit neighboring activators either over short (<100 bp) or long ranges (>1kb). The mechanistic basis of this dichotomy has been poorly understood; initial suggestions that distinct co-repressors mediate these different activities have been disproved by recent findings showing that both short- and long-range repressors recruit Groucho and CBP. We conducted chromatin-based analyses of endogenous genes in the embryo repressed by Knirps and Hairy, representative short- and long-range repressors. Short-range repression is associated with localized chromatin condensation, increased resistance to nuclelease attack, local loss of acetylation marks, and interference of binding by some activators. Long-range repression, in contrast, is correlated with widespread loss of acetylation marks, but little change in histone density or chromatin accessibility, and no change in activator occupancy. Strikingly, effective repression is found in each case, although RNA polymerase occupancy eliminated by long-range repression, but not by short-range repression. Short-range repressors appear to locally target the cis regulatory element to which they are bound, leading to loss of a positive signal for transcriptional elongation, while long-range repression is associated with loss of polymerase access to the gene. The mechanistic insights derived from these studies provide an essential context with which to interpret global chromatin states associated with developmental gene regulation. Our studies also provide new perspectives on the properties of highly conserved transcriptional cofactors and pathways in metazoans.

843C

The recent advent of whole-genome methods and quantitative imaging and analysis techniques provides the first opportunity to investigate the role of population dynamics on the evolution of gene regulatory mechanisms in animal development. The first line of research on the mesoderm specification where the highly evolutionary conserved transcription factor Snail is responsible for repressing a range of different genes in order for coordinated and reliable specification of the mesoderm to occur. Preliminary studies suggest that the Snail repressor inhibits the release of RNA Polymerase II (Pol II) at the core promoter. Once released, Pol II completes transcription even when subjected to active repression by Snail.

844A
Characterizing Functions of the Groucho Central Domains in Transcriptional Repression. Pak Kwong1, Wiam Turkji-Judeh2, Albert Courey1,2. 1) Department of Chemistry and Biochemistry, UCLA; 2) Molecular Biology Interdepartmental Program, UCLA.
POSTER: Regulation of Gene Expression
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

Groucho (Gro) is a transcriptional regulatory factor that represses a wide range of genes throughout Drosophila development. Gro does not have a DNA binding domain, but rather is recruited to the template via protein-protein interactions. Once recruited, Gro directs long-range gene silencing. However, the detailed mechanism of Gro-mediated repression remains elusive. Gro family members have a five-domain structure: two highly conserved regions, the N-terminal Q and C-terminal WD-repeat domains; and three less conserved central regions, the GP, CCN, and SP domains. We have shown that the central regions have critical roles in repression: deletion of the GP domain abolishes repression; and deletion of the SP domain enhances repression. To further illuminate the functions of the central regions, we are employing both high throughput and biochemical approaches. Using affinity chromatography in conjunction with multidimensional LC-MS, we are identifying novel interacting proteins. In addition, since the GP domain was previously shown to interact with the histone deacetylase Rpd3, we are creating Gro variants that will allow us to resolve the function of the GP domain in Rpd3 binding from its function in nuclear localization.

845B
miR-10 recognize target sequences of 3'UTR of Scr in D. melanogaster. Jannet E. Salinas-Hernandez, J.C. Moreno, A. Esparza, S. Elizondo, D. Resendez. Biología Celular y Genética, Instituto de Biología de Nuevo Leon, Monterrey, N.L., México miRNAs are non-coding RNA that have an important function in post-transcriptional gene regulation and in particular modulate the expression of developmentally important transcription factors including Hox genes. The sequence of miR-10 is conserved in many vertebrate Hox complexes which is located within the Drosophila Antennapedia gene complex between the Hox genes Deformed and Sex combs reduced (Scr). Since has been proposed as a direct miR-10 target here, we present evidence that miR-10 affects Scr expression level both in vitro and in vivo in D. melanogaster. Scr 3' untranslated region (3'UTR) containing predicted target sites for miR-10 were cloned into pMIR-Luc vector and co-transfected into a cell culture lines in presence and absence of synthetic miR-10. Our results showed that miR-10 decreased in 50% the activity of the reporter gene 3'UTR-Luc in the quantitative assay. We also assessed the expression of the GFP-Scr-3’UTR sensor in the presence of ectopic DaRed-miR-10- containing. When driven by dII-G41 and dpe-G41 in imaginal discs, Scr sensor express levels were specifically diminished in those cells expressing the miR-10 transgene. Detailed analysis of the DsRed-daRed-R10 and GFP-Scr-3’UTR expression profiles suggests repression of the Scr sensor by ectopic miR-10 miRNA is dose-sensitive. Taken together these data showed in vitro and in vivo evidence that miR-10 specifically recognize target sequences in the Scr 3’UTR, repressing gene expression.

846C
Cis-regulatory analysis of the snail locus demonstrate two early embryonic enhancers share repressors. Angeliki Stathopoulos, Leslie Dunipace, Anil Ozdemir. Div Biol, MC 114-96, Caltech, Pasadena, CA. snail encodes a transcriptional repressor that is influential for delineating the mesoderm/mesectoderm/neurogenic-ectoderm boundary in the embryo. The snail boundary is quite sharp, and therefore we undertook a search for repressors that define this pattern. We created a ~25 kbp transgene containing sequence from the snail locus, able to complement the mutant to viability. We analyzed the significance of identified Twist ChIP-seq binding to the snail locus. A promoter proximal enhancer identified previously is contained in the ~2 kbp sequence upstream of the snail gene; this sequence can support expression comparable to the endogenous pattern when assayed in a standard reporter gene assay. However, we noticed that the pattern supported was not as sharp as the endogenous gene. The Twist ChIP-seq experiment identified binding to both snail promoter proximal regions as well as to a region greater than 10 kbp upstream of the gene, within the intren of a flanking gene. We show that this distal regions functions to support expression of snail that is comparable to endogenous pattern. The distal enhancer contains sites for the repressor Huckebein and an uncharacterized repressor that refines the snail pattern in dorsal-lateral regions; presumably as a result of looping interactions, these repressors function in a dominant fashion to silence the promoter proximal enhancer. In summary, reporter gene analyses conducted in the context of the native gene locus demonstrate that enhancers that support snail expression in the early embryo, that they are not redundant (the distal enhancer is required to support viability but the proximal enhancer is dispensable), and that the distal enhancer contains repressor activity that is shared as it functions to silence the promoter proximal enhancer as well as the distal enhancer. Assaying the function of cis-regulatory sequences in the context of the native gene locus can provide important insights into cis-regulatory mechanisms.

847A

Gene expression in eukaryotes is largely regulated by regulatory transcription factors that bind cis-regulatory elements of genes in a sequence-specific manner and promote or antagonize transcription, acting as activators or repressors, respectively. Repressors function primarily by recruiting accessory proteins, corepressors (CoRs), that antagonize transcription largely by modifying chromatin structure. Although in theory a repressor may only need to recruit a single CoR, studies of most repressors, for example, the E26 Transformation-specific Sequence (ETS) transcription factors regulate gene expression programs to direct differentiation of a variety of tissues and organs, and when perturbed contribute to tumor progression in mammals. The Drosophila ETS protein Yan is a transcriptional repressor that functions downstream of the receptor tyrosine kinase signaling pathway to regulate the differentiation of many cell types. Yan can self-associate to form a head-to-tail polymeric structure that is required for its in vivo function. Although Yan has been the focus of much research, gene regulatory factors used by Yan to regulate gene expression are not well characterized and an understanding of what occurs at the chromatin level is lacking. Following chromatin immunoprecipitation (ChIP) experiments to characterize the genome-wide chromatin binding profile of Yan, we observed that Yan binds to 40% of its putative target genes with one or more small chromatin footprints (Class A), while at the remaining 60% of targets (Class B), Yan spreads along a larger chromatin region. These different binding patterns represent the hypothesis that Yan may regulate these two groups of genes by different mechanisms and in the case of Class B genes, in a manner unprecedented for a site-specific transcription factor. Supporting this hypothesis, our preliminary data suggest that Yan chromatin association patterns are dynamic over time, with particular target genes switching between class A & class B type Yan binding. We are addressing this question by a combination of approaches, including ChIP-chip from fly lines in which Yan mutants are expressed at endogenous levels in a yarm null background to determine whether Yan self-association contributes to the class B chromatin binding pattern. Alternatively, reporter-enhancer constructs will be utilized to see if the Yan binding pattern reflects complex regulation of gene expression by multiple or shadow enhancers. Finally, ChIP-seq from D. virilis and D. simulans will be used to determine the extent to which the Yan chromatin occupancy pattern is evolutionarily conserved.

848B

E26 Transformation-specific Sequence (ETS) transcription factors regulate gene expression programs to direct differentiation of a variety of tissues and organs, and when perturbed contribute to tumor progression in mammals. The Drosophila ETS protein Yan is a transcriptional repressor that functions downstream of the receptor tyrosine kinase signaling pathway to regulate the differentiation of many cell types. Yan can self-associate to form a head-to-tail polymeric structure that is required for its in vivo function. Although Yan has been the focus of much research, gene regulatory factors used by Yan to regulate gene expression are not well characterized and an understanding of what occurs at the chromatin level is lacking. Following chromatin immunoprecipitation (ChIP) experiments to characterize the genome-wide chromatin binding profile of Yan, we observed that Yan binds to 40% of its putative target genes with one or more small chromatin footprints (Class A), while at the remaining 60% of targets (Class B), Yan spreads along a larger chromatin region. These different binding patterns represent the hypothesis that Yan may regulate these two groups of genes by different mechanisms and in the case of Class B genes, in a manner unprecedented for a site-specific transcription factor. Supporting this hypothesis, our preliminary data suggest that Yan chromatin association patterns are dynamic over time, with particular target genes switching between class A & class B type Yan binding. We are addressing this question by a combination of approaches, including ChIP-chip from fly lines in which Yan mutants are expressed at endogenous levels in a yarm null background to determine whether Yan self-association contributes to the class B chromatin binding pattern. Alternatively, reporter-enhancer constructs will be utilized to see if the Yan binding pattern reflects complex regulation of gene expression by multiple or shadow enhancers. Finally, ChIP-seq from D. virilis and D. simulans will be used to determine the extent to which the Yan chromatin occupancy pattern is evolutionarily conserved.
MicroRNAs Facilitate Evolutionary Canalization. Justin J. Cassidy1,2, Richard W. Carthew1,2. 1) Department of Molecular Biosciences, Northwestern University, Evanston, IL; 2) Chicago Center for Systems Biology.

Canalization epitomizes the developmental forces that limit environmental or genotypic variations experienced by a species to produce a uniform phenotype. The molecular systems that underlie this process remain elusive. Some have suggested that microRNAs might be ideally suited for this purpose. These potent regulatory molecules tune gene output to optimum expression levels while their seemingly redundant connectivity within gene networks provides buffering against variation. Recent studies demonstrate these capabilities on developmental and physiological timescales, but so far there have been no direct experimental approaches to address whether microRNAs might stabilize gene expression within a population over evolutionary time.

Here we test the prediction that microRNAs reduce phenotypic variability within a population in the face of selective pressures. To do so, we evoke a classic model of evolutionary canalization - Rendel's artificial selection experiments with Drosophila - in the context of microRNA mutant backgrounds. Like Rendel, we infer the existence of tuning and buffering mechanisms that serve to transiently limit robust responses to selection. Intriguingly, these responses seem to dramatically change in certain microRNA mutant backgrounds. We will discuss these preliminary findings that suggest microRNAs might be one class of molecular players that underlie evolutionary canalization.

850A

Drosophila let-7-C is a direct target of the nuclear hormone receptor EcR. Geetanjali Chawla, Nicholas S. Sokol. Department of Biology, Indiana University, Bloomington, IN.

MicroRNAs (miRs) are a class of small RNAs that regulate the expression of target genes. The Drosophila let-7-Complex (let-7-C) encodes three co-transcribed miRs, miR-100, let-7 and miR-125. Their expression is triggered at the onset of metamorphosis, suggesting transcriptional regulation by the steroid hormone 20-hydroxyecdysone (20E) via Ecdysone Receptor (EcR). Given conflicting published accounts of the relationship between 20E:EcR and let-7-C miRs, we characterized the transcript pri-let-7-C transcript (pri-let-7-C) using semi-quantitative RT-PCR. Pri-let-7-C was first detected at the mid-third larval transition, coinciding with the detection of processed let-7-C miRs as well as known 20E target sgs-3. Pri-let-7-C was also detected in Drosophila embryonic cell lines treated with 20E. Given these results, a comprehensive set of deletions in the let-7-C locus were generated to map DNA segments required for activation by 20E, and let-7-C DNA fragments were also assayed for their ability to confer 20E responsiveness to a luciferase reporter. These approaches identified a highly conserved EcR response element (EcRE) in the first intron of let-7-C. This element was directly bound by EcR and its co-receptor Ultraspiracle (Usp) in gel-shift assays. Furthermore, mutation of the EcR-Usp binding element reduced transcription of a pri-let-7-C reporter, and abolished 20E-mediated activation in cell culture. Our data also uncovered the presence of cis-elements that confer repression and that were distinct from the EcRE. Based on these results, we conclude that the let-7-C locus is a direct target of EcR as well as other currently unidentified trans-acting factors. Our current work is focused on determining the functional significance of the EcR/let-7-C relationship as well as identifying the trans-acting repressors, and the current status of this work will be presented at the meeting.

851B


Capped small RNAs called Transcript-Associated (PASRs) and Termination Associated Small RNAs (TASRs) are found at the 5' and the 3' end of most genes, respectively. The small RNA landscapes of 4 Drosophila cell lines. We will discuss these preliminary findings that suggest microRNAs might be one class of molecular players that underlie evolutionary canalization.

852C

miR-275 is indispensable for blood digestion and egg development in the mosquito Aedes aegypti. Alexander Raikhel, Bart Bryant, Warren MacDonald. University of California, Riverside, CA.

The mosquito Aedes aegypti is the major vector of arboviral diseases, particularly of Dengue fever, of which there are over 100 million cases annually. Mosquitoes, such as A. aegypti, serve as vectors for disease pathogens because they require vertebrate blood for their egg production. Pathogen transmission is tightly linked to repeated cycles of obligatory blood feeding and egg maturation. Thus, the understanding of mechanisms governing egg production is necessary to develop approaches that limit the spread of mosquito-borne diseases. Previous studies have identified critical roles of hormonal- and nutrition-based Target of Rapamycin (TOR) pathways in controlling blood-meal-mediated egg maturation in mosquitoes. In this work, we uncovered another essential regulator of blood-meal-activated processes, the microRNA miR-275. The depletion of this microRNA in A. aegypti females after injection of its specific antagomir resulted in severe defects in blood digestion and egg development, clearly demonstrating that miR-275 is indispensable for these physiological processes. miR-275 exhibits an expression profile that suggests its regulation by a steroid hormone, 20-hydroxyecdysone (20E). In vitro organ culture experiments demonstrated that miR-275 is induced by this hormone in the presence of amino acids, indicative of a dual regulation by 20E and TOR. This report has uncovered the critical importance of microRNAs in controlling blood-meal-activated physiological events required for completion of egg development in mosquito disease vectors.

853A


MicroRNAs are short non-coding RNAs that can post-transcriptionally silence gene expression by binding to target miRNAs. Our research focuses on studying the function of the microRNA miR-8 in Drosophila melanogaster. Previous studies have identified miR-8 as a regulator of Wnt and insulin signaling, neurodegeneration and neuromuscular junctions. We have found that miR-8 is also required for proper adult cuticle pigmentation. Female adult miR-8 mutant flies exhibit decreased pigmentation of the dorsal abdomen, with a pattern of pigmentation similar to control flies grown at higher temperatures. This phenotype is independent of the small size of mutants, as tissue specific loss of miR-8 through expression of a miR-8 “sponge” in the developing cuticle results in a similar decrease in pigmentation. miR-8 can directly target multiple members of the cuticular protein (cpr) family in cell culture and we are currently exploring the role of these and other potential targets in the altered abdominal pigmentation of miR-8 mutants.
POSTER: RNA Biology
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

854B
Identification and characterisation of more than 1000 non-coding transcripts in Drosophila. Andrew Roger Bassett, Robert Young, Charlie Tibbit, Ji-Long Liu, Chris Ponting. MRC Functional Genomics Unit, University of Oxford, Oxford, Oxfordshire, United Kingdom.

Non-coding transcription is an important feature of higher eukaryotic genomes, and correlates with complexity. Here we use RNA-seq data from the modENCODE consortium to identify more than 1000 long non-coding intergenic transcripts in Drosophila (lncRNAs). These comprise around 2% of the genome, and show conservation in sequence across several Drosophila species, suggesting that they play important roles in the fly. Several also show conserved locations within the genome with organisms as distant as mouse, zebrafish and chicken. We show that these non-coding transcripts are tightly regulated in their expression across development, especially at pupal stages suggesting important roles in gene regulation during metamorphosis.

855C
Drosophila Transcriptome RNA-Seq Data Identifies Candidate RNA Editing Sites in 561 Additional Genes. Joseph W. Carlson1, Brenton R. Gravely2, Peter Cherbas1, Susan E. Celinker1, modENCODE Drosophila Transcriptome Project. 1) Lawrence Berkeley National Laboratory, Berkeley CA; 2) University of Connecticut Health Center, Framingham CT; 3) Indiana University, Bloomington IN.

Posttranscriptional modification of RNA through the deamination of adenosine acts to alter messenger RNA molecules from template DNA by the conversion of adenosine to inosine. The process requires the action of ADAR and the enzymes are known to cause behavioural abnormalities. Previous studies identified 54 genes whose transcripts undergo RNA editing, primarily genes encoding signaling components of the nervous system.

The wealth of recent RNA-Seq data from the modENCODE project has enabled an analysis of the transcriptome for additional editing events. We have identified 972 potential edited sites in 597 genes. The majority of these editing events occur in late pupal and adult stages of development, although a significant number are edited beginning in late embryogenesis. We confirmed editing sites in 36 of the published genes, and identified sites in 561 genes not known to be edited. Approximately one quarter of the newly discovered editing sites are confirmed in EST or cDNA data.

As a result of the increased number of editing sites, we are able to perform analyses of the local context of the transcript sequence in the vicinity of the edited position to discover motifs associated with editing. Meme reveals three statistically significant motifs, two of which are strongly positioned at the edited base. Roughly one-third of the candidate sites have a sequence motif within 50 bp of the edited base.

856A
Functional analysis of Drosophila eIF4E in P bodies. Paola Ferrero1,2, Carla Layana1,2, Ezequiel Paulucci1, Rolando Rivera Pomat1,2. 1) Centro Regional de Estudios Genómicos, UNLP- Florencio Varela, Buenos Aires, Argentina; 2) Departamento de Ciencias Básicas y Experimentales, UNNOBA, Pergamino, Buenos Aires, Argentina.

The eukaryotic translation initiation factor 4e isoform 1 (eIF4E-1) is the main of eIF4E isoforms that are expressed in Drosophila. It plays a major role in translation and the absence of eIF4E-1 is lethal. eIF4E-1 is present in the cytoplasm of the cells as soluble protein, in translational complexes and also in P bodies, the cytoplasmic foci involved in mRNA silencing. eIF4E-1 is the only translation factor present in either active or inactive mRNPs. To elucidate the pathways of silencing mediated by eIF4E we have studied the interactions between eIF4E, mRNAs and P bodies components in vivo. We have generated eIF4E mutants on key tryptophan (W) residues by replacing them with alanine (A). We transferred S2 cells with either wild type and mutant eIF4E-1 to evaluate the requirement for PBs formation and localization. Mutations of W117 results in the absence of eIF4E-1 in P bodies, but it does not affect the formation of the foci. Mutations in W residues required for cap-binding did not affect the presence of eIF4E-1 in P bodies. This was also observed in cross-experiments in HeLa cells. Therefore we conclude that protein-protein interactions rather than cap-binding are required for the transition from polysomes to P bodies.

857B
Using confirmed dicistronic gene structures from multiple Drosophild species as a model for understanding eukaryotic translation diseases. Henry C. Hunter, Christopher D. Smith. Biology Dept, San Francisco State University, San Francisco, CA.

RNA secondary structures have been found in humans to act as 5'-UTR regulatory elements to allow proper translation. Mutations or any other alterations to those secondary structures can drastically affect the translation of the mRNAs, causing diseases such as Multiple myeloma, fragile X syndrome, and numerous cancers. Similar RNA secondary structures have been found in multicistronic genes. Encoding multiple genes in a single mRNA, multicistronic gene structures are common among prokaryotes and viruses, but evidence has shown that RNA structures in the mRNA may promote translation. These same genes can appear as standard monocistronic transcripts in distantly related Drosophila species, suggesting that there exist mechanisms that allow genes to merge and become expressed as dicistronic products. While full-length cDNA evidence supports the existence of these non-coding transcripts, it does not provide the proof that dicistronic expression occurs. To elucidate the pathways of silencing mediated by eIF4E we have studied the interactions between eIF4E, mRNAs and P bodies components in vivo. We have generated eIF4E mutants on key tryptophan (W) residues by replacing them with alanine (A). We transferred S2 cells with either wild type and mutant eIF4E-1 to evaluate the requirement for PBs formation and localization. Mutations of W117 results in the absence of eIF4E-1 in P bodies, but it does not affect the formation of the foci. Mutations in W residues required for cap-binding did not affect the presence of eIF4E-1 in P bodies. This was also observed in cross-experiments in HeLa cells. Therefore we conclude that protein-protein interactions rather than cap-binding are required for the transition from polysomes to P bodies.

858C
The piNG-body - a novel non-membrane organelle in the nuage of Drosophila male germ cells. Mikhail V. Kibanov, Ksenia S. Egorova, Sergei S. Ryazansky, Olesia A. Sokolova, Alexei A. Kотов, Oxana M. Olenkina, Anastasia D. Stolyarenko, Vladimir A. Gvozdev, Ludmila V. Olenina. Laboratory of Biochemical Genetics of Animals, Department of Molecular Genetics of Cell, Institute of Molecular Genetics, RAS, Kurchatov sq., 2, 123182, Moscow, Russia.

In many eukaryotic species, germ cells contain specific cytoplasmic granules. During Drosophila oogenesis, these granules form a perinuclear organelle called nuage. Recent studies have accentuated participation of the nuage in piRNA biogenesis and piRNA-dependent silencing. Proteins of the PIWI subfamily, Aub and AGO3, associated with small RNA pathways are common among prokaryotes and viruses, but while much is known about the ovarian nuage, its structure and functions in spermatocytes remain essentially under-investigated. In our work we analyzed the nuage in D. melanogaster testes. Using immunostaining, we demonstrated size heterogeneity of the Vasa-containing cytoplasmatic particles and found a new nuage-associated non-membrane organelle, which was 50 times larger than the ordinary nuage granules, and called it the piNG-body (piRNA Nuage Giant body). This body contained the known ovarian nuage proteins, Vasa, Aub, AGO3, Tud, Bel, Squ, and Cuff, as well as AGO1, the key component of miRNA pathway, but not Dicer1. The piNG-bodies emerged at the primary spermatocyte stage during a period of active transcription. A spatial separation of the proteins inside the piNG-body was revealed. Aub, Vasa, Tud and the others were located at the periphery of the piNG-bodies, whereas AGO3 was found in the core. Mutations in these proteins led to various piNG-body assembly defects accompanied by derepression of testis-specific Stellate repeats. It has been shown recently that functions and protein interactions of PIWI proteins are dependent on the same arginine methylation provided by csu methyltransferase. We found that mutations in csu led to piNG-body disruption followed by up-regulation of Stellate genes.
859A Drosophila RNase Z is an essential gene involved in tRNA processing. Xie Xie, Veronica Dubrovskaya, Edward Dubrovsky. Biological Sciences, Fordham University, Bronx, NY.

Drosophila RNase Z (dRNaseZ) is a juvenile hormone-inducible gene that encodes a member of the ELAC1/ELAC2 protein family with homologs in every living organism. RNase Z proteins possess endoribonuclease activity and are involved in tRNA 3’-end maturation. Given that the biochemical function of RNase Z has been delineated using in vitro, bacterial and cell culture models, its in vivo activity still needs to be established. In this study, we performed a spatiotemporal loss-of-function analysis of dRNaseZ in tissues of a developing fly using RNA interference (RNAi) in combination with the GAL4/UAS system. We found dRNaseZ protein is indispensable for organism viability, as ubiquitous knockdown causes growth arrest and early larval lethality. Northern blot analysis of pre-tRNA molecules revealed that, for all nuclear and mitochondrial RNAi tested, reduction of dRNaseZ protein leads to the accumulation of RNA processing intermediates with the unprocessed 3’ ends. Using tissue-specific GAL4 drivers, we found that dRNaseZ is required for cell proliferation in mitotic wing and eye imaginal discs and cell growth in endoreplicating salivary glands. Although the mechanisms remain unclear, our results support the notion that RNase Z is involved in biological pathways regulating cell growth and proliferation.

860B Drosophila Tis11 and its effects on mRNA expression. Youn-Jeong Choi1, Wei Lai1, Robert Fedic2, James Mason2, Perry Blackshear1. 1) Laboratory of Signal Transduction, NIEHS, Research Triangle Park, NC; 2) Laboratory of Molecular Genetics, NIEHS, Research Triangle Park, NC.

The mammalian tristetraprolin (TTP) family of CCCH tandem zinc finger proteins can bind to AU-rich elements in the 3’ UTRs of mRNAs, leading to their deadenylation and destabilization. Rodent genomes express four members of this family, which are involved in the decay of mRNAs encoding growth factors. In contrast, Drosophila RNase ZL is an essential gene involved in tRNA processing. See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

Here, we identify and characterize MIP2, a candidate RNA-binding adapter for the insulator. MIP2 is a conserved RNA-binding protein and has no effect in non-CNS tissue. Furthermore, ectopic expression of MIP2 in non-CNS tissue reduces activity. Interestingly, unlike ubiquitously expressed core insulator proteins, MIP2 expression is restricted to the central nervous system (CNS). Using both a novel quantitative, tissue-specific assay and in vitro binding assays, we have shown that MIP2 binds specifically to the insulator. These data suggest a novel role for RNA in defining tissue-specific insulator function. 861C Systematic analysis of RNA-protein interactions in Drosophila. John D. Laver1, Karen Fung1, Saddchet Sidhu1, Craig Smibert1, Howard Lipshitz1. 1) Department of Molecular Genetics, University of Toronto, Toronto, Canada; 2) Department of Biochemistry, University of Toronto, Toronto, Canada.

During early Drosophila embryogenesis, post-transcriptional control of gene expression by regulated mRNA translation, degradation, and subcellular localization has an essential role in directing development. Each of these post-transcriptional processes is regulated by the binding of particular RNA-binding proteins (RBPs) to specific sites in mRNA transcripts. One goal in the Liphitz and Smibert labs is to better understand the functions and coordination of RBP activities in early Drosophila embryos by examining RBPs-mRNA interactions on a genome-wide scale. Specifically, we aim to identify the entire complement of mRNAs associated with all RBPs present in early embryos. As proof-of-principle, towards this goal, we are developing synthetic RBPs and using these antibodies to perform RNA co-immunoprecipitations coupled with high-throughput sequencing to identify RNAs bound by these proteins. Synthetic antibodies are selected using phage display technology from artificially-designed antibody libraries, then synthesized in bacteria. They have the advantage over conventional antibodies of being more rapid and cost-efficient to produce, as well as being amenable to high-throughput production, making them particularly suitable for the goal of producing antibodies against a large number of RBPs. To date, synthetic antibodies have been obtained for 10 out of 10 RBPs attempted, with affinities ranging from sub-nanomolar to 450 nM. Of the individual antibodies obtained, 13 of 14 tested, representing five RBPs, are able to provide insights into post-transcriptional regulatory networks in which they have a role.


Chromatin insulators are protein-DNA complexes that influence chromatin organization and gene expression through two functional properties, enhancer blocking and barrier activities. Activity of the gypsy insulator requires the core proteins Suf(Hw), Mod(mdg4)2,2, and CP190. Previous work has suggested a role for RNA in gypsy insulator function; however, the identities of insulator-associated RNA and mechanism by which RNA and the insulator interact have not yet been elucidated.

Here, we identify and characterize MIP2, a candidate RNA-binding adapter for the gypsy insulator complex. MIP2 harbors two RNA recognition motifs and interacts directly with Mod(mdg4)2,2 by yeast two-hybrid and in vitro binding assays. Furthermore, MIP2 co-immunoprecipitates with core gypsy insulator proteins from nuclear extracts. Interestingly, unlike ubiquitously expressed core insulator proteins, MIP2 expression is restricted to the central nervous system (CNS). Using both a novel quantitative, tissue-specific Gal4-inducible insulator assay as well as classical gypsy-dependent phenotypes, we determined that reduction of MIP2 levels improves gypsy insulator activity in the CNS but has no effect in non-CNS tissue. Furthermore, ectopic expression of MIP2 in non-CNS tissue reduces gypsy insulator function.

In order to identify RNA targets of MIP2 and perform RNA immunoprecipitation from nuclear extracts followed by high-throughput sequencing (RIP-seq), RIP-seq using α-Suf(Hw) or α-MIP2 antibodies reveal large numbers of specifically co-purified transcripts, several of which have been verified by RT-PCR. Analyses of bound transcripts with respect to functional classes, motifs, and in vivo function are ongoing. These data suggest a novel role for RNA in defining tissue-specific insulator function.

863B Analysis of tissue-specific requirements for the nonsense mediated mRNA decay pathway. Alex Chapin, Mark M. Metzstein. Human Gen, Univ Utah, Salt Lake City, UT.

The nonsense mediated mRNA decay (NMD) pathway is a post-transcription gene regulatory mechanism, first identified for its ability to target and degrade mutant transcripts harboring premature termination codons. More recently, a role for NMD in endogenous gene regulation has been identified. Transcriptome analysis of NMD-compromised animals indicates that NMD could directly regulate a significant number of native target genes, several of which have been shown to be bona fide NMD targets. NMD components are required for viability in higher metazoan, and it has been proposed that overexpression of endogenous NMD targets is deleterious in NMD mutants. However, there is little direct evidence to connect regulation of NMD target genes to organismal viability. To identify critical NMD targets and to describe the link between target over-expression and inviability in NMD mutants, we have undertaken a multifaceted approach. First, we have conducted a tissue-specific rescue screen. In these experiments various GAL4 drivers with restricted expression patterns were used to drive expression of the NMD core component, Upf2 in a Upf2 null background. From this screen, we have observed that strong expression in the nervous system is sufficient to rescue the L2 lethal phase of Upf2 null animals into L3. These data indicate that larval development may be dependent on NMD-based degradation of neuronaly expressed target genes. We have also undertaken screen for genomic deficiencies that can suppress the sub-viability of a Upf2(20B) hypomorph.
Nonsense mediated mRNA decay (NMD) is a cellular pathway that selectively degrades RNA containing premature termination codons (PTCs). Six evolutionarily conserved genes are necessary for nonsense mediated decay. Analysis of target features and genes necessary for nonsense mediated decay. We have performed forward genetic screens to identify alleles of these genes, as well as potentially novel NMD factors. Mutants were identified by driving ectodermal expression of FLP and an NMD-sensitive fluorescent reporter in the epidermis of third instar larvae, which allows us to identify NMD genes based on mosaic increase in fluorescence. We have screened to saturation both the X and the right arm of the 3rd chromosome. On the X-chromosome we have isolated several alleles of three known NMD genes; Upf1, Upf2 and Smg1. Preliminary analysis of an allelic series of Upf2 indicates interactions between Upf2 and other NMD proteins may be redundant. On the 3rd chromosome we have isolated the first alleles of Smg6, a known NMD gene, and have shown these alleles are sub-viable and stabilize PTC-containing mRNAs. We have also identified a lethal complementation group that fails to complement known NMD genes. We are currently verifying the mutated gene, which may represent a novel factor involved in NMD.

There are six known, evolutionarily conserved, NMD genes in *Drosophila*. We have performed forward genetic screens to identify alleles of these genes, as well as potentially novel NMD factors. Mutants were identified by driving ectodermal expression of FLP and an NMD-sensitive fluorescent reporter in the epidermis of third instar larvae, which allows us to identify NMD genes based on mosaic increase in fluorescence. We have screened to saturation both the X and the right arm of the 3rd chromosome. On the X-chromosome we have isolated several alleles of three known NMD genes; Upf1, Upf2 and Smg1. Preliminary analysis of an allelic series of Upf2 indicates interactions between Upf2 and other NMD proteins may be redundant. On the 3rd chromosome we have isolated the first alleles of Smg6, a known NMD gene, and have shown these alleles are sub-viable and stabilize PTC-containing mRNAs. We have also identified a lethal complementation group that fails to complement known NMD genes. We are currently verifying the mutated gene, which may represent a novel factor involved in NMD.

Analysis of target features and genes necessary for nonsense mediated decay. Jonathan O Nelson, Mark M Metzstein. Dept Human Genetics, Univ Utah, Salt Lake City, UT.

Nonsense mediated mRNA decay (NMD) is a cellular pathway that selectively degrades RNA containing premature termination codons (PTCs). Six evolutionarily conserved factors, Upf1-3, Smg1, Smg5, and Smg6 are necessary for NMD. However, an exhaustive screen for NMD mutations covering the entire *Drosophila* genome has yet to be completed. We utilize a fluorescent reporter construct with an NMD sensitive 3' untranslated region, which gives enhanced fluorescence in an NMD mutant, to identify mutations in NMD components in vivo. This reporter has previously been used to identify alleles of Smg1, Upf1, and Upf2 on the X chromosome, and alleles of Smg6 on the right arm of chromosome 3. We plan to use this reporter to continue with screens covering chromosome 2 and the left arm of chromosome 3 for novel mutations of both known and previously unidentified NMD factors.

In addition to acting as a quality control mechanism against PTC containing transcripts, NMD targets many native mRNAs. NMD is required during development for regulation of many wild-type genes and for larval viability. Identifying which genes are necessary to be regulated by NMD for viability may provide insight into important developmental and physiological pathways, as well as the role of NMD in these pathways. To identify key NMD targets, we identified deficiencies that when heterozygous reduce lethality observed in NMD mutant animals. In addition, we have used RNA-SEQ data of NMD mutants to identify transcripts that are regulated by NMD. By combining these data we identify a candidate loci, *activity-regulated cytoskeleton associated protein 1 (Arc1)*, as a potential key NMD target required for viability. We are continuing to determine if *Arc1* is indeed a necessary target of NMD, and if so, how NMD may facilitate the biological function of *Arc1*, and how the loss of NMD causes lethality.

Identification of cell type-specific mRNA decay networks in *Drosophila*. Maxine Umeh, Mike Cleary. School of Natural Sciences, University of California, Merced, Merced, CA.

Standard mRNA profiling approaches do not reveal the role of mRNA decay in determining transcript levels and are often performed using mRNA from a heterogeneous population of cells. Regulation of mRNA decay is particularly important in the nervous system, where the unique structure of neurons requires mRNAs to be selectively stabilized in axon terminals, far from their site of synthesis, and the generation of cellular diversity by neural progenitors requires the programmed decay of mRNAs that regulate proliferation or differentiation. We are using “TU-tagging” and related techniques to perform neuron-specific mRNA decay measurements in *Drosophila*. In TU-tagging experiments, cell type-specific expression of the uracil salvage enzyme UPRT is coupled with exposure to 4-thiouracil (TU) which is converted to 4-thioUMP and incorporated into nascent RNAs only in UPRT-expressing cells. TU-tagged mRNAs can then be selectively purified from whole embryos or larvae. We have developed methods in which TU exposure followed by treatment with an excess of uridine and/or exposure to drugs that inhibit transcription allows “pulse-chase” analysis of transcript stability. This approach has been applied to S2 cells, embryos and larvae in experiments aimed at defining global mRNA decay kinetics. We have also found that a related method, labeling of nascent RNAs in all cells (independent of UPRT) with ethylid-uridine, allows in situ visualization of RNA synthesis and global analysis of transcript stability. Our goal is to identify “mRNA decay networks” comprised of the following information: the decay rates of all mRNAs expressed in neural cells, the mRNA targets of trans-acting mRNA decay regulatory proteins, and the cis-elements that target neural mRNAs for coordinate decay.

Identification of a cap-dependent mRNA localization pathway for the early oocyte. Risa Broyer, Elena Monfort-Prieto, James Wilhelm. Section on Cell and Developmental Biology, UC San Diego, La Jolla, CA.

10% of mRNAs are estimated to be localized to the developing oocyte during oogenesis. One proposed pathway for mRNA transport into the oocyte involves the recognition of a stem-loop mRNA localization signal by the Eglitarian/Bicaudal D (Egl/BicD) complex which acts as an adaptor for the microtubule motor dynein. However, the fact that only a small number of transcripts have recognizable RNA elements that can bind the Egl/BicD complex has left open the question of whether all mRNAs use a common mechanism for mRNA localization to the oocyte or if there are multiple independent oocyte localization pathways. In order to address this question, we have screened for mutations that disrupt the localization of *oskar* (*osk*) mRNA to the early oocyte. This screen identified a novel allele of the translational repressor *cup* as having defects in osk mRNA localization to the oocyte. In this mutant, the oocyte is properly determined and the localization of grk mRNA to the oocyte is unaffected indicating that the localization defect is not due to disruption of oogenesis or the microtubule cytoskeleton. We have also found that a number of RNP components that are normally localized to the oocyte fail to be transported in *cup* mutants suggesting that a large class of RNP complexes require *cup* for mRNA localization. Consistent with this result, we have also identified additional mRNAs whose localization to the oocyte is *cup*-dependent. Since grk mRNA possesses a canonical Egl binding site, while *osk* mRNA does not, our studies suggest the existence of multiple mRNA decay pathways into the oocyte. Furthermore, these results help explain a long-standing mystery regarding *cup* function - alleles specifically defective for translational control have the weakest effects on oogenesis. Our data support the model that Cup is both a translational repressor and an mRNA localization factor and that its role in mRNA localization is the more critical function for oogenesis.
The germ plasm is assembled at the posterior of the developing oocyte through the localization of maternal mRNAs and proteins. These germ plasm components are necessary for pole cell formation during embryogenesis and are critical for the development and maintenance of the future germ cells. The earliest step of germ plasm formation is the localization of oskar mRNA to the oocyte posterior during mid-oogenesis, which results in spatially-restricted synthesis of Oskar protein. Posteriorly-localized Oskar protein then nucleates the accumulation of other germ cell components, including Vasa protein and nanos mRNA. Previous studies have concluded that localized germ plasm components are maintained at the posterior cortex via an actin-dependent anchor. Surprisingly, we have observed previously uncharacterized movement of germ plasm components during late oogenesis. By using two photon microscopy to perform high resolution time-lapse imaging of live oocytes, we have visualized directed transport of both nanos mRNA and Vasa protein. Preliminary studies using drugs to depolymerize cytoskeleton filaments support a role for both microtubules and actin in mediating this movement. We are currently quantitating the dynamic behavior of germ plasm components and will further elucidate the mechanism underlying their movement by characterizing their transport in mutants in which dynein, kinesin, or myosin V activity is reduced or eliminated.

The correct transport of oskar mRNA is important in the determination of germ cell fate and posterior polarity in the embryonic development of Drosophila melanogaster. Several biological mechanisms act on oskar mRNA after transcription, including nuclear export, active cytoplasmic transport, translational repression, localization at the posterior of the oocyte via anchoring, translational de-repression and RNA decay. Translation of oskar mRNA is repressed during transport and de-repressed only when posteriorly localized. Its premature translation causes developmental defects implying the importance of translational control prior to localization. Armitage (armi), an RNA interference (RNAi) protein that acts as a trans-acting factor, is involved in oskar’s translation repression during transport. Previous studies have shown that mutations in armi interfere with the correct expression of Oskar protein at the posterior pole. We investigated whether the mutations in armi initially affect the correct spatial and temporal localization of oskar mRNA in vivo, thus giving rise to the misexpression of Oskar protein during oogenesis. The localization of oskar mRNA in a transgenic fly that co-expresses mutated armi and Staufen-GFP, a double-stranded RNA-binding protein important for the proper localization of oskar mRNA to the posterior, was generated, and then visualized in vitro via fluorescence in situ hybridization (FISH) with molecular beacons. Our results indicate that oskar mRNA fails to localize to the posterior pole of the oocyte when mutated armi is being expressed, while Staufen protein is distributed around the oocyte’s cortex, without distinct retention at the posterior. We also observed that the microtubule organization is disrupted, thus giving rise to a central, but not posterior localization of oskar mRNA. This suggests that Armitage and perhaps other proteins belonging to RNAi pathways are involved in mRNA transport and localization processes in the Drosophila oocyte via effects that extend to the microtubule cytoskeleton organization during development.

Trailer hitch is a localization factor for a novel class of RNPs that contain the translational repressor Hubcap. Several studies have identified these complexes in various systems, such as in the early embryo and in Drosophila melanogaster, where they are involved in the control of maternal gene expression. In Drosophila oogenesis, a core complex comprised of the RNA helicase, Me31B, the eIF4E binding protein, Cup, the Y box RNA binding protein, Yps, and the Lsm family RNA binding protein, Trailer Hitch (Tral) is believed to be bound to the majority of maternal messages and regulate their translation and localization. However, there has been no systematic attempt to determine if there are other equivalent complexes in Drosophila that regulate maternal messages. In order to identify additional regulatory complexes for maternal transcripts, we have used a proteomics approach to identify all of the Tral-associated proteins in the early embryo. This approach identified a previously uncharacterized protein, Hubcap, which is a direct binding partner of Tral. Our studies of Hubcap revealed that Hubcap is a translational repressor that acts via binding to eIF4E and that it defines a novel localized ribonucleoprotein (RNP) complex. Furthermore, the localization of Hubcap complexes is completely dependent on tral, while the localization of other complexes is not. Biochemical studies of Hubcap complexes in tral mutant ovaries revealed that the localization defect is not due to a failure to assemble the Hubcap RNP. However, additional studies revealed that Hubcap RNPs fail to oligomerize into large “transport particles” in the absence of tral. These results argue that tral is a localization factor for a specific class of RNP complexes and that it promotes the localization of Hubcap RNPs via oligomerization into transport-competent supramolecular complexes.

RNA interference: Does R2D2 witness Dicing? Tracey A Lincoln, Phillip D Zamore. Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA.

RNA interference (RNAi) is an evolutionarily conserved cellular defense against foreign or parasitic genetic elements. This regulatory system ensures genetic stability, loss of which in the soma, may lead to cancer, and, in the germ line, birth defects. In Drosophila melanogaster, the proteins Dicer-2 and R2D2 are essential for the RNA interference (RNAi) pathway. These proteins form a stable heterodimer in vitro, and without Dicer-2, R2D2 is unstable in vivo. Yet in vitro analysis indicates that Dicer-2 cannot carry out one of its functions—cleaving long dsRNA—in the absence of R2D2. The second role of Dicer-2—loading small interfering RNAs (siRNAs) into Argonaute 2, the catalytic component of the RNA-induced silencing complex (RISC)—requires R2D2. The goal of this study is to identify the amino acids required for binding these two proteins so as to design mutant flies in which I can test whether R2D2 must be bound to Dicer-2 during dicing or, rather, must only be present at subsequent steps in the production of an active RNAi enzyme complex. To this end, I generated a library of 2 x 10^7 Dicer-2 mutants and used a reverse yeast two-hybrid screen to select variants of Dicer-2 that are unable to bind R2D2. Seven hundred mutants of Dicer-2 were identified in this screen. I carried out biochemical tests, which include performing in vitro dicing assays on lysed yeast expressing individual Dicer-2 mutants, to isolate a form of the protein that retains its ability to cleave long double-stranded RNA (dsRNA) into siRNAs, the guides that direct RNAi. I will generate transgenic flies that express this mutant Dicer-2 as well as another already-identified mutant of Dicer-2 that binds R2D2 but is extremely impaired in its ability to cleave long dsRNA, and, through deep sequencing techniques and phenotypic assessments, I will determine whether RNAi can occur in a system where processing dsRNA into siRNAs and loading these siRNAs into functional complexes are decoupled.


In Drosophila, the machinery of small interference RNAs is involved in two levels of arm-race co-evolution of parasites and hosts: 1) defense against invasion of viruses into the host, and 2) defense against transposable elements (TEs) into the genome. A substantial number of endogenously expressed siRNAs are produced from TE s, and they repress activities of TEs in somatic cells. However, little is known about the endogenous siRNAs that are produced outside TE regions. Based on the extensive sequencing results of small RNAs in previous studies, we identified 65 highly conserved endogenous siRNAs. The highly conserved siRNAs on average have more conserved target genes than non-conserved siRNAs, suggesting that natural selection has retained the functional targets of the highly conserved siRNAs. About half the highly conserved siRNAs are located in the aporter locus, and these siRNAs and the protein product of aporter have co-operative effects on the associated targets. We found target genes of the highly conserved, moderately conserved, or non-conserved siRNAs are significantly over-represented in the up-regulated genes, and under-represented in the down-regulated genes in AGO2-depleted S2 cells. We observed strong synergetic effects between the endogenous siRNAs and miRNAs. Finally we discuss the evolutionary patterns of the siRNAs among Drosophila species and
compare it to the evolutionary patterns of miRNAs. Our results indicate the wide-spread repression effects of siRNAs on the transcriptomes. To our knowledge, this is the first time that the regulatory effects of the endogenous siRNAs have been elucidated at such a large scale.

873C

Dumpy, the largest euchromatic gene in Drosophila, encodes a gigantic protein located in the extracellular matrix and has 78 coding exons. Dumpy mutations affect one or more of three different phenotypes: wing shape (oblique or dumpy mutants), tendon cell - cuticle attachment (vortex or dp6 mutants) or viability (lethal or dp9 mutants). We recently sequenced 48 dumpy mutants and all are almost nonsense mutations or lesions that generate downstream nonsense codons, even in those mutants affecting only one or two of the three phenotypes. We hypothesize that such mutations mark alternatively spliced exons while dp69 mutations, which affect all three phenotypes, mark constitutive exons in the gene. To test this hypothesis, we have used both RNA-Seq and RNAi. We developed a modification of RNA-Seq to enrich for dumpy transcripts and have cataloged the exon junctions from several different developmental stages. We have confirmed earlier RT-PCR identifications of alternatively spliced transcripts and found many additional examples of exon skipping, and possibly exon shuffling. Also, with the exception of three anomalous exons, nos. 11, 15, and 16, exons tagged by dp69 mutations show no evidence of being skipped. We have tested many different Gal4 drivers on RNAi’s directed against exons 6, 15, 24, 34, and 68. Drivers expressed ubiquitously, as expected, kill the flies, but at different developmental stages depending on the particular exon being inactivated. Drivers active in the wing disc produce viable adults but with oblique wings. The results, again with the exception of exon 15, are also consistent with our hypothesis that dp69 mutations mark constitutive exons.

874A
Structure/function analysis of PPS: Does PPS contribute to Sex-lethal splicing autorregulation by regulating transcription? Ashley Kendig, Helen Salz. Dept Genetics, Case Western Reserve University, Cleveland, OH.

Alternative splicing of Sex-lethal (Sxl) is important for both sex determination and dosage compensation. Although transcription occurs in both sexes, protein expression is limited to females because the inclusion of the translation-terminating 3rd exon is blocked. The mechanism leading to male exon skipping is autoregulatory, and accomplished by a mechanism in which the SXL protein interacts with core spliceosomal proteins, including the U1 snRNP protein Sams-fille (SNF), to antagonize exon inclusion. In studies begun by screening for proteins that interact with SNF, we identified PPS (CG6525) as a novel component of the machinery required for Sxl male exon skipping. The results of our ChIP analysis shows that, while SXL and SNF are recruited co-transcriptionally to their predicted binding sites, PPS has a distinct pattern of accumulation along the Sxl gene, including occupancy at the SdPm promoter region. Furthermore, PPS contains 4 signature motifs which are more suggestive of a function in transcription elongation and chromatin modification, than in splicing. Thus, PPS may act in concert with the transcription machinery to promote male exon skipping. On the other hand, it is possible that PPS has multiple independent functions. To distinguish between these scenarios, we are carrying out a comprehensive structure/function analysis with a new collection of EMS-induced alleles. This collection now includes 12 mutations identified through a standard non-complementation screen, and an additional 11 EMS-induced mutations identified by the Seattle Fly-TILL reverse genetic service. Through these studies, which are still ongoing, we have yet to identify any separation of function alleles, strengthening the possibility that PPS serves to link transcription to regulated splicing.

875B

The Drosophila rnp-4f gene encodes a splicing assembly factor that dimerizes U4- and U6-snRNPs during splicesosome formation. 5'-UTR pre-mRNA intron processing results in two major isoform classes, “long” (unspliced) and “short” (alternatively spliced). The long isoform has a secondary structure in which an intron pair with adjacent highly evolutionarily conserved exons 2 to form a stable 177-nt stem-loop. The stem-loop structure is also evolutionarily conserved. DIG hybridization has shown that both the long isoform and dADAR, a class of editase enzymes which catalyze deamination of adenosine to inosine and which utilize double-stranded RNA as substrate, are located in the developing CNS. It is known that rnp-4f long isoform mRNA levels diminish by 30% in a dADAR null mutant, showing that dADAR may be a component of a trans-acting splicing silencer. RNA electrophoretic mobility shift assay (REMSA) was carried out to see if any embryo extract proteins bind specifically to the conserved exon 2 sequence or the shape of the rnp-4f 5'-UTR stem-loop which plays a role in binding of proteins, a combined REMSA and mutational analysis was carried out. The results show that the band shift in the conformation mutant is similar to that in wild-type, while the sequence mutant has significantly reduced band shift, suggesting that the conserved 5'-UTR exon 2 sequence also plays an important role in binding of proteins. These results provide a basis for understanding molecular mechanisms behind expression control of the Drosophila splicing assembly factor rnp-4f gene, with special reference to the role of dADAR, during intron splicing regulation.

876C
The Control of Lipid Metabolism by Splicing in Drosophila. Alexis A. Nagengast1,3, Nicole Chickears2, Thomas Carr2, Timothy Rudolph2, Justin DiAngelo3. 1) Dept Biochemistry, Widener Univ, Chester, PA; 2) Dept Biology, Widener Univ, Chester, PA; 3) Dept Chemistry, Widener Univ, Chester, PA; 4) Dept Biology, Hofstra Univ, Hempstead, NY.

The fat body of Drosophila controls overall energy metabolism by regulating long-term storage of triglycerides in structures called lipid droplets and therefore serves a function similar to the liver and adipose tissues in mammals. Recent genome-wide RNAi screens in Drosophila tissue culture cells have identified mRNA splicing factors such as the Serine-Arginine (SR) domain containing proteins B52 and U2AF-50 as playing a role in lipid droplet formation; these decreased expression results in the production of fewer lipid droplets. Using conditional RNAi knock down experiments with GALL-UAS effector in the fat body of larvae, we have identified several early splicing factors that control lipid storage in vivo. We have observed a visibly lean phenotype with decreased expression of U1-70K, U2AF-50, Snp19 and the SR proteins S9G and SRp54 in the fat body. The lean phenotype corresponds to a significant decrease in triglyceride levels as measured by quantitative colorimetric assays. To further understand this defect in lipid storage, we are taking a candidate gene approach to identify potentially alternatively spliced genes important for lipid metabolism. Through these experiments we hope to gain insight into the mechanisms underlying tissue-specific splicing in the fat body and how the alternative splicing of important lipid metabolic genes leads to proper fat storage.
The Half-Pint protein plays a direct role in the regulation of alternative splicing and acts as a repressor in combination with Transformer-2. SHANZHI WANG¹, SHIHUANG SU¹, ERIC WAGNER², WILLIAM MATTOX¹. ¹) GENETICS, UT MD ANDERSON CANCER CENTER, HOUSTON, TX; ²) BIOCHEMISTRY AND MOLECULAR BIOLOGY, UT HEALTH SCIENCE CENTER HOUSTON, TX.

Half-pint (Hfp) is a bifunctional protein that acts in both transcription and splicing. Both Hfp and its mammalian homologue, PUF60, are known to regulate c-myc transcription and are involved in selection of alternative splice sites in other RNAs. In Drosophila, hfp mutants display reduced numbers of programmed cell division in the germline resulting in mature 8 cell cysts. Previous studies suggested that Hfp affects cell division in the ovary by disrupting the ovarian tumor gene and that it also has effects on splicing of other mRNAs, however it is unclear if these effects are direct and the mechanism by which Hfp affects alternative splicing is unknown. In an RNAi screen for factors influencing the regulation of splicing by the sex determination factor Transformer 2 (Tra2) we identified Hfp as a required co-repressor. Hfp mutants fail to carry out germline specific repression of M1 intron splicing in Tra2 RNA, a target of the Tra2 protein. However, loss of Hfp did not affect the activation of Tra2-dependent doublesex splicing, suggesting that Hfp plays a transcript-specific role in Tra2 dependent splicing repression. This contrasts with previous studies on the PUF60 orthologue that indicate its role is to promote splice site recognition. In further studies we have determined that Hfp directly associates with the target RNA and that repression depends on specific sequences in the M1 intron that also are known to interact with the Tra2. Taken together our studies suggest that Hfp directly participates in the regulation of pre-mRNA splicing in the germline and that it is part of Tra2-dependent negative regulatory complex that is distinct from the splicing enhancer complexes responsible for activation of sex-specific splice sites in dsx and fru pre-mRNA.
The correct maintenance and differentiation of germ line stem cells (GSCs) is essential for fertility and fecundity. Numerous studies have begun to unravel the extrinsic and intrinsic pathways that regulate the balance between GSC maintenance and differentiation in female and male GSC niches. We have identified a new gene involved in regulating the differentiation of female germ line stem cells, CG10990, an ortholog of Programmed Cell Death 4 (Pdcd4) and referred to as dPdcd4, is expressed in germ line stem cells and early differentiating daughter cells. Functional analyses in females demonstrate that dPdcd4 functions to promote the differentiation of germ line stem cells. We found an enhancing genetic interaction between dPdcd4 and bag-of-marbles (bam), the key regulator of differentiation in female GSCs, indicating these genes have related functions in the female germ line. Additionally, mammalian Pdcd4 is known to bind to and inhibit eIF4A, which has recently been reported to promote GSC maintenance in Drosophila females by antagonizing BAM. We found a significant suppressive genetic interaction between dPdcd4 and eIF4A, supporting the inference that dPdcd4 functions in a genetic pathway with eIF4A and bam. We propose a model where dPdcd4 functions in cytoblasts to relieve the inhibition of BAM by eIF4A, and thereby promote differentiation.

SEX-LETHAL downregulates nanos in the female germline to promote differentiation. Johnnie Chau, Helen K. Salz. Dept Gen, Case Western Reserve Univ, Cleveland, OH. SEX-LETHAL (SXL) is a female-specific RNA binding protein that controls somatic sex determination and dosage compensation by alternative splicing and translation inhibition of its target genes. SXL also functions in the female germline where it facilitates the transition of a germline stem cell (GSC) to a committed daughter cell. In the female germ line that lacks SXL protein, germ cells fail to differentiate. The undifferentiated germ cells continue to proliferate forming a germline tumor. Here, we provide evidence that SXL promotes differentiation by directly downregulating nanos (nos), a GSC self renewal factor. We show that NOS protein expression is sex-specifically regulated. In female germ cells, NOS protein expression is detected in GSCs and severely reduced or absent in the differentiating daughter cells that specifically express Bag-OF-Marbles (BAM). In contrast to the female germ line, NOS protein remains high in both the GSCs and in the differentiating BAM expressing cells of the male germ line. Three lines of evidence suggest that SXL is necessary for this sex-specific expression pattern. First, there is an inverse correlation between SXL and NOS expression in the absence of SXL, where SXL-deficient germ cells express both NOS and BAM protein. Lastly, we show that the SXL protein associates with nos mRNA which has multiple SXL binding sites in ovarian extracts. Experiments are underway to address how SXL downregulates NOS expression.

Stat primarily regulates adhesion to the niche in male germine stem cells, and not the undifferentiated state. Judith L. Leatherman1, Steve DiNardo2. 1) School of Biological Sciences, University of Northern Colorado, Greeley, CO; 2) Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA.

Adult stem cells are maintained by niche signals, and the Drosophila testis is a premier model system to study these stem cell-niche interactions in vivo. This niche is comprised of a group of cells called the hub, around which cluster two stem cell populations: the germline stem cell (GSC) and somatic cyst stem cells (CySCs). STAT activation has been proposed as the primary pathway for self-renewal of both stem cell populations (Tulina and Matunis, 2001; Kiger et al., 2001). To sort out the contribution of STAT activation to each stem cell type, we selectively depleted stat only in the germline. As expected, the CySC population was maintained since it retained functional stat. Surprisingly, the presence of the CySC population non-autonomously prevented loss of germline cells. These stat-depleted germline cells continued to support spermatogenesis, and GSC-like cells were maintained indefinitely, producing descendants that populated all the normal stages of germ cell differentiation. Thus, germline Stat depletion does not affect self-renewal of the germline. Interestingly, the stat-depleted GSC-like cells lost contact with the hub, and clustered instead next to CySCs, which now surrounded the hub. This suggests that germline STAT activation normally promotes GSC attachment to hub cells, thereby anchoring germline cells to the niche. Thus, in previous analyses, we suspect that stat mutant GSC clones were “lost” primarily due to loss of adhesion to the hub, and not principally because they lost stem cell character. Finally, to identify the nature of the CySC signal that supports GSC renewal, we examined the BMP pathway, previously demonstrated to be required for GSC maintenance (Shivdasani et al., 2003; Kawase et al., 2004). We found that CySCs cause neighboring germ cells to strongly activate the BMP signaling pathway, suggesting that, like the Drosophila ovary, BMP signaling may be the primary pathway leading to male GSC renewal.

Environmental and Genetic Control of Germline Stem Cell Diversions. Benjamin B. Parrott, Alicia Hudson, Regina Brady, Cordula Schulz. Dept Cell Biol, Univ Georgia, Athens, GA.

To realize the full therapeutic potential of stem cells, we must uncover the fundamental properties that govern their behavior in normal tissues as well as in those tissues affected by disease. In contrast to regulators of cell fate dynamics in tissues maintained by adult stem cells, we know relatively little regarding how organisms control the number of terminally differentiated cells they produce. Here we report that, in the Drosophila testes, Germline Stem Cells divide at significantly higher frequencies than their transit amplifying daughters. In addition, we show the frequency of Germline Stem Cell divisions depends on the levels of sexual activity that an animal experiences. These findings suggest that stem cell divisions are differentially regulated than their daughters, and that stem cells themselves respond to the demand for terminally differentiated cells by modulating their division frequency.

Furthermore, Germline Stem Cell division frequency depends on genetic factors. In previous work we showed that EGF signaling in Drosophila testes is required for germ cells to differentiate and adopt later stage cell fates. Mutations in the EGF locus, spitz, result in the accumulation of early-stage, undifferentiated germ cells resembling a germ cell tumor. We now show that Germline Stem Cells in animals with attenuated EGF signaling display a two-fold increase in their division frequency, suggesting a mechanism by which stem cells contribute to tumorigenesis. In addition, we show that two novel genetic suppressors of the spitz phenotype, seven-up and homothorax, specifically suppress the cell fate component of the phenotype without suppressing the hyper-proliferation of GSCs. These data provide evidence that EGF functions in two genetically distinct pathways to regulate cell fates and GSC divisions. Thus, this work provides a fundamental link between stem cell biology and the role they may play in various cancers.

Comeback, a putative AAA-ATPase, and regulator of germline stem cells? Yue Qian, Ye Wang, Cordula Schulz. Cellular Biology, University of Georgia, Athens, GA.

The stem cell fate decision, determined by intrinsic cues and external signals coming from the cellular microenvironment, plays an important role in metazoan development. Our recent study of Drosophila germline stem cells (GSCs) has discovered that female flies bearing a mutation in a novel gene, comeback (coba), are viable but defective in oogenesis, and usually cannot generate an egg. DNA sequencing of coba indicates a stop codon in a predicted AAA-ATPase. Previous studies implicated the AAA-ATPase family of proteins in multiple cellular activities, including regulation of spindle disassembly, protein folding, and vacuole transport. Fluorescent immunostaining of the control and coba mutant ovaries revealed the accumulation of germline cells at the anterior tip of the ovaries in a structure called germarium. Based on the expression of markers, the accumulating cells appear to be GSCs and/or their immediate daughters. This infers that coba is likely to regulate the decision between stem cell fate and differentiation, and/or may be essential for stem cell daughters to initiate differentiation. We are interested in understanding the mechanism through which coba regulates the development of Drosophila GSCs.
POSTER: Stem Cells

883A
Asymmetric cytokinesis and midbody inheritance during Drosophila germline stem cell division. Viktoria Salzmann, Amita Tiaboonchai, Yukiko M. Yamashita. Life Sciences Institute, University of Michigan, Ann Arbor, MI.

The balance between stem cell self-renewal and differentiation is critical to maintain tissue homeostasis. Tissue homeostasis is achieved by asymmetric cell division, which is used in many stem cells. Asymmetric stem cell division is achieved by asymmetric segregation of intrinsic fate determinants and/or placement of daughter cells in different microenvironments. In Drosophila tests, germline stem cells (GSCs) normally divide asymmetrically, giving rise to one self-renewing cell and one differentiating cell, called gonialblast (GB). This is accomplished by stereotypical positioning of the centrosome, which sets up the mitotic spindle perpendicular to the hub, the major component of the stem cell niche. We have shown that the mother centrosome is always positioned close to the hub, while the daughter migrates toward the opposite side. It remains unknown whether the mother centrosome and/or daughter centrosome harbor any fate determining factors that contribute to the asymmetric outcome of the GSC division. It is known that cytokinesis is asymmetric even in apparently symmetrically dividing cells and that only one daughter of the division inherits the midbody ring (MR) upon completion of abscission. Here we report that cytokinesis of Drosophila male and female GSCs is asymmetric in that the MR is inherited asymmetrically. In male GSC division the MR is always inherited by the GB. Our studies have shown that the MR is also inherited in female GSCs, the MR is not lethal in males. We have found that the MR is enriched in the area next to the testis tip. Flies mutant for CG2264 exhibited a reduced number of GSCs, yet maintained a normal population of somatic stem cells and hub cells. This specific defect on GSCs can be rescued using the Gal4-UAS system to restore CG2264 expression in mutant testes. Testes of CG2264 mutant flies exhibited reduced staining of pMad, the signal transducer in BMP pathway, whereas activation of the JAK-STAT pathway appeared to be similar comparing CG2264 mutants with controls. The number of GSCs can also be restored when an activated form of the BMP receptor Tkv is expressed in the germline. Current experiments are focusing on dissecting the mechanism by which CG2264 controls the testis niche signal.

884B
CG2264 (pentagone), a novel BMP modulator controlling male germline stem cell maintenance. Qi Zheng1, Yiwen Wang2, Eric Vargas2, Stephen Dinardo2. 1) Department of Biology, School of Arts and Sciences, University of Pennsylvania; 2) Department of Cell and Developmental Biology, School of Medicine, University of Pennsylvania.

It is known that both BMPs and STAT are necessary for the maintenance of germline stem cells (GSCs) in the testes [Kiger et al. 2001, Tulina & Matunis 2001, Shivdasani & Ingham 2003, Kawase et al. 2003, Schulz et al. 2004]. However, our recent work strongly suggests that BMP signaling is the primary pathway leading to GSC self-renewal [Leatherman & Dinardo 2010]. Here we report that CG2264 is a novel BMP modulator controlling male germline stem cell maintenance. Using in situ hybridization and a lacZ reporter line [Vuilleumier et al. 2001], we found that CG2264 was specifically expressed in hub cells, the niche cells in the testes. Antibodies against different epitopes revealed that CG2264 protein was enriched in the area near the testis tip. Flies mutant for CG2264 exhibited a reduced number of GSCs, yet maintained a normal population of somatic stem cells and hub cells. This specific defect on GSCs can be rescued using the Gal4-UAS system to restore CG2264 expression in mutant testes. Testes of CG2264 mutant flies exhibited reduced staining of pMad, the signal transducer in BMP pathway, whereas activation of the JAK-STAT pathway appeared to be similar comparing CG2264 mutants with controls. The number of GSCs can also be restored when an activated form of the BMP receptor Tkv is expressed in the germline. Current experiments are focusing on dissecting the mechanism by which CG2264 controls the testis niche signal.

885C
A novel role for Fizzy/Cdc20 in promoting neural stem cell survival in Drosophila. Chaoyuan Kuang, Cheng-Yu Lee. Life Sciences Institute, University of Michigan, Ann Arbor, MI.

Stem cells generate all differentiated cell types during development and continuously replenish lost cells to aging and injury throughout the lifetime of an organism. Thus, the growth and maintenance of the stem cell population requires proper cell proliferation and cell survival. Drosophila larval brain neuroblasts become reactivated for proliferation following hatching and undergo repetitive asymmetric divisions to generate and to produce differentiated neurons throughout the larval life. We isolated the fzy mutation that leads to amino acid substitution in the WD40 repeats of Fzy and specifically results in premature loss of neural stem cells (neuroblasts) in fly larval brains without affecting morphogenesis of other larval tissues. In contrast, larvae carrying a null mutation of fzy or lacking the core components of the anaphase-promoting complex (APC) show systemic impairment of cell cycle progression. Importantly, a transgene carrying a genomic fragment containing the fzy locus restores neuroblasts in fzy mutant larval brains, suggesting that the fzy mutation likely perturbs a novel function of Fzy in promoting neuroblast survival. Consistently, neuroblasts in fzy mutant larval brains exit from quiescence, but rapidly become lost without a detectable activated Caspase 3 activity. Our preliminary study shows that an increase in the expression of autophagic cell death markers correlates with the loss of neuroblast phenotype in fzy mutant brains. A strong evolutionary conservation of the Cdc20 family protein suggests that Fzy might also promote survival of neural stem cells in higher organisms.

886A
Hippo signaling regulates Drosophila intestine stem cell proliferation through multiple pathways. Fangfang Ren1, Bing Wang1, Tao Yue1, Eun-Young Yun2, Y Tony Ip3, Jin Jiang4. 1) University of Texas Southwestern Medical Center, Dallas, TX; 2) University of Massachusetts Medical School Worcester, MA 01605; 3) National Academy of Agricultural Science, Rural Development Administration, Suwon 441-100, Korea.

Adult stem cells play critical roles in maintaining tissue homeostasis and replenishing lost cells in response to tissue damage. The Drosophila adult midgut contains intestinal stem cells (ISCs) and two types of differentiated epithelial cells, enterocytes (ECs) and enteroendocrine cells (EEs). The proliferation of ISC is under tight control by several conserved signaling pathways including the Notch, Wingless (Wg)/Wnt and JAK/STAT pathways. We demonstrate that the Hippo (Hpo) signaling pathway, an evolutionarily conserved signaling pathways in ISCs to stimulate ISCs proliferation. Therefore, we have revealed a novel non cell-autonomous role of Hpo signaling in restricting ISC proliferation. In addition, we found that Yki is required in precursor cells for injury-induced ISC proliferation in response to the tissue damaging reagent DSS but is dispensable for damaged-induced ISC proliferation in response to bleomycin or infection by Pseudomonas entomophila (PE). These results suggest that DSS stimulates ISC proliferation through a cell-autonomous role of Yki in the progenitors whereas bleomycin and PE can stimulate ISC proliferation through an Yki-independent mechanism.

887B
Loss of Heterochromatin Protein 1a causes chaos in the germline. Michael W. Vitalini, Lori L. Walrath. Biochemistry, University of Iowa, Iowa City, IA.

Complex interactions between stem cells and their surrounding microenvironment, termed the niche, promote stem cell self-renewal. In the Drosophila testis, the niche contains ‘hub’ cells that secrete the JAK-STAT activating ligand Unpaired. Activation of the JAK-STAT pathway, in both germline stem cells (GSCs) and somatic cyst stem cells (CySCs) adjacent to the hub, promotes adherence to the hub and maintains proper spatial organization of the niche. Our studies identified Heterochromatin Protein 1a (HP1a) as necessary for maintaining this spatial organization. In Drosophila, HP1a is essential for the essential gene Su(var)2-5; transheterozygous-null animals die at the third larval instar. We sought to define the functions of HP1a that are essential for viability through genetic complementation experiments using different mutant forms of HP1a. Transgenes encoding each mutant form of HP1a under control of a heat shock promoter were introduced into transheterozygous-null flies that underwent a daily heat shock regimen. A transgene encoding wild-type HP1a (wHP1a) or a transgene encoding HP1a lacking the hinging region was each capable of rescuing to adulthood. All rescued flies were completely sterile, regardless of sex or the transgene present. Dissections revealed that the rescued flies contained deformed gonads. Immunostaining of the gonads from sibling flies containing both an endogenous wild type copy of Su(var)2-5 and a transgene encoding wHP1a, showed that the endogenous gene was expressed throughout the gonads; however, heat-shock induced
expression of the transgene was lost as differentiation progressed from stem cells to mature gametocytes. Staining the testes of rescued male flies for cell-type specific markers demonstrated the presence of GSCs, CySCs and hub cells; however, the spatial organization of these cells was lost, suggesting improper adhesion among the various cell types. Our data demonstrate a requirement for HP1α in the Drosophila germline. We hypothesize that HP1α functions in STAT-regulated gene expression to control proper adhesion within the male gonad.

888C
Orthogonal functions of BAB1 and BAB2 proteins in determining germ stem cell niches in the ovary of D. melanogaster. Mathieu Bartelletti1, Thomas Robin1, Fabienne Chalvet2,3, Nicolas Dos Santos2,3, Emilie Poisot2,3, Delphine Cumenal1, Jacqueline Leroy1, Frédérique Peronnet1, Laurent Théodore2,2, 1) Centre de Génétique Moléculaire - UPR 2167, GIF SUR YVETTE, France; 2) Université Paris-Sud 11, 91405 Orsay France; 3) LGBC, Université Versailles St-Quentin 78035 Versailles, France; 4) UMR7622 - Biologie du Développement CNRS - UPMC Bâtiment C - 7ème étage - case 24 9, quai Saint-Bernard 75005 - Paris, France.

The D. melanogaster ovary is a compound organ made of ca. 20 ovarioles. Morphogenesis of ovarioles starts in larvae after most of the cell proliferation has ended, with the formation of a dense layer of cells, stacks of 8-9 disc-shaped cells that constitute the distal tip of the female germarium. These stacks of cells, known as the TEs, are part of the organizers of the germ cell niches. The only known nuclear factors involved in terminal filament cell specification are BAB1 and BAB2, products of the bric-a-brac locus (Godt and Laski, 1995). bab mutants display rudimentary ovarioles (Coudere et al, 2002). However, in bab simple or double heterozygotes, ovariole number may increase up to 30 ovarioles, with an average of 25 per ovary. Using targeted silencing of bab1 and bab2 in specific territories in the larval ovary, we showed that bab1, which is specifically expressed in TEs, is dispensable for their presence, and that bab2 is required upstream of bab1. However, bab2 is not required in TEs once bab1 is expressed. Finally, silencing of bab1 but not that of bab2 in TEs leads to an increase in ovariole number. We also showed that silencing of ban/lolal whose product interacts with BAB1 but not with BAB2 leads to a similar increase in ovariole number, also found in ban heterozygotes. Two known partners of ban, i.e. Trl and psq, are also required to limit ovariole number. Our results indicate that in the counting of the niches in the drosophila ovary, bab1 and bab2 play separable factors, and that in this process bab1 is part of a regulatory network of nuclear BTB/POZ proteins.

889A
Genetic ablation of the somatic hub cells in the drosophila testis. Phylis Hiete. Cell Biology, Johns Hopkins Univ, Baltimore, MD.

The drosophila testis is a model system widely used for genetic studies because of its simple architecture and easy malleability. Three cell populations are found in the testis apex: germline stem cells (GSCs), somatic stem cells called cyst stem cells (CySCs), and hub cells. The hub produces the ligand unpaired (Upd) that activates the Jak/Stat pathway in both CySCs and GSCs and maintains them in the niche. Since hub cells are post-mitotic, and given their importance in the testis, we wanted to determine if the tissue has a mechanism to replace lost or damaged hub cells. The work of Voog et al., 2008 suggests that CySCs can become hub cells and can serve as a source of hub replenishment in the testis under normal conditions. In our study, we used hub-specific drivers and cell death-inducing transgenes to ablate hub cells in the adult testis. Our results show that at two days after the beginning of hub cell ablation we observe a significant decrease in the number of hub cells. However, the testes maintain their GSCs and CySCs. In order to determine if ablated hub cells can be replaced, we stopped hub cell death induction and allowed the testes to recover. After 15 days of recovery, to our surprise hub cell number did not increase but rather slightly decreased. All recovered testes still had CySCs and GSCs along with germ cell cysts. We are unsure of the reason why the hub cell number does not return to wild type. We are currently doing further experiments to characterize the state of the CySCs and GSCs in these testes.

890B
Insulin signals control the competence of the Drosophila female germline stem cell niche to respond to Notch ligands. Hwei-Jan Hsu1,2, Daniela Drummond-Barbosa2. 1) Inst. of Cellular and Organismic Biology, Taipei, Taiwan, Taiwan; 2) Department of Biochemistry and Molecular Biology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA.

Stem cells reside in niches, a population of specialized cells, that provide both physical contact and diffusible factors to control stem cell self-renewal and proliferation; however, little is known about niche regulation itself. We previously showed that insulin signals control niche size and germline stem cell (GSC)-niche interaction in Drosophila females. Insulin signals modulate Notch signaling to maintain cap cells in the niche, and control cap cell-GSC attachment, likely via E-cadherin. Here, we further dissect the molecular mechanisms underlying these processes, and reveal that Notch ligands produced within the niche stimulate Notch in cap cells, and insulin signals act via phosphoinositide 3-kinase and FOXO to control the competence of cap cells to respond to Notch ligands to regulate niche size. Insulin signals, however, control cap cell-GSC attachment independently of Notch. These results are potentially relevant to many systems in which Notch signaling modulates stem cells, and demonstrate that complex interactions between local and systemic signals are required for proper stem cell niche function.

891C
Apoptic restricts the stem cell population in the testis by inhibiting the JAK/STAT signaling pathway. Michelle A. Starz-Gaiano, Archana Murali. Biological Sciences, University of Maryland Baltimore County, Baltimore, MD.

How stem cells are maintained within a “niche” or microenvironment is a central question to understanding homeostasis or repair of adult tissues. The Drosophila testis provides an exemplary context for examining the genetic and molecular signals that balance stem cell self-renewal and differentiation. In the testis, germ line stem cells (GSCs) and somatic cyst stem cells (CySCs) are sustained by their association with niche cells, called the hub. Several laboratories have shown that activation of the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway is necessary for maintenance of types of stem cells. We have found that a required feedback inhibitor of STAT signaling in ovaries, called Apoptic (APT), is also highly expressed in the somatic cells of the testis. When apt is overexpressed in the soma, there are fewer CySCs, while overexpression in the germ line has no effect. In apt loss of function mutants, we observe more Zfh-1-positive CySCs, and an expanded domain of GSCs. Thus, the additional CySCs in apt mutants may permit GSCs self-renewal by acting as a secondary niche or otherwise altering the architecture of the microenvironment. The apt phenotype is distinct from those due to mutations in other STAT targets or regulators, such as smc3/csk. In ovaries, apt mutant cells display altered adhesion and morphological properties, and parallel changes may explain the delayed CySCs differentiation in the testis. We propose that APT acts cell-autonomously as a transcriptional regulator to restrict STAT signaling in the niche and maintain CySCs, and non-autonomously to organize the GSCs within the distal tip of the testis. This suggests a complex interplay between stem cells and their niche as well as between two different types of stem cells to maintain the appropriate number of each.

892A
Molecular and genetic studies on the posterior signaling center of the Drosophila lymph gland: factors controlling specification and maintenance of the hematopoietic progenitor niche. Jessica Renee Stoller-Conrad, Tsuyoshi Tokusumi, Yumiko Tokusumi, Robert A. Schulz. Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA.

The Drosophila hematopoietic organ, the larval lymph gland, is being used as an effective model for the genetic study of hematopoiesis. The lymph gland is made up of three distinct zones: the cortical zone (CZ), the medullary zone (MZ), and the posterior signaling center (PSC). The CZ is a region made up of three differentiated hematocyte types: plasmatocytes, crystal cells, and rarely, lamellocytes. The MZ is composed of prohemocytes, which have yet to differentiate into one of these three cell fates; the PSC is the hematopoietic progenitor niche which maintains the prohemocyte population of the MZ. The Hedgehog (Hh) signaling molecule is known to function in many diverse developmental processes, and is also known to be expressed in the PSC. In addition, hh deficient lymph glands lack prohemocyte maintenance. To study hh gene expression in the PSC, we characterized the hh enhancer region responsible for hh gene expression in niche cells. Our results have shown that the GATA factor Serpent (Srp) is necessary for hh expression in PSC cells, while Suppressor of Hairless (Su(H)) and U-shaped (Usb) transcriptional regulation prevent hh expression in the MZ and CZ. Together, these results
POSTER: Stem Cells
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

demonstrate that Srp, Su(H), and Ush work together in the regulation of hh gene expression and niche cell function in the Drosophila PSC. Current research in our lab employs the use of the newly created hhP/GFP cell-specific marker in several genetic screens to identify novel genes involved in niche maintenance and PSC specification.

893B
A novel side-by-side fused egg chamber phenotype in pak mutant ovarioles indicates a role for Pak in cyst encapsulation. Stephanie Vlachos1, Ryan Conder2, Todd Nystul3, Nicholas Harden1. 1) Department of Molecular Biology & Biochemistry, Simon Fraser University, 8888 University Drive, Burnaby, BC, V5A 1S6 Canada; 2) Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Dr. Bohr Gasse 3, A-1030 Vienna, Austria; 3) University of California, San Francisco, 513 Parnassus Ave, San Francisco, CA 94143.

Females transheterozygous for alleles of pak, a serine/threonine kinase that is an important component of signaling by the small GTPases Rac and Cdc42, are female sterile, with defects in actin cytoskeletal organization and apicobasal polarity in the follicle cells covering egg chambers. About 40% of pak mutant ovarioles exhibit an additional, novel phenotype of side-by-side fused, age-matched egg chambers. To our knowledge this striking “siamese twin” ovariole phenotype, with each egg chamber within the fused pair entirely surrounded by its own monolayer of follicle cells, has not been previously reported. We used clonal analysis and tissue-specific expression of a Pak RNAi transgene to determine in which cells loss of Pak led to the side-by-side egg chamber phenotype. Using various Gal4 lines specific for the somatic cells of the germarium to drive Pak RNAi, we have been able to reproduce the pak fusion phenotype suggesting a role for Pak in the gerarium during cyst encapsulation and positioning of the follicle stem cell niche.

894C
Live imaging cell migration within the Drosophila gonad. Lindsey Wingert1, Stephen DiNardo2. 1) Cell and Molecular Biology, University of Pennsylvania, Philadelphia, PA; 2) Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.

The Drosophila testis is an excellent system for studying stem cell-niche interactions. The hub (nische) cells residing at the apical tip of the testis provide germline stem cells (GSCs) and cyst stem cells (CSCs) with signals promoting self-renewal and attachment, thus allowing them to maintain production of sperm for the lifetime of the animal. Much has been discovered about the key signaling pathways involved in steady state maintenance. However, very little is known about how the architecture of the adult germline stem cell niche is achieved. Recent studies have shown that hub cells are specified among a pool of somatic gonadal precursors (SGPs) due to Notch signaling (Kitade and Kobayashi, 2010, Proc Natl Acad Sci USA. 107(32):14241-6 and Okegbe and DiNardo, submitted). We are interested in the process by which these specified hub cells arrive in an aggregate at the anterior pole of the gonad during late embryogenesis(Le Bras and Van Doren, 2006, Dev. Biol. 294:92-103). We suspect this occurs by selective adhesion to other hub cells along with a signal driving their attachment to the anterior tip where they will remain throughout testis elongation and morphogenesis (Tarentzapf et al., 2007, Nat Cell Biol 9:1413-1418). To investigate these processes, we will image the movement of hub cells within a coalesced gonad as they sort out from other SGPs and migrate to their final position. There are various drivers and reporters we can utilize and it is likely these cells will exhibit morphological differences distinguishing them from other cell types within the gonad. Elucidating the mechanisms by which the stem cell niche is established will be fundamental for advancing stem cell research.

895A
Characterization of Midgut Stem Cell- and Enteroblast-Specific Gal4 Lines in Drosophila. Xiankun Zeng, Chhavi Chauhan, Steven Hou. Mouse Cancer Gen Program, NCI Frederick, Frederick, MD.

The homeostasis of Drosophila midgut is maintained by multipotent intestinal stem cells (ISCs), each of which gives rise to a new ISC and an immature daughter cell, enteroblast (EB). Considering its high cell turnover rate, the midgut has been a fertile ground for investigating the ISC daughter. In the Gal4-UAS system, once a tissue-specific transgene is expressed in the Drosophila midgut, there are no ISC- or EB-specific Gal4 lines available. Here we report the generation and characterization of DI-Gal4 and Sex(H)GBE-Gal4 lines, which are expressed specifically in the ISCS and EBs respectively. Additionally, we demonstrate that DI-Gal4 and Sex(H)GBE-Gal4 are expressed in adult midgut progenitors (AMPs) and niche peripheral cells (PCs) separately in larval midgut. These two Gal4 lines will serve as invaluable tools for navigating ISC behaviors.

896B
High-threshold Notch signaling promotes commitment of stem cell daughters in the adult midgut. Allison J. Bardin1, Carolina Perdigoto2, François Schweisguth2. 1) Department of Developmental Biology and Cancer, Institut Curie, Paris, France CNRS URA3215 Inserm U934; 2) Department of Developmental Biology, Institut Pasteur, Paris, France CNRS URA2578.

In adult tissues, stem cell pools are maintained by a balance of self-renewal and differentiation choices. The misregulation of such choices can lead to the depletion of stem cell pools or hyperplasia. In the Drosophila intestine, Notch signaling mediates differentiation choices of the intestinal stem cell (ISC) daughters controlling enteroendocrine and enterocyte fates. How Notch signalling output is translated into these decisions is unclear. Our results indicate that in addition to controlling terminal differentiation, Notch signaling acts on the commitment step of the ISC daughter. In a genetic screen, we identified the gene GDP-mannose 4,6-dehydratase (Gmd) as a specific regulator of the commitment decision but dispensable for terminal differentiation choices. Previous work has implicated Gmd as a modulator of fringe-dependent Notch signaling. However, our data indicate that in the intestine Gmd acts in a novel, fringe-independent manner to limit stem cell number likely by promoting high-level Notch signaling. Using additional genetic contexts to modulate Notch signaling levels, we find that daughter cell decision to properly commit to differentiation requires a high-level Notch signalling, whereas terminal differentiation of a committed daughter cell can occur with lower-level signalling. A requirement of stem cells to undergo high-threshold signalling events in order to differentiate may represent a general mechanism by which stem cell pools are protected from loss through differentiation.

897C

Tissue homeostasis needs a tightly regulated balance between cell death and proliferation. In the Drosophila intestine and assessed changes in the stem cell population by medium-throughput imaging. First, we have analyzed whether Evi/Wh and the secretion of Wg is required for ISC proliferation. We will present results on the evaluation of novel components required for ISC maintenance and proliferation.

319
The role of the JAK/STAT pathway in ovarian follicle stem cell self-renewal. Natana Field, Cynthia Vied, Daniel Kalderon. 1) Department of Biological Sciences, Columbia University, New York, NY; 2) Department of Biomedical Sciences, Florida State University, Tallahassee, FL.

It has been shown that the follicle stem cells (FSCs) of the Drosophila ovary require the activities of multiple signaling pathways and adhesion molecules for normal self-renewal. Abnormally high levels of Hedgehog (Hh) signaling have a particularly striking effect, resulting in FSC duplication and accumulation of excess follicle cells. We tested whether changes in activity of other signaling pathways, such as JAK/STAT, produce similar effects. Overexpression of Hopscotch, the Drosophila homolog of JAK, resulted in apparent production of excess FSCs that migrated anteriorly. In some extreme cases we observed FSCs in contact with the germline stem cells and an apparent loss of escort cells. Loss of JAK/STAT signaling resulted in loss of FSCs, highlighting the Hedgehog and JAK/STAT pathways as critical regulators of FSC behavior. In fact, overexpression of Hopscotch suppressed the decrease in FSC persistence seen when Hedgehog signaling was abolished through loss of Smoothened. Additionally, we are examining whether the JAK/STAT pathway regulates FSCs through integrin-mediated adhesion, as was observed in testicular stem cells. We examined integrin protein expression in ovaries that had either a loss or gain of JAK/STAT signaling. No changes in integrin genes were observed in cells that lacked JAK/STAT signaling. The anteriorly migrating cells that overexpressed Hopscotch expressed integrin at roughly normal levels, but integrin expression was no longer confined to interfaces with the basement membrane along the walls of the germarium. We are currently investigating expression levels of integrin and other adhesion molecules associated with changes in activity of other pathways, as well as interactions between these pathways.

The Hippo pathway regulates intestinal stem cell proliferation during Drosophila adult midgut regeneration. Rachel L. Shaw, Alexander Kohlmaier, Cedric Polevsoile, Cornelia Vaterstetten, Bruce Edgar, Nicolas Tapon. 1) Apoptosis and Proliferation Control Laboratory, London Research Institute, London, United Kingdom; 2) ZMBH-DKFZ Alliance, Im Neuenheimer Feld 282, D-69120 Heidelberg, Germany; 3) Centre de Biologie du Développement, UMR5547, CNRS/Université Paul Sabatier Toulouse III, 118 route de Narbonne, 31062 Toulouse, France; 4) Division of Basic Sciences, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. N., Seattle, WA 98109, USA.

Pluripotent intestinal stem cells (ISCs) are critical for the maintenance of the adult posterior midgut. ISC proliferation is dynamically regulated in response to various environmental challenges and can be controlled by signals emanating from surrounding enterocytes (ECs) and/or visceral muscle cells, as well as by systemic growth regulators such as insulin-like peptides. ISC divisions are asymmetrically, producing a new stem cell and a partially differentiated enteroblast (EB). Notch activation in EBs promotes their differentiation into either the absorptive ECs or into secretory cells known as enteroendocrine cell (EEs). While insulin/IGF signaling (IIS) activity in ISCs promotes their proliferation, we find that the same pathway also regulates apicopodification and growth of differentiating EBs, and influences lipid and glycogen metabolism in terminally differentiated ECs. The cellular response to IIS activation thus changes in the ISC lineage as cells progress toward terminal differentiation. How these different responses to IIS activation are achieved, and how the response of ISC, EB and ECs changes during the commitment and differentiation process, remains unexplored. Our preliminary results suggest a critical role for the Tuberculous Sclerosis Complex (TSC) in diversifying the response of the ISC lineage to insulin signaling. TSC is an inhibitory component of TOR signaling, and TOR is required downstream of the insulin receptor to promote cell growth. Our results suggest that Tor signaling regulates growth of EBs, but does not influence proliferation of ISC. We observe high levels of TSC2 expression in ISCs, and a transient reduction of TSC2 in EBs during the commitment and differentiation process, suggesting that the differential expression of this protein determines the specific response of ISCs and EBs to IIS activation. Our data further indicate that this transient repression of TSC2 expression is caused by elevated Notch signaling activity in EBs. The molecular mechanism of this interaction is currently under investigation.

Stem Cell Therapy to Fight Gastric Cancer. Natana Field, Cynthia Vied, Daniel Kalderon. 1) Department of Biological Sciences, Columbia University, New York, NY; 2) Department of Biomedical Sciences, Florida State University, Tallahassee, FL.

Stomach cancer is the second most frequent cause of cancer-related death worldwide. Thus, it is essential to unfold the properties of gastric stem cells, which include their self-renewal or what the targets of Chinmo are in these cells. BTB domain proteins can recruit co-repressors to chromatin and repress transcription in a locus-specific manner or can act as adaptors for Cullin-3 (Cul-3) E3 ubiquitin ligases. To distinguish between these possibilities, we made clones of a cul-3 null allele and found that cul-3 null mouse is able to recover 72 after 7 days. These results suggest that Chinmo regulates transcription and not ubiquitination. Furthermore, the fact that Zfh1 functions as a transcriptional repressor raises the possibility that Zfh1 and Chinmo act together in a complex to repress target genes, which will be tested by co-immunoprecipitation studies.
Finally, in an effort to identify targets of Chimmo, we have designed an RNAi screen for suppressor of a chimmo gain-of-function phenotype (i.e. expansion of GSCs and CySCs) away from the niche.

903C
Fragile X protein controls neural stem cell proliferation. Matthew A. Callan1, Clemens Cabernard2, Jennifer Heck1, Chris Q. Doe2, Daniela C. Zarnescu1,3,4. 1) Molecular & Cellular Biology, University of Arizona, Tucson, AZ; 2) Institute of Molecular Biology, Howard Hughes Medical Institute, University of Oregon, Eugene, OR; 3) Department of Neuroscience, University of Arizona, Tucson, AZ; 4) Graduate Program in Genetics, University of Arizona, Tucson, AZ.

Fragile X syndrome (FXS) is the most common form of inherited mental retardation and is caused by the loss of function for Fragile X protein (FMRP), an RNA-binding protein thought to regulate synaptic plasticity by controlling the localization and translation of specific mRNAs. To determine whether FMRP is also required in early brain development we examined the distribution of cell cycle markers in dfmr1 mutant brains compared with wild-type. Our results indicate that the loss of dfmr1 leads to a significant increase in the number of mitotic neuroblasts and BrdU incorporation in the brain, consistent with the notion that FMRP controls proliferation in neural stem cells. To determine the role of FMRP in neuroblast division and differentiation, we used Mosaic Analysis with a Repressible Marker (MARCM) approach in the developing larval brain and found that single dfmr1 neuroblasts generate significantly more neurons than controls. Developmental studies suggest that FMRP also inhibits neuroblast exit from quiescence in early larval brains, as indicated by misexpression of Cyclin E. Our results demonstrate that FMRP is required during brain development to control the exit from quiescence and proliferative capacity of neuroblasts as well as neuron production, which may provide insights into the autistic component of FXS. Current experiments are aimed at determining whether Cyclin E is directly regulated by FMRP, causing the early reentry into the cell cycle. We are also testing a possible role for FMRP in glia, to determine the contribution of these neuroblast support cells in diseases such as Fragile X.

904A
Redox regulation by Keap1 and Nrf2 controls intestinal stem cell proliferation in Drosophila. Christine E. Hochmuth1, Benoît Biteau1, Dirk Bohmann2, Heinrich Jasper1. 1) Department of Biology, University of Rochester, River Campus Box 270211, Rochester, NY, 14627, USA; 2) Department of Biomedical Genetics, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY, 14620.

The intracellular redox state is emerging as a central determinant of stem cell function, but the underlying regulatory mechanisms are poorly understood. In Drosophila, intestinal stem cells (ISCs) respond to oxidative challenges and inflammation by increasing proliferation rates, a phenotype that is part of a regenerative response, but can lead to hyperproliferation and epithelial degeneration in the aging animal. Here we show that Nrf2, a master regulator of the cellular redox state, specifically controls the proliferative activity of ISCs, promoting intestinal homeostasis. We find that Nrf2 is constitutively active in ISCs, and that repression of Nrf2 by its negative regulator Keap1 is required for ISC proliferation. We further show that Nrf2 and Keap1 exert this function in ISCs by regulating the intracellular redox balance. Accordingly, loss of Nrf2 in ISCs causes accumulation of reactive oxygen species and accelerates age-related degeneration of the intestinal epithelium. Our findings establish Keap1 and Nrf2 as a critical redox management system that regulates stem cell function in high-turnover tissues.

905B
A role for Caprin during oogenesis. John C. Reich, Ophelia Papoulas. Molec Cell & Dev Biol, Univ Texas, Austin, TX.

Vertebrate Caprins are RNA binding proteins implicated in translational regulation. Previously, our lab has identified Drosophila Caprin as a protein that functions in the timing of the mid-blastula transition (MBT). During this dynamic-developmental transition, Caprin genetically interacts with fgr to regulate translation of specific RNAs involved in modulating the cell cycle. Caprin null mutant flies lacking a functional copy of fgr also show a dramatic decrease in egg production over time. In order to determine Caprin’s role in egg production, we examined Caprin mutant ovaries. Caprin mutant mothers produced a small percentage of egg chambers with an inappropriate number of nurse cell nuclei, some of which contained two oocytes. This percentage increases in Caprin null flies lacking a functional copy of fgr, suggesting these two genes may function together during oogenesis. This phenotype seems to originate in the gerarium. Geraria from Caprin mutant mothers appear to have an increased number of nuclei and resemble tumorous geraria in the disorganization of the densely packed cells. The percentage of geraria with this phenotype increases in older mutant females, suggesting a stem cell defect. There are three types of stem cells in the gerarium: germinle stem cells (GSC), escort cells, and somatic stem cells (SSC). Each population gives rise to distinct cell types. We believe that germinle cells are being produced correctly, a defect in SSC’s leads to defects in cyst encapsulation. Consistent with a SSC defect, we also observe an irregular stalk cell number and organization in a small number of wild-type ovarioles. We are currently using cell specific markers and genetic interactions to determine the molecular basis for these phenotypes.

906C

Adult stem cells are essential for the regeneration and repair of tissues in an adult organism. Stem cell populations must be carefully regulated to prevent tissue degeneration or overproliferation and cancer. To achieve proper balance between self-renewal and differentiation, stem cells rely on signals from their surrounding microenvironment or niche. The Drosophila testis contains an excellent model stem cell niche consisting of both germline stem cells (GSCs) and somatic support cells called cyst stem cells (CySCs), both attached to a cluster of somatic hub cells. In the Drosophila central nervous system, the Roundabout (Robo) family of axon guidance receptors are activated by the secreted ligand Slit and function to repel axons away from the midline. Robo signaling is also involved in cell migration and cell adhesion in multiple tissues. By screening for genes enriched in the testis apex, we found that Robo2 is expressed in the hub and early somatic cells including CySCs, and that Slit is enriched in the hub. We then used mosaic analysis to determine a cell type requirement for pathway components and found that robo2 is required cell autonomously in CySCs but not in GSCs. Further experiments to determine the role of this signaling pathway in the stem cell niche are ongoing.

907A
Zfrp8 and Hematopoiesis. William Tan, Svetlana Minakhina, Ruth Steward. Waksman Institute, 190 Frelinghuysen Rd, Rutgers University, Piscataway, NJ 08854.

The lymph gland is the hematopoietic organ of the Drosophila larva. We are studying zfrp8, an essential gene that functions in hematopoiesis. Loss of zfrp8 results in hypertrophy of the lymph gland, increased hemocyte proliferation and lamellocyte differentiation. We have shown that zfrp8 is crucial for the maintenance of hematopoietic stem cells (HSCs) in the lymph gland. To determine the tissue requirement of zfrp8, we have created transgenic UAS-zfrp8 fly lines. We have screened a number of GAL4 drivers and show that transgene expression under either a ubiquitous or medullary lymph gland specific driver rescues mutant phenotypes. To study the molecular function of Zfrp8 we have also created fly lines expressing NLS-, NES- and mCD8- tagged Zfrp8, where the protein is restricted to the nucleus, cytoplasm or plasma membrane. We have identified two genetic interactors of zfrp8, bam and lgl(2), and characterized their lymph gland phenotypes. Bam and lgl(2) are known to regulate stem cell maintenance in the germline and neuroblasts. The lymph gland phenotypes suggest that both genes have regulatory functions in hematopoietic stem cell maintenance as well.
POSTER: Techniques and Functional Genomics

See page 16 for presentation schedule. Poster board number is above title. The first presenter is the author.

908B
Microarray and CAGE analysis of gene expression from larval RNA. Eric Bremer1, Mitchell Dushay2. 1) Precision Biomarker Resources, Inc, Evanston, IL 60201; 2) Biology, Illinois Institute of Technology, Chicago, IL 60616.

CAGE identifies Transcription Start Sites (TSSs) of capped mRNA transcripts and yields counts of how many transcripts initiate at each TSS in the RNA sample. As previously reported, we are studying a CAGE library from whole larvae. In this library, only 10% of CAGE peaks overlapped with annotated TSSs, while 40% were found within genes, and the remainder could not be assigned to genes. This revealed a more complex transcriptional landscape than expected. An important question was how this complexity could be related to other measures of gene expression such as microarrays. Microarrays are typically biased towards the 3’ end of mRNA as opposed to the 5’ end in CAGE. For those CAGE tag clusters that are unambiguously linked to genes and correspond to annotated gene transcripts, we have compared CAGE-assessed gene expression levels to microarray analysis of similar RNA. We found a greater than 70% overlap in detected gene expression between CAGE and Affymetrix Drosophila 2.0 arrays when CAGE peaks that were found within genes were included. This suggested that transcripts identified by the internal CAGE peaks are detected by microarray. Conversely, there was very little concordance when only CAGE peaks linked to annotated TSSs were used. The number of CAGE peaks per gene tended to correlate with the level of gene expression as measured by microarrays. These findings show the curious result that successful CRM discovery was achieved using as input TFBSs not demonstrably functional in the identified CRMs. Our data underscore the importance of thorough empirical validation of computational predictions even when results seem to be in line with expectation.

909C
Erroneous attribution of relevant transcription factor binding sites despite successful prediction of cis-regulatory modules. Elizabeth R. Brennan1,2, Marc S. Halfon1,2. 1) Department of Biochemistry, SUNY at Buffalo, Buffalo, NY; 2) NYS Center of Excellence in Bioinformatics & Life Sciences.

Although accurate spatial and temporal regulation of gene expression is of fundamental importance for all animals, the vast majority of cis-acting transcriptional regulatory sequences in metazoan genomes remain uncharacterized. Computational approaches undertaken by a number of groups, including our own, have begun to show success in identifying these cis-regulatory modules (CRMs, “enhancers”). A common approach to computational CRM discovery is to search for colocalization and/or clustering of the binding sites for the specific transcription factors believed to regulate the CRMs being sought. However, in many cases these transcription factor binding sites (TFBSs) are not subsequently subjected to experimental testing to validate their role as important regulators of identified CRMs. We have undertaken an extensive examination of the role of TFBSs as criteria to computationally identify several CRMs. We mutated sets of TFBSs in the context of the entire CRM and directly compared activity of each mutated CRM to its corresponding wild-type sequence by dual reporter gene assays in the same transgenic embryo. Surprisingly, we found that TFBS mutagenesis had a significant effect—a complete loss of CRM activity—in only one out of eight constructs, and only minor effects in two additional constructs (loss of expression in a limited portion of the overall expression pattern for one and gain of reporter gene expression in a small subset of cells in the other). Five sets of TFBS mutations had no apparent effect on CRM activity at all. These findings show the curious result that successful CRM discovery was achieved using as input TFBSs not demonstrably functional in the identified CRMs. Our data underscore the importance of thorough empirical validation of computational predictions even when results seem to be in line with expectation.

910A
Tools for Enhancer Discovery and Analysis. Thomas Brody1, Amar Yavatkar1, Mukta Kundu1, Jermaine Ross2, Alexander Kuzin1, Ward F. Odendal1. 1) Neural Cell-Fate Determinants, NINDS/NIH, Bethesda, MD; 2) Division of Intramural Research Information Technology Program.

We have developed three web-accessed resources for discovery and analysis of cis-regulatory sequences. The first, EvoPrint, superimposes multiple eBLAT reads of 12 Drosophila species to identify evolutionarily hardened sequences within the species of interest. EvoPrint analysis of in vivo identified cis-enhancer regulatory motifs reveals that they are made up of conserved sub-pattern clusters (CSCs). A second resource consists of a genome-wide CSC database of over 100,000 CSCs, identified in EvoPrints that span nearly 100% of the Drosophila eukaryotic genomic DNA. The CSC database represents the first attempt to catalog the conserved sequences at the genomic level that are essential for cis-regulation. Third, cis-Decoder alignment algorithms identify repeat sequences within CSCs and search the CSC database for related clusters based on their shared elements. We demonstrate multiple uses for these resources in discovery and analysis of cis-regulatory sequences: 1) The ability to switch reference genomes allows for discovery of boundaries demarcating independent enhancers by the sequence length variation in non-conserved regions separating CSCs, thus providing a rationale for subdivision of enhancers into functional units. 2) CSC database searches using cis-Decoder algorithms detect families of co-regulating enhancers based on their sharing of conserved sequence elements. 3) cis-Decoder provides one-on-one alignments between identified enhancers to provide an understanding of enhancer substructure. 4) cis-Decoder provides for the identification of putative sub-pattern enhancers regulating tissue specific determinants, thus elucidating an unexpected complexity to gene regulation in specific lineages in developmental evolution. We use one of the late temporal network enhancers of cad as an example of our methods for discovery of co-regulating enhancers, including enhancers regulating expression of vrl, naba1, grin and thr.

911B
Cross-Species Expression Profiling Identifies Functional Genes in Drosophila Eye Development. Bryce Daines1, Yumei Li1, Hui Wang2, Xiaobo Zhou1, Graeme Mardon1, Rui Chen1. 1) Molec & Human Gen, Baylor College Med, Houston, TX; 2) Human Genome Sequencing Center, Baylor College Med, Houston, TX; 3) Bioengineering and Bioinformatics Program, The Methodist Hospital Research Institute, Houston, TX.

A broadly important and difficult problem in biology is the prediction of novel genes which function within a particular developmental process. Microarray expression profiling has been broadly used to identify transcripts expressed under specific developmental conditions. While microarrays can accurately predict temporal and spatial expression patterns, experience suggests that these data are not sufficient to consistently predict gene function.

We address the limitation of existing microarray-based approaches by using a comparative genomics approach. Using the Drosophila eye as a model system, cross-species expression profiling was performed. This approach, which integrates traditional microarray expression profiling with cross-species RNA-sequencing and additional high-throughput computational algorithms, dramatically improves the ability to predict functional genes. Based on our initial functional assay using RNAi, a 4.5x fold improvement in accuracy over attempts based exclusively on microarrays has been observed.

It is expected that this comparative genomics approach can be broadly applied to any developmental or biological process and model organism. In many cases, our approach may even be appropriate for use in non-model organisms and humans.

912C
SigWiki: A collaborative genomic resource for machine-readable descriptions of regulatory DNA signatures. Albert J. Erives1, Victoria Boggiano1, Alexandra Arnold1, Curtis J. Hansen1, Alexander R. Lloyd2, Grace A. Nauman1, Kerrie K. Nguyen1, Katie Ann Paden1, Dov A. Pechenik2, Shirulte Sajadi1, Arvis Sulovari1, Anna L. Tyler2. 1) Dept Biological Sci, Dartmouth Coll, Hanover, NH; 2) Dept Genetics, Dartmouth Medical School, Hanover, NH.

All biological cells encode heritable information via the sequential order of different nucleotides within a nucleic acid molecule. This information takes the form of a gene when it evolves essential parts that perform two functions: 1) condition-sensitive regulatory induction, and 2) an inducible molecular role. The genic DNA sequences that encode the conditions for gene activation are known as regulatory DNAs. Regulatory DNAs represent a research frontier that has been opened by the routine availability of whole genome assemblies. However, while much is known about the small percentage of the genome in the inducible or transcribed protein-coding sequences of genes, almost nothing is known about the total number, types, familial relationships, or functional structure of regulatory DNAs. Here we describe a new semantic wiki medium, SigWiki, for cataloging families of co-regulated DNAs within a genome by describing the functional grammars and signatures that define them. The structured data format allows researchers to query the database in
POSTER: Techniques and Functional Genomics
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

arbitrarily complex lists that update automatically as the database grows. The SigWiki regulatory genomic resource thus has the capacity to generate testable molecular hypotheses of specific gene regulatory mechanisms. We describe how we have used SigWiki in the laboratory and in the classroom to document functional families of regulatory modules for Drosophila.

913A
Gene annotation of 90 kb genomic DNA sequence of Drosophila mojavensis dot chromosome. Amber L. Harlan, Joshua F. Machone, James E.J. Bedard. Department of Biology and Earth Sciences, Adams State College, Alamosa, CO.

As part of the Genomics Education Partnership undergraduate student research initiative, we have annotated approximately 90 kb of the D. mojavensis dot chromosome, termed contigs 17 and 27. The Drosophila dot chromosome is of particular interest due to its ability to express both heterochromatic and euchromatic characteristics. Blastx comparison of the gene CG1909 in contig 17 of D. mojavensis predicted nine exons when compared to the reference D. melanogaster sequence. After complete annotation, it was found that the D. mojavensis gene contained only eight exons. In the gene Rdfab (Retinoid- and fatty acid-binding glycoprotein) from contig 27 a blastx comparison with D. melanogaster predicted seven exons but completion of the annotation determined only six exons in D. mojavensis. This was due to the exclusion of a ten amino acid sequence that did not meet the requirements of blasts to be presented as an exon. Molecular data suggest that this protein-coding gene functions as a binding receptor of acetylcholine and zinc ion binding. The gene Rdfab is important for retinol binding, lipid transporter activity, receptor binding, microtubule binding, and heme binding. These findings further characterize exon variances within the Drosophila genus and may be used to understand the differences between chromatin packing and gene expression.

913B
Robust estimates for the range of morphogen gradients. Jitendra Kanodia1, Yoo sik Kim1, Kwanghun Chung2, Hang Lu2, Stanislav Shvartsman1. 1) Chemical & Biological Engineering, Princeton University, Princeton, NJ; 2) School of Chemical & Biomolecular Engineering and Parker H. Petit Institute for Bioengineering & Bioscience, Georgia Institute of Technology, Atlanta, GA.

One of the main questions related to pattern formation by morphogen gradients is related to their range, defined as the distance over which morphogens act in developing tissues. Quantitative measurements of protein concentrations using fluorescence-based experiments are an effective tool for estimating the range. However, quantitative measurements are rife with various forms of biological variability and measurement noise which are often unknown. Analysis of this type of data requires rigorous statistical approaches. We describe a straightforward and computationally efficient method based on a combination of studentized t-test and bootstrapping that provides a robust point estimate for the range as well as the 95% confidence interval (CI) of the estimate. We demonstrate the developed method by applying it to the DI morphogen gradient, which patterns the DV axis of the embryo. The estimate obtained for the range of DI is 70% of the DV axis extent with a CI of 53%-83% DV axis size. To validate this estimate, we used a microfluidics-based device for mounting samples and confocal imaging to visualize the DI gradient in 10 independent experiments, each of which led to an independent dataset with dozens of DI gradients. The point estimates of the range of the DI gradients from each of the 10 biological repeats lie within the CI derived from the first experiment. The developed approach is nonparametric and can be applied to any gradient in any tissue without any a priori information about the gradient or the tissue. We demonstrate the general applicability of this method by applying it to other morphogen gradients in the early embryo.

913C
FlyExpress: A Platform for Discovering Co-expressed Genes via Comparative Image Analysis of Spatial Patterns in Drosophila Embryogenesis. Sudhir Kumar1,2, Michael McCutchen2, FlyExpress Consortium2. 1) School of Life Sciences, Arizona State Univ, Tempe, AZ; 2) Center for Evolutionary Medicine and Bioinformatics, Arizona State Univ, Tempe, AZ.

Images containing spatial expression patterns illuminate the roles of different genes during embryogenesis. Overlaps in expression patterns are frequently an initial clue to genetic regulatory interactions. FlyExpress is a web resource that facilitates the discovery of putatively interacting genes during Drosophila embryogenesis. It contains a library of >100,000 standardized expression images from ~4500 genes. All images have been uniformly oriented, aligned, and scaled allowing direct comparison by gene across stages and anatomical views. FlyExpress provides tools to automatically identify co-expressed and, thus potentially co-regulated, genes by searching for other genes with similar expression profiles. Our search tool directly compares expression pattern images and emulates biologists’ practices of manual inspection. In addition, FlyExpress provides global views of gene activity across each developmental stage through Geneswide-Expression-Maps (GEMs). GEMs are two-dimensional heat maps that synthesize individual spatial patterns into genomic summaries. By simple point-and-click, one can query GEMs directly to produce a list of genes expressed in any region of the embryo. Users can also create GEMs for their own list of genes based on available expression data from two high throughput sources (BDGP and FlyFISH). FlyExpress now includes GEMs built using spatial expression data from >2,000 peer-reviewed publications. This provides a novel scientific-literature mining tool that displays all publications reporting gene expression at any given embryo coordinate. Therefore, the FlyExpress platform is designed to meet needs of biologists to identify similar expression patterns, judge the biological relevance of these matches within the complexities and subtlety of the developmental process, generate novel gene interaction hypotheses, and visualize genome-scale summary of gene expression relevant to development.

916A
Introduction of a genomic tag in roX1 through gene conversion. Manuski Apt1, Victoria Moran1, Richard Kelley2, Victoria Meller1. 1) Dept. of Biological Sciences, Wayne State University, Detroit, MI; 2) Dept. of Molecular and Human Genetics, Baylor college of Medicine, Houston, TX.

Engineering Drosophila genes at their endogenous locations is notoriously difficult. Techniques such as ends-in and ends-out recombination are widely used but labor intensive. We have tested a relatively simple and efficient genome engineering technique, based on gene conversion, which may be useful in many situations. As a proof of principle, we introduced a genomic tag of six tandem MS2 loops in the roX1 gene. roX1 is a long non-coding RNA that assembles with the Male Specific Lethal (MSL) proteins. The resulting MSL complex accomplishes dosage compensation of the X-chromosome in male flies. MS2 loops interact with MS2 coat protein (MCP) in the MSL2 bacteriophage. RNAs tagged with MS2 loops are readily visualized with an MCP-GFP fusion protein. We introduced MS2 loops into roX1 using a multi-step strategy. Flies carrying a P-element containing roX1 with MS2 loops inserted in a non-essential region were created. This P-element was moved into roX1 by targeted transposition. When this P-element was re-mobilized from a site in roX1, 10% of all excisions were gene conversions that introduced MS2 loops into the roX1 (roX1<sup>™</sup>). We postulate that this occurs by gap repair using a sister chromatid template. The sister chromatid retains the P-element and supplies a template with MS2 loops. At lower frequency other predicted products of gene conversion that retain P-element sequences, were recovered. The MS2 loops are over 400 bp from the point of insertion, suggesting that repair tracts capable of incorporating large amounts of non-homologous sequence occur frequently. We are currently using roX1<sup>™</sup> to determine the localization of roX1 during early embryogenesis. Our method requires a P-element near the target site, limiting this method somewhat. However, our preliminary studies suggest that this strategy may be generally applicable.

917B
Donor design limits using Zinc Finger Nucleases. Kelly J. Beumer, Jon Trautman, Dana Carroll. Dept Biochem, Univ Utah, Salt Lake City, UT.

Introduction of a double-strand break (DSB) in chromosomal DNA stimulates repair by recombination in the vicinity of the break. Previously we have shown that a class of engineered nucleases with zinc finger DNA-binding domains (zinc-finger nucleases, ZFNs) can make recombiningag DSBs in the Drosophila genome and stimulate gene targeting. We have shown that both targeted mutations and homologous gene replacements can be recovered efficiently in a single generation after injecting a plasmid encoding a “donor” DNA in the presence of RNAs encoding the ZFNs. We are exploring the behavior of a variety of donor configurations at using ZFNs for the rosy locus. We are testing donors with homologies ranging from 100bp to 8kb to determine the limits of donor size, as well as testing the recovery of deletions or insertions at varied locations throughout the chromosomal sequence.
A novel method to generate gene deletions by induction of DSB in the transgene. Maria V. Kim, Artem Tkachuk, Anna Aristarkhova, Mikhail Savitsky. Institute of Gene Biology, RAS, Moscow, Russian Federation.

In Drosophila, chromosomal deletions are important tool to study gene function. Many of the deletions available were generated with use of irradiation and chemical mutagens. The phenotypic analysis of deletions generated by these methods is very often complicated due to their large size and accompanying mutations. Moreover many of the deletions are not mapped precisely. Modern techniques using transgenic technologies address these problems. Chromosomal aberrations can occur as a result of imprecise excision of a P-element or between P-elements presented at different sites on homologous chromosomes. Another method involves site-specific recombination between FRT sites in trans. Here we report a new method to generate chromosomal deletions using inducible double-strand breaks within the transgene. We have created a P-element-based vector containing miniwhite and EGFP under 3xP3 promoter, surrounded by I-SceI sites. An induction of I-SceI in transgenic flies leads to the removal of EGFP and the neighboring regions wich is also associated with the loss of one of the P-element’s termini. Flies with modifications can be easily selected for they do not have mosaic eyes in presence of the transgene source. This substantially simplifies the screening for the derivatives that carry deletions in the adjacent genomic region. The precise molecular characterization is then performed by PCR-analysis and sequencing. Using this method we obtained partial deletions of genes inv2 and Phar.

Defining off-target cleavage in a pair of zinc finger nucleases. Kusumika Mukherjee, Dana Carroll. Biochemistry, University of Utah, Salt Lake City, UT.

Studies on Zinc Finger Nucleases (ZFN) have shown that they can be toxic in organisms. This is potentially due to ZFN cleavage at multiple off-target sites. In applications of ZFNs in human gene therapy, this off-target cleavage is intolerable. We are attempting to develop a procedure to identify these off-target sites in Drosophila. We can then analyze every new ZFN pair for potential off-target cleavage and select and redesign it to work more efficiently. We propose to capture ends created by ZFN cleavage and subject them to deep sequencing using Illumina methodology. ZFN cleavage produces a 5'-4-base overhang at the targeted cleavage site, which is likely to be random at the off-target cleavage sites. We have designed adapters with 5'-4-base overhangs which are compatible with Illumina methodology, to capture all ends produced by ZFN cleavage. Analysis of the DNA sequences obtained will be done against Drosophila genome, to identify and characterize off-target cleavage sites for each particular ZFN pair. Towards the development of such a procedure, we have started with a well-characterized ZFN pair which targets rosy in Drosophila. Previous work in our lab has shown that rosy ZFN pair is efficient and relatively non-toxic, thus likely to have few off-target cleavage sites. This makes this ZFN pair ideal to establish the procedure. In preliminary experiments, we are able to efficiently capture the targeted ends in an in vitro system. We are working to optimize the procedure, first to capture expected ends in a genomic context, and then to capture ends created by off-target cleavage. Finally, we will apply the procedure to genomic DNA that has been cleaved in vitro. Our results will be presented.

Brain transcriptional regulatory network predicts behaviorally-related functions for conserved transcription factors. Seth A Ament1,2,3, Sritam Chandrasekaran1, James A Eddy1, Sandra Rodriguez-Zas1, Bruce R Schatz1, Nathan D Price1,2, Gene E Robinson1. 1) Institute for Genomic Biology, University of Illinois, Urbana, IL; 2) Howard Hughes Medical Institute; 3) Department of Molecular and Cellular Biology, University of California, Berkeley, CA.

The brain transcriptome is highly responsive throughout life to a variety of stimuli associated with behavior; evolutionary changes within transcriptional regulatory networks (TRNs) are thought to underlie phenotypic differences between species, but this has been little studied outside of developmental contexts and in microbes. We reconstructed a genome-scale predictive model of a TRN in the brain of the honey bee - the largest of its kind for the brain of any organism - which predicts the expression of 2176 genes using a novel algorithm, conserved transcription factors (TFs) known from studies in Drosophila, and an extensive collection of gene expression profiles from the whole brains of individual bees performing a variety of social behaviors. Because it is likely that social behaviors in the bee arose from antecedents present in the common ancestor of bees and flies, we hypothesize that TFs linked to honey bee social behavior may also regulate related, solitary behaviors in the fly. We focused on a set of 35 highly-connected TFs (with >50 predicted target genes) that were predicted by the TRN model to regulate feeding-related social behaviors in the bee, including several TFs previously linked to neuronal plasticity and hormonal signaling in the fly, such as Creh, ftz-f1, and broad. In ongoing work, we are using additional transcriptomics experiments to determine whether these TFs are also associated with nutritional status in the brains of flies. We will then characterize the transcriptional targets for some of these TFs in flies and test the hypothesis that they regulate feeding in flies and feeding-related social behavior in bees. We anticipate that this fusion of molecular systems biology in the bee with behavioral and neural genetics in the fly will elucidate relationships between genes, brain, behavior, and evolution.


At the Berkeley Drosophila Genome Project (BDGP), we have established a gene expression resource for Drosophila development that contains 2D spatial and temporal embryonic expression patterns. Annotating the patterns using a standardized, controlled vocabulary (CV) based on ontology, as well as standardized virtual representations of the patterns. Our database currently contains over 100,000 annotated images showing expression patterns generated using in situ hybridization of staged whole-mounted embryos for over 55% of the protein-coding genes in the Drosophila genome including all currently known and proposed sequence specific functional elements. To accelerate our imaging effort and move the project towards high throughput systems biology, we have started to develop a completely automatic imaging platform. We have fitted a microscope with a camera, automated stage and stage loader and added a customized version of MicroManager to the cross-platform, open source, ImageJ based biological image processing package Fiji (http://fiji.sc). This enables us to automatically image sets of slides and extract the embryos. We plan to further enhance Fiji with image processing algorithms to create and manage standardized representations (Triangulated Images) for computational analysis and comparison of embryonic expression patterns.

The use of next-generation sequencing to functionally dissect the functions of the Drosophila larval midgut. Philip Bitterham, Stephen Pearce, Phillip Daborn. Genetics Dept, Melbourne University, Parkville, Victoria, Australia.

The Drosophila larval midgut is a complex tissue involved in many biological processes. The midgut has a number of functionally specialised regions, being characterised into three major compartments (anterior, middle and posterior) based on cell morphology and physiology. Genetic analysis reveals a more complex picture however, with thirteen compartments of gene expression being identified. We explored the changing expression environment of the larval midgut using RNA-Seqencing. Using GFP labelled genetic compartments as a guide, the midgut was dissected into eight regions. A number of genes were identified with compartment specific expression patterns, providing insights into the functional role of these midgut regions. Surprisingly, many changes in gene expression were observed even between morphologically identical compartments. The larval midgut may therefore be more highly functionally specialised than previously thought.

Morphogen gradients quantified by sub-single embryo RNA-seq. Peter A. Combs1, Susan E. Lott2, Michael B. Eisen1,2. 1) Biophysics Grad Group, Univ California, Berkeley, CA; 2) Department of Molecular and Cell Biology, Univ California, Berkeley, CA; 3) Howard Hughes Medical Institute, Univ California, Berkeley, CA.

Genome-scale techniques have been invaluable at illuminating multi-gene interactions, at the expense of spatial information, while any technique that respects spatial dependence
works for only a handful of genes at a time. This is particularly troublesome for studying Drosophila patterning, which has detailed and precise spatial dependence among a network of genes. Previous work from our lab has shown that single Drosophila provide ample material for RNA-seq. Here we extend this work to quantify gene expression within a single embryo. We used a combination of cryo-sectioning and Illumina sequencing to measure gene expression along the anterior-posterior axis of single D. melanogaster embryos. The sample preparation protocol was specifically optimized for small volume samples, and yielded enough DNA to perform high density sequencing of 50 micron thick samples. Our analysis focuses on the expression of various morphogens along the AP axis.

924C

Transposable elements (TEs) are a ubiquitous feature of genomes, contributing to the evolution of genome size and structure. In Drosophila species, there are thousands of TE sequences present in the published reference sequence, the majority of which are at low frequencies in natural populations, presumably because novel insertions are generally deleterious. A systematic method of identifying TEs in an individual Drosophila species and its host genome. We have developed a method to systematically identify TE insertions using high-throughput, paired-end (PE) sequencing. Paired-end data allows for the identification of regions of the genome where uniquely mapping sequence information is coupled with sequences that align to TEs. By sequencing a genome to high enough coverage, novel insertion events can be indicated by dozens or even hundreds of short sequences. Given enough local coverage of the insertion event, the point in the genome where the TE inserted can be determined to within a few base pairs if not exactly. Insertions located near other insertions can also be distinguished. We have obtained PE sequence data for nine D. melanogaster isofemale lines sequenced to about 45x sequence coverage per genome. We have detected hundreds of TE insertions per isofemale line present in the reference sequence. Furthermore, in most cases we have had enough sequence coverage in the local area of the novel breakpoint that we have been able to completely reconstruct the insertion breakpoint. Preliminary data analysis indicates that 30 - 40% of these events occur in genes. While we have tested this pipeline in D. melanogaster this pipeline could be used to detect novel insertions in any species for which there is an appropriate reference sequence; this does not require TE annotations for the genome of interest. Other applications applications of this technique, including identifying mutant TEs created via TE mutagenesis or identifying novel duplications of highly duplicated gene families are also possible.

925A
Next generation sequencing to identify novel X-linked EMS induced mutations. Ana Clara Fernandes1,2, Gert-Jan Hulselmans1, Jan Slabbaert1,2, Sabine Keumen1,2, Valerie Uyttendevoen1,2, Jaroslaw Kasprzowicz1,2, Stein Aerts1, Patrik Verstreken1,2, 1) K.U.Leuven, Center for Human Genetics; 2) VIB, Department of Molecular and Developmental Genetics.

To identify novel X chromosome linked mutations that affect neuronal communication we have performed a chemical mutagenesis (EMS) screen. We re-engineered the eyFlp-system, allowing us to generate homozygous female flies with eyes homozygous for an EMS treated X chromosome. This system allowed us to screen 13 thousand lethal chromosomes using electoretinogram (ERG) recordings. We isolated 120 mutants that show ERG defects when homozygous in the eye. Given that the cost of whole genome next generation sequencing (NGS) is rapidly decreasing, we tested if this brute-force approach is feasible for fast identification of lesions. We sequenced 7 heterozygous mutant genomes (mutant/Xiso) and the isogenized wild type genome (Xiso). Mapping the sequence data to the reference genome reveals an average coverage between 4 and 10 for the 8 sequenced genomes. To identify mutations we used a minimum of 3x coverage per base and found between 76 and 202 heterozygous base changes per Mb on the X chromosome, most of which are a single G to T/A transitions, typically capped by EMS. By sequencing 15 TEs as well as help to elucidate their effects on nonsense change. Given this ‘whole chromosome’ strategy did not allow us to unequivocally identify one or a few mutations we combined NGS with fast recombination mapping of lethality using molecularly mapped P-elements and duplications. Although each allele maps to a single ~1 Mb locus, indicating the mutants harbor only one lethal lesion, NGS still reveals numerous non-synonymous mutations within these regions. Thus, additional mapping experiments using complementation tests with known lethal alleles or using rescue experiments with smaller duplications were necessary to identify the genes mutated. In conclusion, NGS reveals numerous EMS-induced changes on mutated chromosomes but it is only efficient at readily identifying phenotype-causing mutations with additional mapping and complementation experiments.

926B
Evaluating performance for RNA-seq with external RNA controls. Lichun Jiang1, Carlo Artieri1, Yu Zhang1, Nicolas Mattiuzzo1, David Sturgill1, Renhua Li1, John Malone1, Marc Sait1, Brian Oliver1. 1) Developmental Genomics Section, LCDB, NIDDK, National Institutes of Health, Bethesda, MD 20892; 2) Biochemical Science Division, National Institute of Standards and Technology, 100 Bureau Drive, Mail Stop 8313, Gaithersburg, MD 20899, USA.

RNA-seq, high-throughput short-read sequencing of cDNA, is revolutionizing our ability to profile transcriptomes. The ideal method to evaluate the reliability of RNA-seq is to analyze a known set of external RNA controls. We employed a pool of 96 well-characterized RNAs provided by External RNA Control consortium (ERCC) to test the efficiency of RNA-seq in accurately measuring transcript abundance in the control RNA pool alone, or while using the pool as a ‘spike-in’ control with Drosophila melanogaster mRNA. Excellent linearity was observed with output as compared to known input when profiling the control RNA pool (p=0.95, P<2.2x 10^-16), it indicates dynamic abundance range of 220 and a lower detection limit of 10^-7 the mass of input. This linearity was also observed when mixing control RNAs with a complex biological sample. We found read counts among technical replicates fit a negative binomial distribution model better than a Poisson model. Comparing simulated reads mapped to external RNA controls with reads from real RNA-seq data, we found that non-uniform read coverage leads to inaccurate detection and quantification of low abundance transcripts. We explored reasons for the non-uniform read distribution by fitting the read count data with a general linear model and found that local sequence features partly explain non-uniform read coverage across the genome. Overall, our study shows that RNA-seq is a useful technique for measuring transcriptome abundance.

927C
RNASeq Reveals Novel Regulatory Network Genes in Drosophila Eye Development. Landey E. Nfonsam1, Joann Mudge2, Faye Schilkey2, Ryan Kim2, Jennifer Curtiss1. 1) Biology, New Mexico State University, Las Cruces, NM; 2) National Center for Genome Resources (NCGR), Santa Fe, NM.

The vertebrate Pax6 homolog called eyeless (ey) drives Drosophila eye specification. Ey regulates expression of other eye specification genes that form a complex regulatory network with Hh, Dpp and N signaling pathways in a manner that is still not fully understood. We harnessed the power of massively parallel sequencing to further dissect the Drosophila eye gene network. We first used the Gal4-UAS system to confirm that co-expression of Ey with Dpp, Hh or N is more efficient at generating ectopic eye tissue compared to Ey alone. Next, we used Illumina RNASeq and Alphagene database analysis software to profile the transcriptional pattern of ectopic eye tissues generated in the wing from targeted expression of ey, dpp, hh, N, ey+dpp, ey/hh or ey/N. Approximately 10.5 million reads of 36 bp length were generated per genotype. Reads matched ~86% of all Drosophila genes. At ≥3x, 341genes up-regulated across all genotypes were validated by Agilent array. Principal Components Analyses and 2-way hierarchical clustering revealed the expression pattern resulting from ey/hh to be closest to the eye control relative to other genotypes investigated. We used DAVID Bioinformatics and GOstat/FlyBase to group validated ey/hh genes into functional categories and identified genes with predicted or unknown functions. We identified 24 genes as possible novel targets of ey/hh. Actin filament binding, cell adhesion, nucleic acid/protein binding, protease activity, ATP binding, microtubule motor activity and lipid biosynthesis were represented among candidate genes. Target Explorer and cisTargetX bioinformatic tools were used to identify 8 possible direct targets of ey+hh. We plan to confirm direct targets using reporter gene and gel-shift assays, and to probe the functions of these genes during Drosophila eye development. Our results demonstrate the facility of RNASeq in revealing expression patterns of multiple genes in a single run and in providing the opportunity to screen for novel candidate genes.

POSTER: Techniques and Functional Genomics
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.
POSTER: Techniques and Functional Genomics
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

928A

Genome sequencing for the Drosophila ModEncode project: Progress on the Genome Sequencing of D. biarmipes, D. bippinata, D. elegans, D. ecruacilis, D. ficsushila, D. kikkawai, D. rhopalos, D. tahakashi, and the Oregon R strain of D.melanogaster. Steven Scherer1, Stephen Richards1, Shalini Jhagian1, Yuan-Qing Wu1, Kim Worley1, Artyom Kopp1, Michael Eisen1, Peter Cherbas1, Richard Gibbs1. 1) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX; 2) Department of Evolution and Ecology, University of California at Davis, Davis, CA; 3) Howard Hughes Medical Institute and Department of Cell and Molecular Biology, University of California at Berkeley, Berkeley, CA; 4) Department of Biology and Center for Genomics and Bioinformatics, Indiana University, Bloomington IN.

The model organism ENCyclopedia Of DNA Elements (modENCODE) Project is an effort to identify all sequence-based functional elements in the Caenorhabditis elegans and Drosophila melanogaster genomes. As part of this work, we are adding additional Drosophila genomes to the already available 12 species to improve the ability to discover and refine functional elements from comparative genomic data. Eight additional species have been chosen to span a range of evolutionary distances from D. melanogaster, as well as the specific Oregon R strain used in ModEncode experiments. To enable facile assembly, Artyom Kopp laboratory performed inbreeding to about 15 generations for all of these species, greatly reducing the complexity of sequence datasets for assembly. We are taking a 454 approach, with a minimum of 15X Fragment sequence coverage, and 30X “clone” coverage in both 3kb and 30kb paired end data. In addition, Illumina RNAseq-data is being collected for all of the species to allow high quality automated annotation to be performed. Initial assemblies of fragment and 3kb pe data using Newbler 2.0 gave excellent statistics, for example the D. biarmipes assembly had a contig N50 length of 92kb, and Scaffold N50 of 690kb, with the largest scaffold of 5Mb. The Oregon R line used in many ModEncode experiments is re-sequenced on the Illumina platform. Raw sequence and assembly data will be available via the BCM-HGSC website, appropriate NCBI databases and flybase.

929B

PoPoolation: a toolbox for population genetic analysis of 2nd generation sequencing data from pooled individuals. Christian W. Schloetterer1, Robert Koller1, Pablo Orozco-ter Wengel1, Nicola De Maio1, Ram Vinay Pandey1, Viola Nolte1, Andreas Futschik2, Carolin Kosiol1. 1) Inst f Populationsgenetik, Vetmeduni Vienna, Wien, Austria; 2) Department of Statistics, University of Vienna, Vienna, Austria.

Recent statistical analyses suggested that sequencing of pooled samples provides a cost effective approach to determine genome wide population genetic parameters. Here we introduce PoPoolation, a toolbox specifically designed for population genetic analysis of sequence data from pooled individuals. PoPoolations calculates estimates of \theta_c and Tajima’s D that account for the bias introduced by pooling and sequencing errors, as well as divergence between Genome-wide analyses can be graphically displayed in a sliding window plot. PoPoolations is written in Perl and R and it builds on the commonly used data formats.

930C

Tracking and Automated Behavior Recognition of Drosophila with a Multi-Resolution Camera System. Reza Ardekani1, Anurag Biyani1, Ravi Prakash1, Sergey Nuezdin1, Simon Tavare1,2. 1) Department of Biological Sciences, University of Southern California, Los Angeles, CA; 2) Department of Applied Mathematics and Theoretical Physics, University of Cambridge, Cambridge CB3 0WA, UK.

Studying the behavior of either an individual or a group of Drosophila has been an interest of many researchers. To conduct these studies, acquiring information about position of each individual as well as their body posture over the time is crucial. While several groups have tried to automate this data acquisition, the problem of keeping track of several flies for a long period of time in 3D space is still challenging. Recently, we have developed an approach that enables us to detect and keep track of multiple flies in a 3D arena for a long period of time, using multiple synchronized and calibrated cameras. Our multi-resolution camera system provides us high spatial and temporal resolution at the same time. To track fast moving targets like Drosophila, it is essential to have high temporal resolution, which is obtained using four high frame rate (i.e. 30fps) cameras. Moreover, visibility of details of the fly’s body, which is important for better understanding of flies’ behavior, is achieved by two extra cameras that work at a lower frame rate but much higher resolution. Flies are detected in each view using a background subtraction technique. Once correspondence between views is established, actual 3D positions of flies in space are reconstructed and then tracked over time. Measuring the trajectories of multiple individual flies over time enables high-resolution measurement of complex behaviors and social interactions, including aggression, courtship and mating. Instances of these behaviors can be computationally flagged by the trajectory analysis and then validated or examined in more detail in the high-resolution video system. Overall, this system presents a powerful method for tracking complex social interactions in an ethologically-relevant 3D environment.

931A

The Bloomington Deletion and Duplication Projects. Kevin Cook, Russell Garton, C. Adam Brown, Thomas Kaufman, Kimberley Cook, Dept Biol, Indiana Univ, Bloomington, IN.

Over the past several years, the Bloomington Drosophila Stock Center has hosted two projects to improve gene mapping resources. The intention of the first project was to improve coverage and subdivision of the fly genome with molecularly defined chromosomal deletions. We have now conducted the last of the screens to produce a total of 848 deletions and we will present the analysis of the current distribution of breakpoints. The second project has as its goal improved coverage and subdivision of the fly X chromosome with duplications of X segments carried by Y chromosomes. We will present our progress to date in completing this project, which should, by the time of the conference, provide coverage of more than 90% of X chromosome genes.

932B

Expanding the Utilities Of G-MARET In Drosophila; Taking It A Step Ahead. Indrani Gupta, Ryohei Yagi, Konrad Basler. Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland.

Communication between two functionally distinct cell populations is an integral feature occurring in wide variety of biological processes in multicellular organisms. To better understand such cell communication the ability to experimentally manipulate the two communicating cell populations independently is needed. In Drosophila this could, for example, be done with two independent binary transcriptional systems such as Gal4/VAS and LexA/lexA operator. We recently developed a versatile Gal4 enhancer trap system termed Gal4-based Mosaic-inducible And Reporter exchangeable Enhancer Trap (G-MARET). It has two modes: The initial Gal4 can be exchanged unidirectionally with, for example, LexA transactivator (LexA TA). The exchange may be complete or partial, in the latter case genetic mosaics are produced that are composed of cells expressing either Gal4 or LexA TA (Yagi et al., 2010). This ability to generate a mosaic allows one to either induce or manipulate, independently, two cell populations in a defined area, e.g. for studying regeneration, both populations (dying and regenerating) can be manipulated separately. Here we describe a P-element based screen to establish a large collection of G-MARET insertions, P(Gmrw)s, which show Gal4 expression in specific patterns in wing discs. We mobilized P(Gmrw) and monitored Gal4 expression of the resultant insertions with UAS-GFP. As expected from the fact that P(Gmrw) is a derivative of P(Galw), a Gal4 enhancer trap known to have higher mobilization activity than P(Gaw) (Gerlitzi et al., 2002), it was mobilized efficiently and a huge number of new insertions were generated. We will present the latest results of our analysis of P(Gmrw)s collection and applications demonstrating how we can use them for investigating biological phenomena.

933C

High-throughput analysis of morphogen gradients using a microfluidic device. Yoosik Kim1, Kwanghun Chung2, Jitendra S. Kanodia1, Emily Gong2, Stanislav Y. Shvartsman1, Hang Lu1. 1) School of Chemical and Biological Engineering and Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ 08544 USA; 2) School of Chemical and Biomolecular Engineering and Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332, USA.

Quantitative analysis of developmental systems requires information about the regulatory regions of genes comprising the network and the spatial distribution of patterning signals. The dorsoventral (Dv) patterning system of Drosophila embryo is arguably one of the best understood systems with regard to its sequence-specific transcriptional
regulation, but the information on the distribution of patterning signals is currently lacking, mainly due to the technical difficulties associated with imaging the spatial distribution of proteins and transcripts along the DV axis of the embryo. To enable high-throughput analysis of the DV patterning signals, we developed a Microfluidic Embryo Trap Array (META), a device in which hundreds of embryos can be oriented vertically in a matter of a few minutes. Here, we present the design and the physical principles behind this device and demonstrate how it can be used to quantify morphogen gradients in fixed embryos and monitor nuclear divisions in live embryos. This design allows us to image a large number of embryos to statistically analyze the patterning signals in fixed embryos. Using META, we quantified the spatial extent of Dorsal morphogen gradients and demonstrated how this gradient and the distribution of its signaling and transcriptional targets can be quantitatively compared between wild type and mutant backgrounds.

934A
Production and Purification of Nora Virus ORF 3 Protein. Andrew Prossoki, Darby Carlson, Ethan Cordes, Brad Ericson, Kimberly Carlson. Biology Department, University of Nebraska at Kearney, Kearney, NE.

Nora Virus is a single-stranded RNA virus and is also a novel picorna-like virus that is able to infect Drosophila melanogaster in both naturally occurring and laboratory-evolved populations. It is unlike most other picorna-like viruses in that it has four open reading frames (ORFs), in contrast to one long ORF found in traditional picornaviruses. The coding potentials of the ORFs are not well characterized, but ORF 3 is believed to encode the capsid protein. The site of virus replication is thought to be in the midgut of the host. To begin to characterize the site of replication and viral titers over time, protein studies utilizing a monospecific antiserum need to be performed. The purpose of this study was to generate monospecific antiserum to Nora Virus by cloning ORF3 in a solubility vector with an N-terminal His tag and to express the recombinant protein. The His-tagged recombinant Nora ORF 3 protein was purified and injected into mice to make monospecific antiserum. The resulting antiserum will be used for protein characterizations of Nora virus and its relationship with D. melanogaster. The project described was supported by the NIH grant number P20 RR016469 from the INBRE Program of the National Center for Research Resources.

935B
A genetic mosaic screen for enhancer-trap FLP-induced intersectional GAL80/GAL4 expression in Drosophila imaginal discs. Brittany N Smith1, Rudolph A Bohm2,3, William P Welch1, Lindsey K Goodnight1, Bing Zhang1, John P Masly1. 1) University of Oklahoma, Department of Zoology, Norman, OK; 2) Brandeis University, Department of Biology, Waltham, MA.

The Drosophila imaginal discs provide an ideal model to study developmentally important processes such as patterning, differentiation, cell signaling, and organogenesis. Although the GAL4-UAS system has been an important tool for gene manipulation in developing imaginal tissues, the expression patterns of most GAL4 drivers are often too broad to effectively target small clusters of cells. Recent efforts to dissect neural circuitry have produced a large collection of tools that combine existing technologies that expand the specificity of GAL4 expression (Bohm et al., PNAS 107:16378-16383). In particular, these tools combine existing GAL4 expression drivers with tissue-specific enhancer-trap Flippase (ET-FLP-x2), and FRT-dependent GAL80 constructs, which allows for “flip in” GAL80 repression of GAL4 expression. Here, we characterize the ET-FLP-x2 collection for their expression patterns in third larval instar imaginal discs. We find several insertions that express in imaginal discs with both symmetric and asymmetric expression patterns; we also find sexually dimorphic expression patterns in several imaginal discs including sex-specific expression patterns in the genital imaginal. Most expression patterns we observe are reproducible and vary little among individuals, consistent with previous characterization of ET-FLP-x2 expression in nervous system tissues. The ET-FLP-x2 lines thus provide researchers the ability to perform fine-scale cellular dissolution in imaginal discs.

936C
Monitoring DNA Accessibility and Epigenetic Status in Live Animals with Fluorescent Reporters. Can Zhang1, Michael Novo1, Nianwei Lin1, YiQing Tan2, Huabei Jiang2, Lei Zhou1. 1) Molecular Genetics and Microbiology Dept, University of Florida, Gainesville, FL; 2) Biomedical Engineering Dept, University of Florida, Gainesville, FL.

Epigenetic regulation, by limiting the accessibility of DNA and the gene expression, play an important role in biological processes such as stem cell maintenance and cellular differentiation. Most biochemical methodologies for measuring epigenetic modification rely on homogenizing large amount of cells, which is inapplicable for monitoring dynamic epigenetic changes at cellular level in live animals. In this study, we explored the application of using a fluorescent reporter in monitoring and quantitative assessment of epigenetic status in vivo. Using the “Ends-out” homologous recombination, we knocked an ubiquitin-DsRed reporter into IRER, a previously identified, epigenetically regulated enhancer region residing in the chromosome locus 75C1-2 (Zhang and Lin et al., 2008). A restricted and dynamic DsRed expression pattern was observed throughout animal development, suggesting that the expression of ubiquitin-DsRed accurately reflects the DNA accessibility of IRER. Fluorescence-activated cell sorting (FACS) was performed to separate DsRed positive (+) and negative (-) cells from larvae. Chromatin Immunoprecipitation analysis of the sorted cells verified that both the DsRed reporter and IRER in DsRed (-) cells are enriched for repressive histone markers such as H3K27Me3 and H3K9Me3, demonstrating that DsRed can only be expressed from cells with an open conformation of IRER. This reporter line allowed us to monitor epigenetic changes in IRER in response to histone modifications. RNAi-mediated tissue-specific knock down of HDAC3 or Su(var)3-9 was able to induce ectopic DsRed in wing discs. In addition, by adapting a fluorescence molecular tomography (FMT)-based method, it is possible to quantitatively measure DNA accessibility in live animals by measuring the fluorescence recovery after photobleaching.

937A
pTubHA4C: a new vector for constitutive expression in Drosophila. Yan Zhang1, Stephanie Arcia1,2, Pedro Fernandez-Funez1,2, Diego Rincon-Limas1. 1) Neurology, University of Florida, Gainesville, FL; 2) Science for Life Undergraduate Program; 3) Neuroscience, University of Florida, Gainesville, FL.

Control of gene expression relies on a variety of strategies, including gene overexpression, gene rescue, and gene silencing. The binary UAS/GAL4 system has become a popular choice for genetic manipulation. However, there are cases in which ubiquitous expression is desirable independently of GAL4. Traditionally, constitutive expression has been achieved by cloning cDNAs under control of ubiquitous promoters such as the Actin5C or Hsp70 genes. Unfortunately, the Actin5C promoter displays heterogeneous expression, and the Hsp70 promoter requires heat induction, which may negatively impact certain experiments. Looking for more homogeneous expression, the promoters of 01-Tubulin, Armadillo or EF-1αF1 were isolated. Since the first intron of these genes contain essential regulatory information, they were used together with the first exon containing the ATG and a fragment of the second exon for ligation of the cDNA. Thus, expression under these promoters requires the creation of a fusion with their own amino acids. Additionally, exon 2 contains few suitable restriction sites, complicating cDNA cloning. To overcome these limitations we created pTubHA4C. This plasmid was designed for expression of coding sequences in Drosophila under control of a simplified Tubulin promoter. For this, we cloned and fused the critical regulatory regions of the promoter and intron 1 of 01-Tubulin, producing a shorter, optimized Tub promoter. Then, we incorporated an optimized polylinker to offer flexible cloning options. Finally, we added two C-terminal tags, hemagglutinin (HA) and tetracysteine (4C), to provide flexibility for detection and analysis of the tagged proteins. In particular, the 4C tag technology allows fluorescent labeling of engineered proteins with a small peptide less likely to alter the structure of the protein. To demonstrate the utility of this vector, the photosensor Phytochrome B from Arabidopsis was expressed and monitored with both HA and TC tags.

938B
The isPIN Project. James D. Baker1, John Bixby2, Willie Buchar3, Maria Blulina1, Xiaodong Cai1, Sophie Deng1, Vineet Gupta1, Neil Johnson1, Tatsuo Kagesawa1, Daichi Kamiyama1, Michael Kim1, Vance Lemmon1, Tiffany Li1, Mitsunori Oghara1, Hasitha Samarajeeva1, Nima Sharifi1, Robin Smith1, Gavriel Tsechpenakis1, Grace Zhai1, Akira Chiba1. 1) Dept Biol, Cox Sci Ctr, Univ Miami, Coral Gables, FL; 2) The Miami Project to Cure Paralysis University of Miami, School of Medicine Miami, FL; 3) University of Miami, College of Engineering, Coral Gables, FL; 4) Miami Institute of Renal Medicine, School of Medicine, University of Miami, Miami, FL; 5) Physics Department, University
of Miami, Coral Gables, FL; 6) Molecular and Cellular Pharmacology Miller School of Medicine Rosenstiel Medical Sciences Building, Miami, FL; 7) Department of Computer Science, University of Miami, Coral Gables, FL; 8) Computer & Information Science Dept., Indiana University-Purdue University Indianapolis Indianapolis, IN.

Our brains are composed of over one hundred billion neurons of diverse functions making over 1 trillion connections. Each neuron contains millions of proteins. Exactly how individual proteins interact to form the complex signaling networks that support life has never been examined directly in live animals. The project will offer the first systematic survey of in situ protein-protein interaction networks, or isPIN. It employs advanced genetics, sophisticated interaction networking technology, and high-performance computation. While the current phase of the project focuses on identified neurons under normal conditions, future likely experiments include stress or disease-affected states as well as non-neuronal cells. By beginning our work on proteins with human orthologs we are bridging basic biology to advanced medicine. Ultimately, the project aims to add information rich contexts to the field of proteomics. Visit: www.ispinproject.org.

939C

Transgene Design of isPIN. Maria Bulina1, James Baker1, Kenneth Wan2, Charles Yu2, Susan Celniker2, Akira Chiba1. 1) Biology, University of Miami, Coral Gables, FL; 2) Lawrence Berkeley National Laboratory, Berkeley, CA 94720.

Individual proteins are each tagged in four-ways: with EGFP on the N-terminus, mCherry on the N-terminus, EGFP on the C-terminus, and mCherry on the C-terminus. They are expressed under the control of cell-specific GAL4 drivers and examined for localization and interactions within model neurons in vivo. The isPIN project is open for collaboration. We will accept your protein of interest (POI) and quantify its interaction with any protein from our protein library, or between an interacting protein pair you supply. Contact: maria@ispinproject.org.

940A

Informatics of isPIN. Sophie T Deng1, Vance Lemmon2, Willie Buscher2, Robin Smith1, Mitsunori Oghara3, Hasitha Samarajeewa1, Akira Chiba1. 1) Biology, University of Miami, Coral Gables, FL; 2) Neurosurgery, University of Miami, Miami, FL; 3) Computer Science, University of Miami, Coral Gables, FL.

Currently, we are focusing on 288 neuronal proteins and up to 41,472 of their interaction combinations within the model neurons. By subdividing the results, up to 48 context-specific proteomic maps will be created for these proteins. The results are then compared to predictions from available literature. GeneGo, for example, predicts that, of 288 "humanized" proteins, 41% have direct-association partners through 'binding', 'covalent association', 'cleavage', 'phosphorylation' and 'dephosphorylation' mechanisms. Our project will be able to not only confirm some of these interactions in real-life but also reveal when and where they occur within the neurons.

941B

In vivo Imaging of isPIN. Tatsuo Kagesawa1, Maria Bulina1, Sophie Deng1, Michael Kim2, James Baker1, Akira Chiba1. 1) Biology, University of Miami, Coral Gables, FL; 2) Pharmacology, University of Miami, Miami, FL.

Localization and interactions of each protein are examined within the model neurons using confocal microscopes. FRET between EGFP and mCherry is interpreted as direct protein association. Two cell types, three time points, four subcellular compartments, and two conditions produce 48 distinct contexts of cell and developmental biology. We focus on primary motoneurons in the CNS (aCC) and sensory neurons in the PNS (ddaC) which have well-studied complex morphology. First, we determine the localization of each protein in the model neurons by using eve'-GAL4 and ppk'-GAL4 drivers. Next, we determine the dynamic interactions between protein pairs within the model neurons. We perform recombination to have both EGFP-tagged and mCherry-tagged proteins, at 2L and 2R respectively, present on the same homologous chromosome. By crossing these recombinant transgenics to the GAL4 drivers, we quantify the FRET between each protein pair.

942C

isPIN: in vivo Imaging. Tatsuo Kagesawa, Maria Bulina, Sophie Deng, Michael Kim, James Baker, Akira Chiba. University of Miami, Coral Gables, FL., USA.

Localizations and interactions of each protein are examined within a model neuron in vivo. FRET between GFP and RFP is interpreted as direct protein association. Two cell types, three time points, four subcellular compartments, and two conditions produce 48 distinct contexts of cell and developmental biology. We focus on primary motoneurons in the CNS (aCC) and sensory neurons in the PNS (ddaC) which have well-studied complex morphology. First, we determine the localization of each protein in the model neurons by using eve'-GAL4 and ppk'-GAL4 drivers. Next, we determine the dynamic interactions between protein pairs within the model neurons. We perform recombination to have both GFP-tagged and RFP-tagged proteins, at 2L and 2R respectively, present on the same homologous chromosome. By crossing these recombinant transgenics to two GAL4 drivers, we examine the FRET between each protein pair.

943A

Quantification of isPIN. Daichi Kamiyama1, Chittaranjan Buranachai2, Robert Clegg2, Akira Chiba1. 1) Biology, University of Miami, Coral Gables, FL; 2) Physics, University of Illinois, Urbana, IL.

FRET (Forster, or fluorescent, resonance energy transfer) offers a visual indication of molecular distance and behavior. Direct association of two interacting proteins brings them typically within 8 nm from each other. When they are each tagged with spectrally matched fluorescent molecules, this nanoscopic distance induces FRET. Because the tagged proteins are not artificially tethered to each other as in many of the popular bioprobes, FLIM (fluorescent lifetime imaging microscopy) is the preferred method of FRET quantification. The new 3D FLIM system used in this project is a fast in vivo molecular imaging system. It allows live FRET detection between molecular partners in their native cellular environment. Whereas the localization of each protein is assessed through four tags, the interactions among them are quantified through eight FRET-able tags.

944B

A Sample Analysis of isPIN. Nima Sharifai, Maria Bulina, Daichi Kamiyama, Akira Chiba. Biology, University of Miami, Coral Gables, FL.

Recent work has shown that Cdc42, a key protein in initiating dendrites, requires interaction with a group of proteins containing a Cdc42-Rac-interactive binding (CRIB) motif. We employ two complementary approaches to characterize the protein-protein interaction networks responsible for dendrogenesis. First, we use iProbe to identify the endogenous CRIB effectors of Cdc42 within the model neurons. Second, we use the new protocol to reveal the site-specific interactions between Cdc42 and each CRIB effector. The results will illustrate the potential of the isPIN project.

945C

Model Neurons of isPIN. Gavriil Tsechpenakis1, Hasitha Samarajeewa2, Miahcel Kim3, Akira Chiba2. 1) Computer Science, Indiana University-Purdue University, Indianapolis, IN; 2) Biology, University of Miami, Coral Gables, FL; 3) Pharmacology, University of Miami, Miami, FL.

We apply computer-assisted automations through the following steps. First, when acquiring the 3D FLIM image data, the most time-consuming part of the data collection, we use a 'smart-scan' protocol available as a custom software module from 3i. Randomly oriented embryos and/or larvae in each well of the 24-well plate will be first scanned quickly with a 40x objective lens. Second, we align each set of 3D images from individual model neurons to a standard model through automated image alignment. Third, we consolidate all aligned 3D data into the averaged 1D presentation. The results are expressed as a mean±s.e.m. FRET efficiency per voxel for a given neuron.
POSTER: Techniques and Functional Genomics
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

946A
The BDGP Universal Proteomics Resources. Kenneth H. Wan1, Charles Yu1, James Baker2, Maria Boulima2, Ben Booth1, Joe Carlson1, Akira Chiba3, Ann Hammonds1, Gurusha Kuthetru2, Julian Minteris1, Robert Obar1, Soo Park1, Jean-François Rual2, Richard Weissmann1, Bo Zhai1, Susan Celniker1. 1) Dept Genome Biol, Lawrence Berkeley Natl Lab, Berkeley, CA; 2) Dept of Cell Biology, Harvard Medical School, Boston, MA; 3) Univ. of Miami, Coral Gables, FL.

The Berkeley Drosophila Genome Project (BDGP) is generating a comprehensive cDNA resource, the Drosophila Gene Collection (DGC), with a goal of providing a clone for all 13,794 annotated protein-coding genes. The DGC Gold Collection contains more than 10,000 full-length cDNA clones that encode proteins with a perfect match to a FlyBase annotation. This represents 68% of the annotated protein-coding genes, or 55% of the protein-coding transcripts. The Gold Collection continues to expand as cDNAs representing new and alternative transcript models are discovered from the modENCODE project. The Gold clones are being used to construct the Universal Donor Clone collection, a source of high quality open reading frames (ORFs). The ORFs are subcloned into the pDNR-Dual vector to generate sets of expression-ready clones, one with the native stop codon, for tagging at the amino terminal end; another without the native stop codon, for tagging at the carboxy terminal end. Over 75% of the Gold Collection ORFs are available without the native stop codon (XO clones), and over 50% are available with the native stop codon (XS clones). The donor clone ORFs can be moved into an acceptor expression vector by homologous recombination. The BDGP has developed a variety of acceptor vectors for expressing N-terminal or C-terminal fusion proteins with different tags and for use in multiple biological systems. We produced two types of expression clones: one that allows expression in tissue culture with a metallothionein inducible promoter (pMK33-based vectors) with either a carboxy-terminal TAP or FLAG- HA tag; and another set designed for expression in transgenic flies using the attP landing site and the UAS expression system (pUAST-based vectors) with TAP, FLAG-HA, mCherry or eGFP tags. The clones are available without restrictions to all researchers.

947B
A New Technique for Incorporating Small Mutations In Large Genes: Mutagenesis via Serial Small Mismatch Recombineering (MSSMR). Julie Jacobs, Xiaojing Hong, Daniel Eberl. Department of Biology, University of Iowa, Iowa City, IA 52242, USA.

Very few methods are available to clone large (>20kb) genes, and even fewer are compatible with site-specific mutagenesis and cloning. We have developed a technique that uses recombineering protocols to create mutations as small as a single base pair in genes of any size. Mutagenesis via Serial Small Mismatch Recombineering (MSSMR) allows cloning of wild type and mutant constructs in the same recombination reaction. The clones are constructed directly in transformation vectors, so there is no need to subclone products after mutagenesis. MSSMR products contain seamless mutations with no undesired genetic material left behind (as in cassette insertion and replacement approaches), and unlike site-directed mutagenesis no post-transformation crosses are needed to establish mutant clones. Because the technique is based on recombineering, there is little chance for PCR-induced errors. Efficiency rates are comparable with or better than other mutagenesis strategies. We made separate constructs with designer mutations in 1, 2 and 3 amino acids in the middle of a 30 kb gene using two sequential recombineering reactions. By using a vector with phiC31 targeting abilities, we were then able to create transformants carrying the mutations at identical chromosomal locations in each line, so each mutation could be compared directly to the wild-type construct. While ideal for manipulation of large genes, MSSMR is compatible with mutagenesis at any location in genes of all sizes.

948C
Manipulating Gene Expression Using Recombineering. Hitish Kathuria, Parul Khurana. School of Natural Science and Mathematics, Indiana University East, Richmond, IN 47374. hikathur@iue.edu.

Recombineering (recombination-mediated genetic engineering) is a powerful method for fast and efficient construction of vectors. Traditionally, only large size, low-copy number BAC have been successfully manipulated using recombineering. The BDGP has engaged in a variety of acceptor vectors for expressing N-terminal or C-terminal fusion proteins with different tags and for use in multiple biological systems. We produced two types of expression clones: one that allows expression in tissue culture with a metallothionein inducible promoter (pMK33-based vectors) with either a carboxy-terminal TAP or FLAG-HA tag; and another set designed for expression in transgenic flies using the attP landing site and the UAS expression system (pUAST-based vectors) with TAP, FLAG-HA, mCherry or eGFP tags. The clones are available without restrictions to all researchers.

949A
A novel method for mosaic gene expression with Cre/loxP system in Drosophila embryos. Naotaka Nakazawa1, Kiichiro Taniguchi1, Takashi Okumura1, Reo Maeda1, Kenji Matsuno2,1. 1) Department of Biological Science and Technology; Tokyo University of Science; Noda, Chiba, Japan; 2) Research institute for science and technology; Tokyo University of Science; Noda, Chiba, Japan.

Many techniques have been developed for studying the development of Drosophila melanogaster. Mosaic analysis is a powerful tool to assess the functions of genes in a subset of the cells in an organism. Although some methods for mosaic analysis have been developed, it is still difficult to generate mosaic cells in the most of Drosophila embryonic tissues.

Here, we report a novel method to generate mosaic embryos during early embryogenesis using a modified Cre/loxP system. In this method, we constructed a novel cassette of loxP combined with Actin5C promoter and GAL4 cDNA, designated as pAct5C-loxP-gypsy-loxP-Gal4 (pAct5C-Gal4), and generated transgenic flies carrying this construct (Act5C-Gal4). In these flies, GAL4 expression is suppressed by gypsy insulator. Cre recombinase (Cre) provided in dual hop70-Mos1 promoter-dependent manner induces site-specific recombination in the two loxP sites (Siegel et al., 1996). When site-specific recombination between the loxP sites is induced by Cre, the removal of gypsy insulator results in the activation of GAL4 expression.

After the recombination, we detected the GAL4 expression using UAS-Redstinger. The clonal expression of Redstinger was observed in almost 100% of embryos from embryonic stage 10. In the hindgut epithelium at embryonic stage13, about 50% cells in each embryo expressed Redstinger. Using Actin5C-Gal4 in a mutant background, we can easily make mosaic embryos composed of mutant cells with and without the overexpression of a gene disrupted in this mutant. In these mosaic embryos, phenotypes of cells homozygous for the mutant were rescued in the mutant cells overexpression the gene responsible for the mutation. Thus, our novel system is useful to study the cell-autonomy of gene functions during the early embryogenesis of Drosophila.
Mapping of signaling networks through synthetic genetic interaction analysis by RNAi. Thomas Horn1, Thomas Sandmann1, Bernd Fischer2, Wolfgang Huber3, Michael Boutros1. 1) Signaling & Functional Gen, German Cancer Res Ctr, Heidelberg, Germany; 2) Genome Biology Unit, EMBL, Heidelberg, Germany.

Studies in various model organisms have revealed the existence of pervasive genetic interactions between independent genetic loci with large effects on numerous phenotypes. The characterization of these genetic interactions is therefore an essential prerequisite towards understanding the tremendous complexity of biological systems, the underlying evolutionary processes or the molecular mechanisms of genetic disease.

Large-scale RNAi screens in cultured cells have successfully identified novel components of diverse signaling pathways, but revealed little about the interactions between the different components. To explore the underlying network connectivity, simultaneous perturbations of multiple components are required. RNAi offers the opportunity to simultaneously reduce the expression of any chosen pair of genes, allowing to systematically sample large numbers of distinct, biologically relevant conditions.

Here, we report a robust method to identify genetic interactions in tissue culture cells through combinatorial RNAi and automated microscopy. By performing more than 70,000 pairwise perturbations of signaling factors, we identified >600 interactions affecting different quantitative phenotypes of Drosophila cells. Computational analysis of this interaction matrix allowed us to reconstruct signaling pathways and to identify Cka (CG7392) as a novel, conserved positive regulator of Ras/MAPK signaling. Large-scale genetic interaction mapping by RNAi is a versatile, scalable approach for revealing gene function and the connectivity of cellular networks. We are currently applying this method to map the genetic interaction space of chromatin biology.

The TRiP: The Transgenic RNAi Project at Harvard Medical School. Donghui Yang-Zhou1, Laura Holderbaum1, Jianquan Ni1,5,6, Norbert Perrimon1,2, Lizabeth Perkins6,1. 1) Harvard Medical School, Dept. of Genetics, Boston, MA 02115; 2) Howard Hughes Medical Institute; 3) Pediatric Surgical Research Labs, Mass. General Hospital, Boston, MA 02114.

The Drosophila Transgenic RNAi Project (TRiP) started in 2006 when the HHMI/Janelia Farm Visitor Program supported a pilot project between the laboratories of N. Perrimon, C. Zuker and G. Rubin. As of 2008, with the backing of the NIH/NIGMS, the TRiP has the goal to generate 6,250 transgenic RNAi lines by using phiC31-targeted integration combined with the Gal4/UAS system. Our RNAi lines are designed to fill in the phenotype gap and help researchers overcome issues associated with pleiotropy. The VALIUM (Vermilion-AttB-Lexp-Intron-UAS-MCS) series of vectors was generated for introducing RNAi into the genome. Our first generation of vectors, VALIUM1 and VALIUM10 proved effective for transgenic RNAi (Ni et al., 2008. Nature Methods; Ni et al., 2009, Genetics) and a total of 2500 TRiP stocks have been generated in these vectors. One limitation of the early VALIUM vectors, as is also the case with other RNAi vectors that use long dsRNAs, is that they do not work in the female germline. Recent work using a short hairpin RNAs (shRNAs) approach has overcome this limitation. The second generation of the TRiP combines the superior VALIUM20 vector with these shRNAs, delivered through the microRNA pathway, to produce effective knockdown in both the germline and soma. New TRiP lines are now being generated using this novel strategy. In addition we have developed the VALIUM22 vector, which shows potent knockdown of germline expressed genes. Genes to be targeted for production are selected based on the Bloomington Drosophila Stock Center (BDSC) mandate of one mutation per gene, the needs of screeners at the Drosophila RNAi Screening Center (DRSC), and the needs of the Drosophila community for in vivo phenotypic analyses. The community is invited to review the list of available TRiP stocks on our website (www.flyrnai.org/TRiP-HOME.html) and email us to nominate any gene of interest that is not yet available. All VALIUM1 and VALIUM10 stocks can be ordered through the BDSC.

A genome-scale shRNA resource for transgenic RNAi in Drosophila. Rui Zhou1,6, Jian-Quan Ni1,5,6, Benjamin Czech1,2, Lu-Ping Liu1,5, Laura Holderbaum1, Donghui Yang-Zhou1, Hye-Seok Shin1, Dominik Handler2, Philip Karpowicz2, Richard Binari2, Matthew Booker1, Julius Brennecke1, Lizabeth A. Perkins1, Gregory Hamon2, Norbert Perrimon1. 1) Department of Genetics, Harvard Medical School, HHMI, Boston, MA; 2) Cold Spring Harbor Laboratory, HHMI, Cold Spring Harbor, NY; 3) IMBA-Institute of Molecular Biotechnology, Vienna, Austria; 4) Pediatric Surgical Research Labs, MGH, Harvard Medical School, Boston, MA; 5) Gene Regulation Laboratory & Tsinghua Fly Center, Tsinghua University, Beijing, China; 6) Equal contribution.

Transgenic RNAi has proven a powerful method for investigating gene functions in vivo. For use in Drosophila, genome-wide resources, based on the expression of long hairpin (long-hp) RNAs, have been made available and are currently used widely. However, these reagents are limited in that they are unable to induce effective knockdown in the female germ line. Thus, different approaches are needed to enable analysis of oogenesis and of maternally deposited mRNAs that are important for early embryogenesis. We have found that short hairpin RNAs (shRNAs), modeled on an endogenous microRNA, are extremely effective at reducing gene expression during oogenesis. To illustrate the usefulness of this approach, we compared the effects of shRNA knockdown to mutations known to impact the Piwi-interacting RNA (piRNA) pathway that is required to control transposons in the germ line. We are currently building genome-wide resources comprising both shRNA expression libraries and animals carrying inducible shRNA transgenes, which will be made available to the research community. In the meantime candidate genes to be targeted for production are selected based on the Bloomington Drosophila Stock Center (BDSC) mandate of one mutation per gene, the needs of screeners at the Drosophila RNAi Screening Center (DRSC), and the needs of the Drosophila community for in vivo phenotypic analyses. The community is invited to review the list of available TRiP stocks on our website (http://www.flyrnai.org/TRiP-HOME.html) and send us an email to nominate any gene(s) of interest that are not yet available.
POSTER: Techniques and Functional Genomics
See page 16 for presentation schedule. Poster board number is above title. The first author is the presenter.

953B
Identifying gene mutations in Drosophila melanogaster with inverse PCR: A thematic academic laboratory experience. Dawn M. Hemmerle, Gerald B. Call. Department of Pharmacology, Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ.

Typically, genetics laboratory classes deal with separate subject matters that provide little to no continuity to student learning. Laboratory subjects often range from Mendelian to molecular to population genetics with no apparent connection. Since molecular techniques are commonly used in modern genetics laboratories we decided to focus our curriculum on them. Indeed, techniques such as PCR, sequencing and restriction digests are commonly taught in the academic genetics laboratory. When combining these techniques with the scientific method, it provides an invaluable educational experience for students. We created a cohesive curriculum based on the single theme of utilizing inverse PCR to identify the location of transposon insertions in Drosophila. This curriculum taught students all necessary techniques for them to perform inverse PCR through a series of laboratory periods, and provided an opportunity for them to identify mutations in ambiguous insertion sets obtained from the Bloomington Stock Center. Student data identified over 25 insertions that either verified previous or corrected erroneous information. To ascertain the impacts of this curriculum as a valid research experience, students participated in both pre- and post-course surveys. Data suggested that students appreciated the ability to develop critical thinking, analytical and troubleshooting skills. This thematic approach directed the students’ efforts and gave them a goal that provided motivation for active learning and participation in the academic laboratory. In addition, students developed an appreciation for accuracy and attention to details as subsequent laboratory experiments built upon previous laboratory techniques and results. By utilizing discovery-based learning in the academic laboratory, undergraduate students were provided an opportunity to contribute directly to the Drosophila research community, and have an increased educational experience.

954C
FIRST: Fellowships in Research and Science Teaching - A Postdoctoral Training Opportunity Blending Bench Research and Undergraduate Teaching at Minority Serving Institutes. Jacob Daniel Kagey1, Seth M. Kelly1, Arri Eisen2, J.K. Haynes3, Douglas C. Eaton4. 1) Department of Cell Biology, Emory University, Atlanta, GA; 2) Department of Biology, Emory University, Atlanta, GA; 3) Department of Biology, Morehouse College, Atlanta, GA; 4) Department of Physiology, Emory University, Atlanta, GA.

The FIRST (Fellowships in Research and Science Teaching) program at Emory University in Atlanta, Georgia is part of the NIH sponsored IRACDA (Institutional Research and Academic Career Development Award) initiative. The FIRST program provides postdoctoral fellows with a unique training experience that combines laboratory research at Emory University or Morehouse School of Medicine with teaching at one of three historically black colleges within the Atlanta University Center (Morehouse College, Spelman College, or Clark Atlanta University). The objectives of the FIRST program are four-fold. Most importantly, FIRST aims to increase the number of well-qualified minority students entering graduate and/or professional school. Second, the program supports the implementation of innovative teaching techniques at MSIs (Minority Serving Institutes) while creating an environment where students are introduced first-hand to fellows’ research projects. Third, FIRST allows fellows to conduct high quality research in an outstanding academic environment and promotes both teaching and research collaborations between research-intensive institutions and MSIs. Finally, FIRST strives to develop future faculty who are well versed in both contemporary education/pedagogical and biomedical research strategies. A recent comparison of FIRST fellows with traditional post-docs found that FIRST fellows have comparable publication rates and are funded by the NIH at comparable levels to their traditional counterparts. Furthermore, FIRST fellows were also just as successful at obtaining academic jobs as traditional post-docs. Overall, the FIRST program provides a unique training opportunity combining extensive bench research with pedagogical training and mentorship, both of which extensively prepares fellows for a variety of positions following the fellowship.

955A

I have designed a semester-long laboratory project for an intermediate level Genetics course that complements and reinforces the key concepts covered during the lecture portion of a typical undergraduate laboratory course. The goal of the laboratory project is to have each student generate a new allele of a random gene by using P element based insertional mutagenesis. During the first half of the semester, when I cover mechanisms of inheritance in lecture, the students set up a series of crosses to mobilize a P element located on the X chromosome and isolate lines that contain the element in a new location in the genome. On weeks when we are waiting for the flies to breed, we have discussions during lab of published studies that have used transposable element based mutagenesis to study gene function. In the second half of the semester, when I cover topics related to molecular genetics in lecture, the students map the location of the P element in their new fly line by Inverse PCR. At the end of the semester, students present the location of the element in their new line to the class and propose a study using their fly line to explore the function of a nearby gene. This lab project has allowed me to repeatedly make connections between the lecture and lab portions of the course, giving the students a richer experience that integrates across the entire course. Student response to the project has been overwhelmingly positive, with many commenting on the interconnectedness of topics and the excitement of working on an independent project with a novel outcome.

956B

I teach a junior/senior level undergraduate genetics course, the first portion of which is devoted to transmission genetics. My students diligently work their problem sets and learn to recognize the “tricks of the trade” such as the use of out-crosses, test-crosses and reciprocal crosses, as well as complex inheritance patterns such as recessive epistasis or complimentary gene action. The problem is, most of this knowledge is based on “book learning”, as the real-world examples of some of these more unusual modes of inheritance are often illustrated by the shape of squash fruit or chicken feather color. To give my students a more investigative experience with these inheritance patterns, they work through a laboratory that keys on the fact that several different fly genotypes result in a white-eyed phenotype. Utilizing different combinations of scarlet, brown, cinnamon and white mutations, as well as the judicious use of balancers, key crosses from a six-by-six matrix (The Matrix) are taken over and scored to completion by students. After determining the actual ratios of white-eyed and “non-white-eyed” progeny by Chi-squared analysis, students then work (hard) to synthesize the entire matrix into a workable prediction of the parent flies’ genotypes.

The Genomics Education Partnership (GEP) is a distributed group of institutions that give undergraduates the opportunity to participate in genomics research through sequence improvement, annotation, and comparative evolution leading to scientific publications. Our current focus is on the largely heterochromatic Müller F element (dot chromosome). We seek to determine what genetic characteristics can distinguish heterochromatic and euchromatic domains. Comparison of the dot in D. melanogaster (Dmel) and D. virilis (Dv) demonstrated higher repeat density, larger gene size, lower codon bias, and a higher rate of gene rearrangement as compared to reference euchromatic domains. Analysis of orthologs that moved between heterochromatic and euchromatic domains shows that these genes adopt the properties of their local environment. An analysis of the D. mojavensis dot compared to those of Dmel and Dv shows it to have a higher level of repetitive sequence, while other heterochromatic properties remain essentially the same. Didactically, students appreciate the ability to contribute to ongoing research and show knowledge gains in analyzing genes and genome organization. Students who have participated through a class setting show learning and personal gains comparable to students involved in a full-time summer research experience. Funded by HHMI & NIH.

MAPPING AND CLONING 100-YEAR-OLD MUTATIONS IN AN UNDERGRADUATE COURSE. Eric P Spana, Daniel C Chun, Yi Dong, David Jung, Jason Klein, Si Won Oak, Zachary B Powell, David B Rothschild, Brandon Ruderman, Arun Sharma, Alvin H Shi, Bo Sun, Andrea Stewart. Biology Dept, Duke Univ, Durham, NC.

Over 90 years ago, Calvin Bridges and Thomas Hunt Morgan published detailed descriptions of mutations on the 1st (1916), 2nd (1919) and 3rd (1923) chromosomes. Our class has mapped and is attempting to identify four mutations described in these publications that are not cloned, have stocks available, and display the phenotype as described. The mutants we have chosen are curved, speck, spread and tilt. Identified in 1911 by Bridges, curved (c) has wings that are concave in an outward direction. Through complementation testing, we mapped the location of c1 to a region of ~30 kb encoding 8 transcripts. One gene in this region, Stretchin-Mlck, has mutations of which are reported to be allelic to c1. Morgan identified the first speck (sp) mutant in 1910 as having a heavily melanized spot on the wing hinge. We mapped sp1 and sp2 to a 121 kb interval and identified an insertion in Dopamine N acetyltransferase (Dat) that phenocopies sp. Ubiquitous expression of an RNAi transgene against Dat can produce adults with a strong sp phenotype. We propose that speck is a mutation in the Dat locus. The spread (sprd) mutant identified by Dexter in 1915 holds out its wings at right angles to the body. sprd1 originated on In(3R)C, and we find that stocks of In(3R)C, sprd1 have no wing phenotype. The presence of sprd1 in these genotypes is due to a database annotation error and the sprd1 allele has been lost. A P-element allele, sprd05284 maps to a similar position as sprd1 and has a wing phenotype that is less severe than sprd1. tilt was first described by Bridges in 1915 as having wings that are held out at a wider angle and have a break in the L3 vein. We find that the tilt wing has variable penetrance and frequently lack the campaniform sensilla of L3. Complementation testing places tilt in an interval that includes the Iroquois complex. Molecular and phenotypic characterization of these alleles is underway.
Genetics Society of America Members: Save on ALL Annual Reviews Journals.
Discounted pricing available for GSA Members. Orders should be placed through the offices of the GSA.

Annual Review of Genetics
Volume 44, December 2010 • Available Online & In Print • http://genet.annualreviews.org
ISSN: 0066-4197 • ISBN: 978-0-8243-1244-2 • Regular Personal Copy Price (WORLDWIDE): $84
Editor: Allan Campbell, Stanford University

The Annual Review of Genetics, in publication since 1967, covers significant developments in the field of genetics. These include biochemical, behavioral, cell, and developmental genetics; evolutionary and population genetics; chromosome structure and transmission; gene function and expression; mutation and repair; genomics; immunogenetics; and other topics as related to the genetics of viruses, bacteria, fungi, plants, and animals, including humans.

This journal is ideal for all geneticists, as well as those in the fields of cell and developmental biology, biochemistry, microbiology, and other life sciences.

Annual Review of Genomics and Human Genetics
Volume 11, September 2010 • Available Online & In Print • http://genom.annualreviews.org
ISSN: 1527-8204 • ISBN: 978-0-8243-3711-7 • Regular Personal Copy Price (WORLDWIDE): $84
Co-Editors: Aravinda Chakravarti, Johns Hopkins University, School of Medicine
Eric D. Green, Bethesda, MD

The Annual Review of Genomics and Human Genetics, in publication since 2000, covers significant developments in the field of genomics as they apply to human genetics and the human genome. The journal places particular interest in the areas of genomic technology, genome structure and function, genetic modification, human variation and population genetics, human evolution and, importantly, all aspects of human genetic disease including individualized medicine.

This journal is ideal for genome scientists, human and mammalian geneticists, and physicians, as well as those in the fields of cell and developmental biology and other life sciences.
Speed Up Your Research and Get Results Quicker!

Fast, Efficient Fly Injections and Transgenic Production

Professional Custom Genetic and Molecular Services

- Transgenic production
- Embryo injections
- Non melanogaster injections
- Site directed transgenesis
- Genetic strain and stock production
- Custom mutagenesis and genetic screening
- Mosquito injections
- Stock maintenance

Considering a Large Scale or Genome Wide Transgenic Project?

Contact us about our New Multiplex Transgenesis System!

Our proven techniques allow for accelerated and accurate large scale DNA preparation, injection and transgenic screening.

- Serving over 800 Drosophila labs in 48 U.S. states and 28 countries
- Over four million embryos injected

© 2011 Genetic Services, Inc. All right reserved.
CALL FOR PAPERS

The Genetics Society of America is accepting manuscripts for G3: Genes | Genomes | Genetics, a new peer-reviewed, peer-edited, fully open access journal (inaugural issue to be published in June 2011).

Why G3? To publish the puzzling as well as the novel finding, with an emphasis on experimental design rather than immediate or subjective impact.

G3 is about:
- Data quality and utility
- Rapid publication
- Creating and maintaining links between data and articles
- Open-access publishing with data availability

G3 seeks to publish articles that include:
- Large-scale datasets;
- Population data (e.g. QTL studies);
- New methods and technologies for the production and analysis of large-scale genetic datasets;
- Novel mutant screens, collections, reagents and resources made available to the community for further analysis.

Learn more: http://www.g3journal.org
Submit a manuscript: http://submit.g3journal.org
Contact G3: g3-gsa@andrew.cmu.edu

Editor-in-Chief:
Brenda J. Andrews
Director, The Donnelly Centre for Cellular and Biomolecular Research
University of Toronto
SCHEDULE OF EVENTS, EXHIBITOR LIST, REGISTRANT LIST, AND MORE

AVAILABLE ON YOUR MOBILE SMARTPHONE

m.dros-conf.org

To use the above QR code: Go to your app store or you can go to www.mobile-barcodes.com to find the QR code reader for your mobile phone. Click on the reader to get further instructions and a quick link to download the application. Install the application on your device.