Crumbs and its function in epithelial polarity in Drosophila melanogaster. Sven Klose, Elisabeth Knust. Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

Epithelia are tissues that face either the outside of organisms or their inner lumina. They fulfill various tasks such as secretion and uptake of molecules, communication and barrier function. To afford these tasks a highly organized polarity of the epithelial cells is necessary. This polarity is reflected by an asymmetric distribution of lipids and proteins within the plasma membrane. In many Drosophila epithelia, the Crumbs complex is required to maintain epithelial polarity. It consists of the core components Crumbs, Stardust, DLIn7 and DPATJ and is localized apical to the Zonula Adherens (ZA) in the Subapical Region (SAR). Crumbs is a type 1 transmembrane protein, the cytoplasmic domain of which binds to the PDZ domain of the MAGUK protein Stardust. Embryos lacking Crumbs or Stardust fail to maintain apico-basal polarity of many epithelia and die with severe defects. It has been shown that the membrane-bound intracellular domain of Crumbs (Crbintra) is sufficient to partially suppress the crumbs mutant phenotype in embryos. Additionally, it is known that Crumbs plays an important role in the biogenesis of the ZA. To get further insights into the mechanisms, how the Crumbs complex controls epithelial polarity, following projects have been approached: 1) Investigation of the unusual long signal peptide of Drosophila Crumbs. 2) Establishment of a new assay to perform structure-function analysis by using the genomic region of crumbs in combination with genetic recombineering.

Retromer regulates apico basal polarity during epithelium development. Bo Zhou1, Xinhua Lin1,2. 1) Dev Biol, Cincinnati Children's Hosp, Cincinnati, OH; 2) State key laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Beijing, China.

Epithelial cells are characterized by an apical-basal (A/B) polarization. Apical and basolateral membranes are functionally distinct cell membranes, isolated by adhesion junctions. Four classes of protein complexes are involved in A/B polarity determination. The Crumbs (Crb) complex and the Bazooka complex define the apical region; while, the Lethal giant larval complex and the Yurt/Coracle complex define the basolateral region. Retromer is an evolutionarily conserved membrane-associated protein complex. Retromer is composed of two sub-complexes: Vacular protein sorting35 (Vps35), Vps26, Vps29 form a heterotrimer and provide the cargo selectivity; SNX1 and SNX2 form a homo- or heterodimer and tether the Vps35-Vps29-Vps26 complex to the membrane. Previous studies found that retina primarily mediates an endosomes-to-TGN retrograde transportation of specific transmembrane cargos, such as mammalian cation-independent mannose-6-phosphate receptor. Recently, interests are brought to the roles of retromer during development. Work from our and other labs showed that the retromer regulates Wnt secretion by controlling the stability of Wntless, which binds and escorts Wnt during its secretion. Here, we report our findings that retromer regulates A/B polarity in epithelial cells. Vps35 is a major component in the retromer complex. We generated a vps35 null mutant in Drosophila. Germ-line clone analysis suggests that the apical identity is disrupted in vps35 mutant epithelial cells. Further study shows that among apical determinants, Crb is the primary target affected in vps35 mutants. In wild type cells, we observed a colocalization between Crb and retromer. Based on the working model of retromer, we hypothesize that the retromer complex regulates the recycling and stability of Crb and thus the A/B polarity in epithelial cells.


The formation and regulation of adherens junctions (AJs) are essential for normal development and the misregulation of their components contributes to metastasis. AJs are composed of transmembrane adhesion molecules such as cadherins and nectins, and adaptor molecules like catenins and afadin that directly or indirectly link to the actin cytoskeleton. Previous studies from our lab showed that Drosophila’s afadin homolog Canoe (Cno) play roles in orchestrating AJ-dependent apical constriction during morphogenesis. When Cno was completely removed from embryos, the actomyosin cytoskeleton disconnects from AJs during mesodermal invagination and apical constriction of mesodermal cells is impaired. Surprisingly, Cno is not essential for initial AJ assembly, or for AJ maintenance in many tissues, suggesting that at least in Drosophila, the nectin-afadin system is not essential for cell-cell adhesion. We are currently investigating the mechanism by which Cno activity is regulated. One model we are testing is that intramolecular interactions between different domains create a closed conformation. Rap1 and/or F-actin binding might open up Cno, allowing binding to partners such as nectins and cadherins. ZO-1 is a multidomain scaffolding protein which links the actin cytoskeleton to tight junctions (TJs) and AJs in epithelial cells. Recent evidence suggests a role for ZO-1 in regulating both TJs and AJs. Polychaetoid (Pyd), the Drosophila homolog of ZO-1, is known to genetically and physically interact with Cno and is implicated in regulation of AJs. We have generated a deletion of the Pyd coding sequence. To our surprise, mutants are viable and partially fertile. However, maternal and zygotic knock-down of Pyd led to defects in head involution during Drosophila embryogenesis. We are investigating the possibility of crosstalk between Cno and Pyd, and how they work together to regulate AJs and the cytoskeleton.

Both motor and cargo-binding domains of myosin VI are required for actin stabilization during Drosophila spermatid individualization. Mamiko Isaji, Deborah J. Frank, Kathryn G. Miller. Biology, Washington University, St. Louis, MO.

Stable actin structures are important in many cellular processes and differentiation events, but how these cytoskeletal formations are generated and regulated is not well understood. The final stage of Drosophila spermatogenesis, individualization, serves as a good model for addressing these questions. Meiotic and mitotic divisions without cytokinesis result in cysts of 64 syncytial spermatids, which must be separated into individual sperm. Dense cones of actin form encircling the axoneme in each of the spermaticds. The 64 cones travel synchronously down the axonemes, excluding cytoplasmic contents and organelles, and remodeling the cell to generate individualized sperm. The motor protein myosin VI localizes to the fronts of these cones and is essential for their maintenance and function. To understand myosin VI’s mechanism, we have used domain deletions and site-directed mutagenesis to test which features of the molecule are important. Our experiments have revealed that both the motor domain and the cargo-binding tail are essential for localization, but neither is sufficient. This is in contrast to finding of others that the tail is sufficient for localization in mammalian cells. Although myosin VI that lacks the cargo-binding tail region can bind to and partially stabilize actin cones, it does not localize to the front, instead binding along the length of the cone. Improperly localized myosin VI cannot fully rescue cone function, shape, or actin density. Several specific cargo-binding sites in the tail that were identified in mammalian myosin VI are necessary for localization and function in Drosophila. Consistent with the fact that the motor and tail domains are well-conserved, mammalian myosin VI is also able to encircle spermatic cones during individualization. Finally, close examination of actin cone shape and localization of actin regulatory proteins is revealing how different domains of myosin VI contribute to its function in generating this stable actin structure.
The cytoskeletal protein spectrin plays a role in lipid droplet genesis and storage. Bianca Diaconescu1, Gloria H. Mazock1, Anthony P. Mahowald2, Ron R. Dubreuil1. 1) Biological Sci, Univ Illinois at Chicago, Chicago, IL; 2) Molecular Biosciences, University of Chicago, Chicago, IL.

Spectrin is a cytoskeletal protein with roles in plasma membrane structure, composition, and morphology. In the course of a UAS-Gal4 screen for effects of knocking down the β subunit of spectrin in specific tissues, a phenotype was observed in the morphology of larval fat body cells. CD8-GFP was used as a marker to characterize the plasma membrane in wild-type and RNAi knocked down fat body. Plasma membrane staining in wild-type larvae appeared thick over the “ecto” surface of the fat body facing the hemolymph. This region exhibited a striking foamy appearance in en face views of the fat body. The same pattern was observed by staining with anti-β-spectrin antibody. When β-spectrin expression was knocked down with RNAi the foamy appearance was lost and the “ecto” surface appeared markedly thinner by CD8-GFP fluorescence. Closer examination by electron microscopy revealed that in wild-type animals the plasma membrane forms deep invaginations that drape around a layer of small lipid droplets found near the cell cortex. Together, these features account for the foamy appearance observed by fluorescent staining. The small cortical lipid droplets are distinct from the familiar large lipid droplets that are easily observable by light microscopy. RNAi knockdown of β-spectrin led to a dramatic reduction in the number of cortical lipid droplets and their associated plasma membrane invaginations. Yet, remarkably, neither the viability nor growth of adults was affected by the loss of the small lipid droplets. Overexpression of β-spectrin in the fat body is toxic, resulting in cell death. Interestingly, the ensuing loss of fat body cells results in a dramatic pileup of lipid droplets in the midgut epithelium. These data suggest a novel role for spectrin in the mobilization of lipids from the midgut to the fat body. We speculate that spectrin modulates one of the pathways responsible for lipid translocation in fat cells.

845B

Moesin, a membrane-associated protein, is involved in regulating the actin-cytoskeleton, and is necessary for epithelial integrity, cell survival, and regulation of Hedgehog signaling in Drosophila. All of these properties require the ability of Moesin to negatively regulate Rho1 activity, but the mechanism by which it does this is still unclear. To address this problem, we conducted a yeast-2 hybrid screen to identify interacting proteins with the potential to regulate Rho activity. In this screen we identified CG17082, which is predicted to encode a RhoGAP, a class of proteins known to regulate Rho by increasing its intrinsic GTPase activity. Co-immunoprecipitation experiments confirm the interaction in Drosophila S2 cells. To further investigate the functional significance of this interaction in epithelial tissues, we have generated an epitope-tagged CG17082 transgene, RNAi transgenes, and antibodies to the CG17082 protein. We have demonstrated that the epitope-tagged CG17082 transgene localizes to the cell cortex and this localization is Moesin dependent. Additional knockdown of CG17082 by RNAi results in increased F-actin, suggesting upregulation of Rho1 activity. We are continuing to investigate the function and localization of CG17082 in Drosophila to determine if Moesin negatively regulates Rho via this GAP.

846C
Epsin-dependent Delta endocytosis by signaling cells is not for the purpose of ligand recycling. Susan M. Banks, Xuanhua Xie, Bomsoo Cho, Ji-Hoon Lee, Janice A. Fischer. ICMB, Univ Texas, Austin, Austin, TX.

The importance of Notch signaling is well established. However, the specific mechanisms required for the signaling process are not yet fully understood. Notch ligands (Delt and Serrate) must be internalized by the signaling cells to activate Notch signaling. The recycling model suggests the ligand is internalized, processed or activated, and then recycled back to the membrane to interact with the Notch receptor. auxilin mutants were isolated as dominant enhancers of the rough eye caused by overexpression of the endocytic factor epsin. Like epsin, auxilin is required specifically in Notch signaling cells. Uncoating of clathrin-coated vesicles results in ligand-containing uncoated vesicles and also the release of free clathrin, and possibly epsin. The requirement for auxilin could be explained by the recycling model; efficient vesicle uncoating by auxilin is required in order for ligand-containing endosomes to fuse with the sorting endosome, and enter an endosomal pathway for activation and ultimate recycling to the plasma membrane. We present strong evidence that the requirement for auxilin by the signaling cells does not reflect a requirement for ligand recycling, and in fact, argues strongly against the recycling model. In flies that lack auxilin, yet contain transgenes that overexpress clathrin and epsin, Delta-containing vesicles would not be uncoated efficiently, and yet Notch signaling is unaffected. This result suggests strongly that the role of auxilin in Notch signaling is to provide free clathrin, and possibly also epsin, for use at the internalization step. Thus, we reason that the recycling model cannot explain the absolute requirement for epsin-dependent endocytosis in the signaling cells. The results of preliminary experiments with Rab5 and Rab11 mutants suggest that neither Rab is required in the signaling cells. As these Rabs would be expected to be needed for ligand to be recycled, these results further support the idea that epsin and auxilin are not required for Notch ligand recycling.

847A
Casein Kinase 2 regulates Hedgehog signaling through Smoothened and Cubitus interruptus. Hongge Jia1, Yajuan Liu1, Ruohan Xia1, Jin Jiang2, Jianhang Jia1. 1) Department of Molecular and Cellular Biochemistry, Markey Cancer Center, University of Kentucky, Lexington, KY 40536, USA; 2) Department of Developmental Biology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA.

Casein Kinase II (CK2) is a typical serine/threonine kinase and has been implicated in many cellular signaling pathways. In this study, we show that CK2 is a positive regulator in the Hedgehog (Hh) signal transduction. We found that knockdown of CK2beta by RNAi induces loss-of-Hh wing phenotype. We further found that CK2beta RNAi attenuates the Hh-induced Smo accumulation and downregulates Hh target gene expression whereas co-expressing CK2alp with CK2beta elevates the level of Smo and induces ectopic Hh target gene expression. We identified the serine residues in Smo that can be phosphorylated by CK2 in vitro. Mutating these serine residues attenuates the ability of Smo to transduce high-level Hh signaling activity. Furthermore, we found that CK2 plays an additional positive role downstream of Smo by regulating the stability of full-length Ci. We propose that CK2 promotes Hh signaling activity by regulating multiple pathway components.

848B

The Hedgehog (Hh) family of signaling molecules organizes spatial patterning in a wide variety of morphogenetic processes in both insects and vertebrates. A property of Hh proteins is to act as morphogens during development: Hh proteins spread from localized sites of production to specify a diverse array of cell fates. We have studied the mechanism of Hh gradient formation at the level of Hh release and spreading in the P
component cells of the wing imaginal disc. Hitherto, Dispatched (Disp), a twelve transmembrane spanning domain protein, is the only protein known to be specifically required for proper Hh secretion (Burke and Basler 1999). iHog (interference hedgehog) is a type I membrane protein with four Ig domains followed by two FNIII domains, a membrane-spanning region, and a cytoplasmic region of unknown function. It has been reported that the iHog gene is required for Hh secretion both in Drosophila embryos and imaginal discs (Yao et al., 2006; Zhen et al., 2010). Here, we have analyzed the role of iHog in Hh secretion and we will show that iHog is necessary for Hh stability in the P compartment and that it is able to interact with Hh only when it is properly secreted by Disp suggesting an undetermined role for iHog in the Hh producing cells. We have also examined the possible relation between iHog and other members of the pathway necessary for Hh release. Additionally we have observed the existence of long thin cellular extensions exclusively located at the baso-lateral part of the wing disc epithelium where Hh strongly attaches to iHog. We will present the relevant data and discuss potential mechanisms for Hh secretion and spreading suggesting an original model implicating a regulatory interaction of iHog with other members of the Hh pathway.

849C Nuclear import of Maize fine streak virus proteins in plant and insect cells. Chi-Wei Tsai1, Fiorella Cisneros2, Margaret Redinbaugh3, Saskia Hogenhout4. 1) Dept Entomology, National Taiwan University, Taipei, Taiwan; 2) Dept Plant Pathology, The Ohio State University-OARDIC, Wooster, OH, USA; 3) USDA-ARS, The Ohio State University-OARDIC, Wooster, OH, USA; 4) Dept Disease and Stress Biology, The John Innes Centre, Norwich, UK.

Maize fine streak virus (MFSV) is an insect-transmitted nucleorhabdovirus. The virus replicates not only in its maize hosts but also in its insect vectors. In both hosts, MFSV replicates in the nucleus and assembles at the inner nuclear membrane, so nuclear import of viral proteins is critical to complete viral morphogenesis. The Importin α/β dependent nuclear import machinery is highly conserved from yeast to higher plants and mammals, so we hypothesized that MFSV employs conserved nuclear import machinery of plant and insect cells enabling it to infect both plants and insects. To address this hypothesis, the role of Importin α in nuclear import of MFSV proteins in plant and insect cells was studied by gene silencing combined with subcellular localization assays. The results showed that silencing of importin α genes inhibited nuclear targeting of the N protein and the N-P complex in plant cells. Similarly, Importin α was involved in nuclear import of the N protein and the N-P complex into the nuclei of insect cells. These results suggest that the MFSV N protein and the MFSV N-P complex are dependent on Importin α for nuclear import in plant and insect cells.

850A Discovering Mechanisms of Trel Mediated Germ Cell Polarization and Dispersal. Michelle G. LeBlanc, Ruth Lehmann. Skirball Institute of Biomolecular Medicine, NYU Medical Center, Department of Developmental Genetics, New York, NY.

Knowing when and how to lose contact with neighboring cells and initiate migration is critical to normal development such as during gastrulation and neural crest migration and to aberrant cell behavior such as cancer metastasis. The exact mechanisms and their regulation in space and time, however, remain poorly understood. I have chosen Drosophila primordial germ cell (PGC) migration as a model to better understand the mechanisms a cell uses to prepare for migration. In Drosophila, PGCs are born at the posterior pole and migrate through the midgut to the mesoderm where they give rise to the embryonic gonad. In order to properly move to their final destination, germ cells must receive migratory cues and initiate a migratory program. The G protein coupled receptor Tre1 (trapped in endoderm) is required for the initiation and transepithelial migration (TEM) of the PGCs out of the gut and into the mesoderm. In wild-type embryos, PGCs coalesce into a cluster in the gut prior to migration. Adhesion proteins such as DE-cadherin as well as the small GTPase Rho1 are down-regulated along the cortex and polarize toward the center of the cluster (tails) as PGCs disperse prior to TEM. In embryos from trel mutant females, protein polarization and TEM do not occur. The mechanism of trel mediated polarity and dispersal is unknown. Here I describe recent experiments to analyze by epistasis the downstream effectors of Tre1. I quantified Rho1 fluorescence to show that its levels differ significantly between wild-type and trel mutant embryos. I am now addressing whether recycling or endocytosis accounts for Tre1-dependent down regulation of Rho1 and DE-cadherin. I plan to use live imaging in conjunction with fluorescently tagged Rho1 to uncover the mechanism by which Tre1 regulates the onset of germ cell migration. Interestingly, invasive migration also requires modulation of adhesion proteins before cells undergo TEM (metastasis); thus, understanding the mechanisms of germ cell migration may provide insight into invasive cell migration. 1. Kunwar et al.(2003). 2. Kunwar et al.(2008).


Pecanex is a highly conserved transmembrane protein with homologs found in multiple organisms ranging from flies (pcx) to humans (Pcnx, Pcnx2 and Pcnx3). pcx was described several years ago in Drosophila as a maternal-effect neurogenic gene, such that when the gene is not expressed maternally, the embryos display hyperneuralization, indicating that its function is to promote epidermal cell fate. Such a phenotype also implicates pcx as a new component of the Notch signaling pathway. In order to identify its function, we performed experiments in two model organisms: mouse and Drosophila. To define its role during hair follicle and epidermal morphogenesis, we analyzed Pcnx expression in developing and postnatal mouse epidermises, and found that expression peaked at E14.0-E14.5 and anagen phase. Immunofluorescence analysis with a Pcnx antibody revealed protein expression in the inner root sheath in mouse and human hair follicle. We also detected Pcnx cDNA expression in human keratinocytes and dermal fibroblasts, which correlates with Pcnx antibody detection in mouse epidermises. We next used Drosophila genetics to place pcx in the context of the Notch signaling pathway and to determine genetic interactions. Drosophila pcx is widely expressed during embryogenesis, in both the imaginal discs and ovaries. We confirmed the hyperneuralization phenotype of embryos laid by homozygous mutant females. Finally, we observed extra bristles in the maxillary palps of pcx mutants, a previously undescribed phenotype. Collectively, these data suggest that pcnx plays an important role in morphogenesis processes, and moreover, that its function is related to the Notch signaling pathway.

852C Changing the level of a cyclin can alter the phenotypes of mutations in strawberry notch and the double mutant dig^{null}^{null} sno^{null}. Catherine A. Coyle-Thompson1,2, Georgina Portillo-Aguilar1, Wayne Givens2, Patrick Ollawa2, Luz Ramoz2, Eric Vasquez2, Flora Retano3,4, Josh Duhal1,2. 1) Dept Biol, California State Univ, Northridge; Northridge, CA; 2) LS/AMP, California State Univ, Northridge; Northridge, CA; 3) Dept Dermatology, Columbia Univ, New York, NY. The strawberry notch (sno) allele alters the development of the eyes, wings and legs as well as other developmental processes. The discs large mutation alters the number of bristles on the eye, wing, head and thorax. The dig^{null} sno^{null} double mutant has an absence of the majority of bristles on the head, thorax and wings. Altering the levels of a cyclin can suppress many of the eye, wing, and leg phenotypes in the sno^{null}.
853A
**Drosophila PP1beta regulates Notch signaling in the posterior follicle cells.** Yi Sun¹, Yan Yan¹, Natalie Denef², Trudi Schupbach¹. ¹) Molecular Biology, Princeton Univ, Princeton, NJ; ²) Howard Hughes Medical Institute.

The embryonic axes of Drosophila are established by interactions between the oocyte and the overlaying somatic follicle cells. Prior to stage 7 of oogenesis, Gurken in the germline signals through EGF R, inducing adjacent follicle cells to adopt a posterior cell fate. These posterior follicle cells then send an unknown signal back to the oocyte to repolarize the oocyte microtubules, resulting in the determination of the anterior-posterior axis. The polarization of the oocyte cytoskeleton also directs the relocalization of the oocyte nucleus and Gurken to a dorsal anterior position, initiating the establishment of the dorsal-ventral axis via another round of bi-directional signaling. From a previous mosaic screen in follicle cells, we identified an allele of flapwing, which encodes the beta isoform of the catalytic subunit of protein Serine/Threonine phosphatase 1 (PP1beta). We show that PP1beta is required for the differentiation of the posterior follicle cells and hence the axis specification in the oocyte. Posterior follicle cells mutant for PP1beta are defective in Notch signaling and show an accumulation of Notch in intracellular compartments. Our results further suggest that PP1beta regulates endocytic trafficking of Notch in the posterior follicle cells via its function in the nonmuscle myosin pathway.

854B
**CG3313 modulates TOR and FOXO pathways to regulate growth, apoptosis, and development in Drosophila.** Dae-Sung Hwangbo¹, Boe Dong Kim, Jeongbin Yim. School of Biological Sciences, Seoul National University, Seoul, Korea.

Insulin/IGF receptor and TOR pathways play key roles in regulating growth across species. In Drosophila, upon activation of insulin-like receptor (InR) by growth signals, dAKT inhibits dFOXO activation to suppress apoptosis. Simultaneously, activated dAKT induces growth- promoting TOR signaling pathways, which result in increased protein synthesis. Although significant progress has been made in understanding how these two branch pathways are regulated to balance between growth and apoptosis, details have not been fully characterized. To better understand the regulation of growth and apoptosis by dFOXO and dTOR pathways, we have performed molecular genetic analysis to find novel proteins regulating these pathways. In this analysis we identified that CG3313, a WD40-repeat protein, plays important roles in mediating TOR pathway.

855C
**A novel gene regulating the level of phosphorylated Akt and total Akt in Drosophila melanogaster.** Heujin Kim, Jeongbin Yim, Jeonghun Yim. School of Biological Sciences, Seoul National University, Seoul, Korea.

Akt, also called protein kinase B (PKB), is a hub protein in a variety of cellular signaling and has a critical regulatory role in diverse cellular processes. These include cellular survival, protein synthesis and cell proliferation. In response to a variety of growth factors such as insulin, Akt is phosphorylated and activated by both phosphoinositide dependent kinase-1 (PDK1) and target of rapamycin complex 2 (TORC2). We found a novel gene which regulates the level of phosphorylated Akt by RNAi-screening in S2R⁺ cells. Homozygous mutants for CG3313 are viable and grow comparably to wild type controls until third instar larva but become arrested and die in the late pupae stage. Ectopic expression of full length CG3313 using tissue-specific drivers results in a strong reduction of size. Size reduction in the eye by over-expression of CG3313 is rescued by co-expression of baculovirus anti-apoptotic protein p35. A strong apoptotic phenotype in the eye by over-expression of dFOXO is also significantly ameliorated by co-expression of CG3313. Further epistasis analysis using both loss- and gain-of-function mutants for CG3313 suggests that CG3313 can interact with both FOXO and TOR pathways. Preliminary results show that CG3313 can also regulate oxidative stress and lifespan in flies. These results suggest that CG3313 is an essential gene that can regulate growth and apoptosis in Drosophila. Studies to characterize the molecular mechanisms how CG3313 functions are currently ongoing.

856A
**Cell-ECM interactions mediated by integrins are required for a proper proliferation-to-differentiation switch.** Maria D. Martin-Bermudo, Laura Cobreros-ruquera. Dept Developmental Biol, CSIC, Seville, Spain.

Coordinating differentiation with exit from the cell cycle is critical for proper organogenesis, yet how this is achieved remains largely unknown. How do the developmental signals triggering differentiation impinge on cell cycle regulators? The development of the follicular epithelium of the Drosophila ovary represents an ideal system to study the mechanisms controlling the transition from cell cycle exit to differentiation. The ovary of the adult Drosophila female is composed of various tubular structures called oварioles within which eggs are formed. Each ovariolo contains a line of egg chambers at different stages of development, from stage 1 to stage 14. Each egg chamber begins as a 16-cell germline cyst surrounded by a monolayer of somatic follicle cells precursors. Up to stage 6, these somatic follicle cells undergo a mitotic division programme giving rise to approximately 1000 follicle cells, which will form a monolayer known as the follicular epithelium. After stage 6, the follicle cells differentiate and undergo three endocycles to become polyploidy. Later in oogenesis, four different loci synchronously initiate a gene amplification event. By using clonal analysis, we show that cell-ECM interactions mediated by integrins are required for proper proliferation-to-differentiation switch. Interestingly, although integrin mutant cells exit mitosis they cannot mature and remain in an undifferentiated state. In addition, integrin mutant follicle cells do not initiate the amplification event. Our results support a model in which integrin mediated signalling regulates cell differentiation by executing the mitotic-to-endocycle transition through the regulation of cell cycle regulators, such as Cyclin B and Dacapo.

857B
**Investigating the Role of Protein O-Glycosylation During Drosophila Development.** Duy T Tran, E Tian, Kelly G Ten Hagen. Developmental Glycobiology Unit, NIDCR, NIH, Bethesda, MD.

Protein O-glycosylation represents a major form of post-translational modification that is conserved across most eukaryotic species. One type of O-glycosylation, known as mucin-type O-glycosylation, is initiated by a family of enzymes that catalyzes the transfer of GalNAc to protein substrates. In Drosophila melanogaster, loss-of-function mutations in one family member, pgant35A, resulted in death early in development and irregularities in epithelial tube formation, indicating that PGANT35A is essential for viability. However, it remains unclear what roles the other
family members may play during development. To define the developmental roles of the remaining family members, we have employed RNA interference (RNAi)-mediated knockdown of individual genes in the fly. Using this approach we have identified other members of the PGANT gene family that are required for viability. These results further demonstrate the importance of this enzyme family in Drosophila. To further define the role of each pgant, tissue and stage-specific RNAi will be used to silence each gene. These experiments will allow us to identify specific tissues and developmental stages where pgant function is required. Our studies aim to elucidate the role of mucin-type O-glycosylation throughout Drosophila development and provide insight into the role of this highly conserved protein modification during mammalian development.

858C

Abelson kinase is required for establishing and maintaining the terminally differentiated state of Drosophila photoreceptor cells. Wenjun Xiong, Ilaria Rebay. Ben May Dept Cancer Res, Univ Chicago, Chicago, IL.

Drosophila Abelson(Abl), homolog of the vertebrate abl oncogene, encodes a widely expressed cytoplasmic tyrosine kinase that influences a variety of morphogenetic processes through regulation of the actin cytoskeleton. For example, in the embryonic central nervous system, Ab1 transduces signals from several axon guidance receptors to the cortical actin cytoskeleton via interactions with the actin modulator Enabled and the Rho-family GEF Trio. Although its functions in fly eye development have not been extensively examined, Abl is expressed in the developing photoreceptors and is required for normal eye development, suggesting it might play an important role in the terminal differentiation and morphogenesis of the photoreceptors. In this study, we examined the role of Abl in patterning the Drosophila retina by analyzing abl loss-of-function phenotypes in larval and pupal retinal epithelia. Our data show that Abl is required for photoreceptor terminal differentiation and morphogenesis, but is largely dispensable for early cell fate specification. Thus loss of abl leads to lack of rhabdomeres, destabilization of AJs and the apical membrane domain, disorderly ommatidial arrangement and incorrect geometric position of cells within the epithelia. At late pupal stages, abl mutant cells appear to dedifferentiate, as judged by loss of photoreceptor and neuronal specific markers, but do not reenter a proliferative state, undergo apoptosis, or assume an accessory cell fate. Mechanistically, dedifferentiation correlates with abnormally elevated Notch signaling activity which suppresses neuronal cell fate during late pupal eye development. Thus we propose that Abl-mediated coordination of morphogenesis and intercellular signaling plays a key role in establishing and maintaining the terminally differentiated state of Drosophila photoreceptor cells.

859A

Sulfated is a Negative Feedback Regulator of Wingless. Jia You1, Tanya Belenkaya1, Xinhua Lin1,2. 1) Dev Biol, Cincinnati Child Hosp Med Ctr, Cincinnati, Oh; 2) State key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China.

Wingless (Wg) is a secreted ligand protein which regulates a number of important patterning events in Drosophila. During wing development, Wg protein forms a concentration gradient to activate expression of target genes in a gradient-dependent manner. Previous studies from our lab and others have demonstrated that heparan sulfate proteoglycans (HSPGs) play essential roles in the formation of the Wg gradient. HSPGs are macromolecules composed of a core protein to which heparan sulfate (HS) glycosaminoglycan chains are attached. Various studies have shown that secreted protein 6-O endosulfatase can modulate the sulfation patterns on the HS chains by removing a sulfate group. Previous studies of 6-O sulfated macromolecules composed of a core protein to which heparan sulfate (HS) glycosaminoglycan chains are attached. Various studies have shown that secreted protein 6-O endosulfatase can modulate the sulfation patterns on the HS chains by removing a sulfate group. Previous studies of 6-O endosulfatase in cultured cells showed it can promote Wg signaling activity. In mammals, two 6-O endosulfatases are encoded by mSulf1 and mSulf2. However, neither mSulf1 nor mSulf2 knock-out mice have an obvious phenotype, which suggests that they function co-operatively. In Drosophila, Sulfated (Sulf1) is the only gene encoding for 6-O endosulfatase. Here we investigated the role of Drosophila Sulf1 in regulating Wg signaling in wing discs. We showed that Sulf1 is expressed specifically in late third instar larvae, and only along the dorsal-ventral boundary and the antero-posterior boundary. Wg signaling is required to activate expression of Sulf1. We generated Sulf1 mutant and found that homozygous Sulf1 mutant flies were viable. They show enhanced extracellular Wg staining and Wg signaling activity. We examined Wg signaling in Sulf1 gain of function and loss of function experiments and found that Sulf1 negatively regulates Wg signaling in vivo. The analyses of genetic interactions indicate that notchum, which is a known negative feedback regulator of Wg signaling, may work synergistically with Sulf1. Taken together these results suggest that Sulf1 is a negative feedback regulator of Wg signaling.

860B

Spindle checkpoint Mad1 and Mad2 dynamics in mitosis. Doruk EMRE, Régine TERRACOL, Anaïs PONCET, Roger KARESS. Institut Jacques Monod, CNRS UMR 7592, Université Paris Diderot, Paris, France.

The major described role of checkpoint protein Mad1 is as a platform for Mad2 recruitment to the kinetochore and as a catalyst for generating Mad2/Cdc20 complexes. The disassembly of this platform by shedding is believed to contribute to spindle checkpoint shut-off. However Mad1 and Mad2 protein dynamics have not been directly compared. It is therefore possible that, besides their shared role in the checkpoint, one or both of these proteins has distinct functions as well. We investigated the specific roles of Mad1 and Mad2 in Drosophila by monitoring their in vivo behaviour throughout mitosis, then by genetic and cellular analyses of mutant allele phenotypes. By monitoring fluorescently-tagged Mad1 and Mad2 proteins, we observe a tight correlation in timing and dynamics from recruitment at kinetochores in prometaphase, to shedding along microtubules at metaphase, and removal from kinetochores prior to anaphase. This suggests that Mad1 removal is the primary means of removing Mad2 from kinetochores.

861C

The function of RBF1 during Drosophila eye development. Milena K. Popova1, Wei He1, Spyros Artavanis-Tsakonas2, Nick Dyson3, Nam Sung Moon1. 1) McGill University, Biology Department. 1205 Avenue Docteur Penfield. Montreal, QC. H3A 1B1; 2) Harvard Medical School, Department of Cell Biology. 240 Longwood Avenue (LHRRB 410). Boston, Massachusetts, USA; 3) Massachusetts General Hospital Cancer Research Center and Harvard medical school, Charlestown, Massachusetts, USA.

RBF1 is the Drosophila ortholog of the Rb tumor suppressor protein. Genetic studies in Drosophila demonstrated that, like Rb, RBF1 is an important regulator of cell cycle progression and survival. In mammals, Rb also participates in the terminal differentiation process, a function that it is yet to be clearly associated with RBF1. Recently, we have conducted a genetic screen to identify factors that, when overexpressed, interfere with eye development in rbf1 mutant flies. We have identified that mis-expression of EMC in rbf1 mutant eyes disrupts cone cell and bristle development while it affects only bristle development in the wild type background. Interestingly, a mammalian homolog of EMC was previously identified as an important factor responsible for terminal differentiation defects in RB knockout mice, raising the possibility that RBF1 might also participate in differentiation process. We are currently investigating the significance of this genetic interaction and the role of RBF1 during Drosophila eye development.
In vivo structure/function analysis of the effector caspase drICE in cell death. Yaning Wu, Dongbin Xu, Jeannine Garnett, Andreas Bergmann. Department of Biochemistry and Molecular Biology, University of Texas MD Anderson Cancer Center, Houston, TX.

Caspases are the executors of programmed cell death and are classified into initiator caspases and effector caspases. DrICE and Dcp-1 are the major effector caspases in Drosophila and share functional redundancy. It is known that DrICE is activated upon proteolytic cleavage into a large and small subunits by the initiator caspase Dcr-1. When activated DrICE then forms a functional heterodimer composed of DrICE and Dcp-1. drICE is involved in cell death program in Drosophila salivary glands. We found genetic interaction between l(2)gl and BR-C, as well as between l(2)gl and zipE(br). Ectopic expression of p127 from UAS-l(2)gl stops cell death and provides new molecular insights for reciprocal cell interactions during dynamic remodeling process. Cell replacement in abdominal epidermis during Drosophila metamorphosis is a simple example for tissue remodeling. In the development of Drosophila adult abdominal epidermis, larval epidermal cells (LECs) are replaced by abdominal histoblasts, which are precursors of adult epidermis. We have analyzed the cell death dynamics of LECs using FRET-based indicator for caspase activation (SCAT3). Caspase activation in the larval epidermis begins from lateral sides and ends at the midline, leading to the systematic lateral-to-medial elimination of LECs. Further detailed analysis suggested that caspase activation in individual LECs is frequently observed at the boundary between LECs and histoblasts, and it may correlate with histoblast interactions. In vivo manipulation of histoblast proliferation by UV laser indicated that histoblast nest expansion is required for caspase activation and determines its frequency in neighboring LECs. These results imply the active communication between LECs and histoblasts regulating initiation of caspase activation at their boundary. We speculate that the boundary interactions triggering caspase activation are specific for remodeling processes, which permits tissue replacement without altering tissue size.

Tumor suppressors and cytoskeletal proteins control chromatin access of transcription and remodelling factors during edysosome-triggered cell death and preceding secretary activity in Drosophila. Robert Farkas1,2, Lucia Mentelova1,2, Daniel Vlcek3, Milan Bene1, Erika Halasova1,2, Peter Danis1,2, Ivan Raska1,2, Bernard Mechler1. 1) Inst Experimental Endocrinol, Slovak Academy Science, Bratislava, Slovakia; 2) Department of Genetics, Faculty of Science, Comenius University, 842 15 Bratislava, Slovakia; 3) Institute of Cellular Biology and Pathology, 1st Faculty of Medicine, Charles University, Albertov 4, 12801 Prague, Czech Republic; 4) Department of Developmental Genetics, Deutsches Krebsforschungszentrum-ZMBH Allianz, IMB 581, 69120 Heidelberg, Germany.

At the onset of Drosophila metamorphosis hormone edysone activates a cell death program that leads larval salivary glands (SGs) to rapidly disintegrate about 14-16 hr after pupariation formation. During this process edysone acts through the edysone receptor (EcR/Usp) 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 depend upon the level of p127l(2)gl, a cytoskeletal tumor suppressor that interacts with nonmuscle myosin II heavy chain (nmMHC) encoded by the zipper (zip) gene. Reduced l(2)glp127 expression delays SG secretory cycle and histolysis whereas overexpression accelerates this process without affecting larval and pupal development. We observed that the p127 and nmMHC regulate chromatin access of BR-C Z, E74 and a series of remodeling factors including Sin3A, Rpd3 and Smrter. In wild-type SGs, these factors bind to chromatin but in l(2)gl- they accumulate in the cytoplasm and the cortical nuclear zone and are unable to associate with chromatin. Similar chromatin exclusion can be achieved by overexpression of nmMHC and occurs also in SGs of developmentally delayed zipE(br)+ larvae. We found genetic interaction between l(2)gl and BR-C, as well as between l(2)gl and zipE(br). Exocytic expression of p127 from UAS-l(2)gl transgene fully rescues l(2)gl-phenotype. (Supported by EEA & NFM SK-0086/3655/2009/0RINFM).

The Interaction between p127l(2)gl and Armadillo leads to a decreased formation of Arm/Tcf, activation of hid expression, and execution of cell death program in Drosophila salivary glands. Robert Farkas1,2, Lucia Mentelova1,2, Silvia Kucharova-Mahmood1,2, Daniel Vlcek3, Milan Bene1, Erika Halasova1,2, Peter Danis1,2, Ivan Raska1,2, Bernard Mechler1. 1) Inst Experimental Endocrinol, Slovak Academy Science, Bratislava, Slovakia; 2) Department of Genetics, Faculty of Science, Comenius University, 842 15 Bratislava, Slovakia; 3) Institute of Cellular Biology and Pathology, 1st Faculty of Medicine, Charles University, Albertov 4, 12801 Prague, Czech Republic; 4) Department of Developmental Genetics, Deutsches Krebsforschungszentrum-ZMBH Allianz, IMB 581, D-69120 Heidelberg, Germany.

During Drosophila metamorphosis larval tissues, such as the salivary glands, are histolyzed whereas imaginal tissues differentiate into adult structures. The disintegration of the larval salivary glands and preceding unconventional secretion are triggered by the steroid hormone edysone and take place 8 to 10 hr and 16 hr after pupariation formation (APF), respectively. We showed that disintegration of the salivary glands requires the presence of the p127 cytoskeletal protein encoded by the l(2)gl tumour suppressor gene and that the timing of histolysis displays a l(2)gl-dose response. We found that in l(2)gl salivary glands the Armadillo (Arm) protein, which is normally associated with the plasma membrane, is relocated to the cytoplasm where it distributes in two hybrid system revealed that p127 and Arm physically interacts together. While ectopic expression of wg, which is known to stabilize intracellular Arm, significantly delays the processes of unconventional secretion and cell death, sgg expression, which encodes the Shaggy/Zeste-white 3 kinase, was found to accelerate this process. RT-PCR analysis showed no expression of hid mRNA in both l(2)gl salivary glands or wt glands ectopically expressing arm or wg.
However, we found that sgg can induce a level of hid expression similar to that in wild type at ~10 hr APF. (Supported by the EEA & NFM Norwegian Fund # SK-0086:3655/2009/ORINFM).

866B

Analysis of factors involved in competitive interactions during growth using a Drosophila cell-culture based assay system. Nanami Senoo-Matsuda1,2, Laura Johnston1, Nobuhito Goda1,2. 1) Dept of Life Science & Medical Bioscience, Waseda University, Tokyo, Japan; 2) CREST, JST, Japan; 3) Dept of Genetics & Development, Columbia University, NY.

Our studies have revealed that developing wing cells in Drosophila melanogaster that differ in expression levels of the growth regulator dMyc can compete, leading to the apoptotic death of the cells with less dMyc (“losers”) and over-representation of cells with more dMyc (“winners”) in the wing (de la Cova et al., 2004). This phenomenon, called cell competition, seems to play a crucial role in the control of organ size. To take a systematic approach to identify the molecular mechanisms underlying cell competition, we have developed a cell-culture based cell competition assay (Senoo-Matsuda & Johnston, 2007). The assay has determined that competition is mediated by diffusible factors produced by winner and loser cells that allow recognition and determine the response to each other’s presence. We have been trying to perform cellular metabolome analysis to identify and characterize factors involved in cell competition. We postulate that cell competition is a broadly used and evolutionarily conserved process of tissue homeostasis that may be exploited by cancer cells during tumorigenesis in humans.

867C


γ-Secretase is an important protease complex for regulating processing of the Amyloid Precursor Protein (APP) and Notch proteins; interrupting its function has substantial effects on cell growth and animal development. The essential components of γ-secretase include: Presenilin, Aph-1, Nicastrin, and Presenilin enhancer 2 (Pen-2). Much is understood on the mechanism of how γ-secretase affects Notch signaling, yet its exact role in directly regulating cell growth is not clear. While removing functions of Presenilin, Nct or Aph-1 disrupts γ-secretase activity and thus Notch signaling, removing Aph-1 function during Drosophila wing development seems to further impact cell survival, possibly through its effect on the Presenilin holoprotein. As for the role of Pen-2, previous studies of C. elegans and of mammalian cells in vitro have demonstrated that lack of Pen-2 causes downregulation of Presenilin heterodimer formation, which serves as the catalytic domain for the γ-secretase function. Its exact role in regulating the γ-secretase is not clear; it is not known whether Pen-2 is crucial for regulating activity of matured γ-secretase and for cell survival. Using MARCM and RNAi techniques we have examined the effect of pen-2-- clones during Drosophila wing development. We first confirmed that RNAi approach sufficiently inhibits Pen-2 activity and subsequently the γ-secretase by monitoring Notch expression in the both the larval wing discs and the adult wing blade. We would like to learn whether removal of Pen-2 function affects cell survival like Aph-1 does. Furthermore, we are now examining the effect of Pen-2 on cell survival by analyzing the pen-2-- clones in the presence and absence of Presenilin.

868A

Regulation of gurken expression by insulin signaling. Scott B. Ferguson1,2, Malachi A. Blundon1, Susan A. Cronin1, Trudi Schüpbach2. 1) Department of Biology, SUNY Fredonia, Fredonia, NY; 2) HHMI and Department of Molecular Biology, Princeton University, Princeton, NJ.

Axial patterning of the Drosophila eggshell and embryo is specified during mid-oogenesis. The TGF-α related morphogen, Gurken, specifies these axes through signaling between the germline and follicular epithelium. The levels of the Gurken protein must be carefully controlled in order to avoid patterning defects. Excess Grk leads to ectopic dorsal fates whereas a reduction in Grk protein levels results in an expansion of ventral fates. These patterning defects are manifest in both the eggshell and underlying embryo. The eggshell phenotype is a very sensitive readout of Grk activity. We have used perturbations of this phenotype as a mechanism to further understand the control of Grk expression and localization. The efficiency of grk translation can be reduced through activation of a meiotic checkpoint. Mutations in DNA repair genes including okra and the spindle-class genes result in an inability to repair double strand breaks formed during meiotic recombination. Females mutant for these spindle-class genes lay variably ventralized eggs as a result of activation of a Mei-41/Chk2 checkpoint, although the means by which this checkpoint affects grk remain to be elucidated. As part of a screen to understand these signaling events, we have found that mutations in the lnk gene suppress the eggshell ventralization of spindle-B females. The human homologue of Lnk modulates insulin signaling and we and others have shown that this role is conserved in Drosophila. TOR acts downstream of insulin signaling and we show that TOR inhibition by rapamycin can also suppress the spindle-B eggshell phenotype. As with other insulin mutations, we have found that lnk mutations slow the rate of oogenesis. In vitro translation experiments with grk reporter mRNAs also suggest that lnk mutations stimulate grk translation. These data support a mechanism by which the ventralized eggshell phenotype induced by checkpoint activation can be suppressed by directly promoting grk translation.

869B

IDGF2 - a homeostatic regulator. Lucie Kucerova1, Peter J. Bryan2, Michal Zurovec1. 1) BC AVCR, Branisovska 31, Ceske Budejovice, Czech Republic; 2) Developmental Biology Center, University of California, Irvine, Irvine, CA 92697, USA.

Imaginal Disc Growth Factors (IDGFs) are family of six chitinase-like Drosophila hemolymph proteins produced by fat body and hemocytes. Unlike chitinases they do not posses hydrolytic activity but are still able to bind carbohydrate moieties. IDGFs were isolated from the conditioned media of Drosophila imaginal disc cells C18+ and embryonic cell line S2. The crystal structure of IDGF2 was elucidated and it was found to promote the growth of Drosophila cells in vitro. We prepared recombinant IDGF2 and control chitinase CG9307, and compared their effects on imaginal disc C18+ cells in tissue culture in vitro by the genome-wide screen using Affymetrix GeneChip Arrays. We identified a number of gene expression changes and verified them in cell culture by northern blot analysis. We also examined the effects of IDGF-overexpression on the transcription of the selected genes in flies in vivo using real-time RT-PCR. Our results show that IDGF2 changes the expression profile of genes involved in development, energy conversion, and other processes, indicating that this secreted glycoprotein may serve as a homeostatic regulator.

870C

Investigating the cytoskeletal roles of APC in spindle orientation, anchoring, and chromosomal instability. John Poulton, Dave Roberts, Mark Peifer. Department of Biology, Univ North Carolina at Chapel Hill, Lineberger Comprehensive Cancer Center.

Adenomatous Polyposis Coli (APC) proteins are tumor suppressors known for their roles in Wnt signaling and cytoskeletal regulation. Recent research identified important cytoskeletal functions of APC including regulating mitotic spindle orientation, spindle attachment, and prevention of chromosomal instability. The mechanisms by which APC regulates the cytoskeleton remain unclear. We hypothesize they are based on APC...
localization and its interactions with microtubules, the actin cytoskeleton, and other cytoskeletal regulators. To further our understanding of APC in cytoskeletal regulation I will use Drosophila APC to address a series of key questions. Which regions of the APC protein participate in its cytoskeletal functions? Is APC function in Wnt signaling related to its cytoskeletal roles? Is localization of APC important for its cytoskeletal functions? What are the relevant partners of APC in regulating the cytoskeleton? Answering these questions will provide a more complete understanding of APC’s role in both normal development and cancer.

871A

The mutation in the adenosine receptor gene influences cell survival in mosaic clones. Roman Sidoryov, Lucie Kučerová, Michal Žuvec. Institute of Entomology, Biology Centre, ASCR, v.v.i., České Budějovice, Jihočeský kraj, Czech Republic.

Adenosine is the key component of cell division and survival regulation, as it participates materially in the DNA and ATP synthesis, its derivative cAMP is an important regulator of cell energy processes. It is known to be released by apoptotic cells; high concentrations of adenosine are toxic to the cell. Also adenosine participates in signaling through PIP3/PKC and adenylyl cyclase/PKA pathways. We obtained a new mutation in the adoR gene by the methods of end-out targeting. It is homozygous viable and has itself no special phenotype in the fly. This leads us to check its possible interactions with the other genes affecting cell division and survival in somatic tissue, and judge the viability of such combination by the frequency of correspondent mosaic clones. Together with a mutation in the tumor suppressor wts, the adoR mutation causes nearly 10-fold decrease (compared to wts+/+ flies with normal AdoR) of the wts tumor frequency with different types of mutagens and spontaneously. This suggests that the mechanism of the effect is mutagen-independent and results in a genetic interaction. The adoR mutation removes wts tumors only when present inside of the clone in two copies. This means it acts recessively and cell-autonomously. The RNAi to WT AdoR works similar to the adoR mutation. The expression of a transgenic WT AdoR rescues the effect of the mutation partially. This confirms specificity of the effect to adoR gene rather than to the genetic background. Expression of dominant-negative alleles of p53 has no positive effect on the wts clone frequency in wts adoR dheterozygotes. This may suggest either that a cross-link between adoR and pro-apoptotic signaling is downstream the p53 or that some other mechanism besides apoptosis is involved into the wts clone removal in adoR mutants. The adoR mutation affects frequency of wts tumors and non-tumorous yellow clones oppositely: whereas it removes wts clones, the frequency of y clones grows up. Thus, adoR is unlikely to be a general negative modifier of the mitotic crossing-over frequency.

872B

Regulatory elements of the Sex combs reduced HOX gene. Monica T. Cooper, James A. Kennison. Program in Genomics of Differentiation, Eunice Kennedy Shriver National Institute of Child Health and Human Development,NIH, Bethesda, MD.

The identity of the adult first leg is specified by the Sex combs reduced (Scr) gene of the Antennapedia complex. This requires cis-regulatory elements for both activation and silencing, as well as numerous trans-acting factors. Silencing of Scr transcription in the second and third leg imaginal cells requires multiple cis-acting elements. We have used targeted-gene-replacement and pairing-dependent silencing of transgenes to search for silencing elements. There are at least two clusters of elements that cause pairing-sensitive silencing of transgenes. These coincide with the regions proposed to interact both in cis and in trans to facilitate maintenance of silencing. Deletion of part of either cluster interferes with the adoR gene expression. The regions proposed to interact both in cis and in trans to facilitate maintenance of silencing.

873C

Coordinated assembly of centromeric proteins CID, CAL1 and CENP-C during mitosis. Barbara Mellone1, Kathryn Grive1, Vladimir Streha1, Isaac Oderberg1, Gary Karpen2. 1) Molecular and Cell Biology, University of Connecticut, Storrs, CT; 2) Lawrence Berkeley National Laboratory. Berkeley, CA; 3) Molecular and Cell Biology. University of California Berkeley, Berkeley, CA.

During DNA replication, canonical histones are segregated semi-conservatively to the two nascent DNA strands. Centromeric chromatin, which contains the unique histone H3 variant, CENP-A, is replenished with timing and mechanisms that are distinct from those of canonical chromatin. Using the SNAP-tag labeling system, we show that Drosophila CENP-A/CID is distributed to daughter cells semi-conservatively. However, unlike human CENP-A, new CENP-A/CID is recruited to centromeres during metaphase, by a mechanism that does not require mitotic exit or an intact mitotic spindle. We previously isolated the essential centromeric proteins CENP-C and CAL1 as being required for CENP-A/CID localization. We show that CENP-C is replenished in a manner similar to that of CENP-A/CID. In contrast, CAL1 is recruited before CENP-A/CID, in prophase, by a mechanism that largely replaces pre-existing CAL1 protein. The unusual timing of CENP-A/CID recruitment and the unique dynamics of CAL1’s centromere association identify a distinct centromere assembly pathway in Drosophila, and establish CAL1 as a key player in centromere replenishment.

874A


Function of dRYBP (Drosophila Ring and YY1 Binding Protein) in the epigenetic regulation of gene expression. Ricardo Aparicio* and Ana Busturia. Centro de Biología Molecular (CSIC-UMA). Universidad Autónoma de Madrid. c) Nicolas Cabrera 1. 28049 Madrid, Spain. Epigenetic regulation of gene expression has been shown to be crucial for the development of organisms by maintaining cellular fates during proliferation. The Polycomb (PcG) and the trithorax (trxG) groups of genes play a pivotal role in this process by, respectively, maintaining the repressed and the activated transcriptional states. Both PcG and trxG genes were first identified in Drosophila melanogaster, due to their role in morphogenesis. However, it is now known that the PcG and trxG genes also have relevant roles in flies and other organisms in a range of biological processes, such as hematopoiesis, stem cell renewal, control of cell proliferation and tumorigenesis. Central to the PcG/trxG epigenetic mediated mechanisms is the recruitment and formation of multicentric protein complexes that interact with chromatin through regulatory sequences known as PREs (Polycomb Response Elements) and TREs (Trithorax Response Elements) found in their target genes. In addition to the core protein components of the PcG/trxG complexes, there exit associated proteins that may play a function controlling the specificity of PcG/trxG function and/or in the different responses to cellular stress. One of these proteins, the dRYBP protein, has been shown to interact with PcG and trxG proteins. Furthermore, dRYBP has been shown to have a role in morphogenesis through its interaction with the homeotic genes and in apoptosis through its interaction with the reaper gene. We are studying the function of dRYBP in the cellular stress response. Additionally, we are also studying the influence of the microRNAs in these processes. The results of these investigations will be presented.

875B

Role of Hpo and cell competition in the development of tumours by lgl mutant cells in Drosophila imaginal discs. Javier Menendez1, Ainhoa Perez2, Manolo Calleja1, Gines Morata1. 1) CBMSO, Nicolas Cabrera 1, Madrid 28049, Spain; 2) Rockefeller University, 1230 York
alleles suggest that ADAR is sufficient to rescue the NMJ defects observed in the Drosophila region (5'UTR). Translation of the predominant, smaller form of dFMR1 (dFMR1-S) begins at a canonical start codon (AUG) and translation of the minor, larger form (dFMR1-L) begins upstream at a non-canonical start codon (CUG). The 5'UTR sequence is well conserved in several Drosophila species suggesting that it is likely important for dFMR1 function. To assess the contribution of dFMR1-L to dFMR1 activity, we generated transgenic flies that express only dFMR1-L or only dFMR1-S. We are currently investigating whether the N-terminal extension contributes to dFMR1 function by using our transgenic lines to evaluate several processes known to require dFMR1 activity (including dendritic pruning, axon guidance, circadian behavior, short term memory, and oogenesis). Our latest results will be presented.

877A The Drosophila Fragile X protein (dFMR1) and ADAR interact and affect neuromuscular junction development. Balpreet Bhogal1, James E. Jepson1, Yiannis A. Savva1, Anna S.-R. Pepper1, Robert Reenan2, Thomas A. Jongens1. 1) Department of Genetics, University of Pennsylvania, School of Medicine, Philadelphia, PA; 2) Department of Microbiology, Cell Biology, and Biochemistry, Brown University, Providence, RI. The fragile X syndrome is caused by the loss of expression of the fragile X mental retardation protein (FMRP). FMRP is a RNA binding protein thought to be important for the translation of its neuronal target transcripts. The Drosophila homologue, dfMR1, is well conserved in sequence and function with respect to human FMRP. Although dfMR1 is known to express two isoforms (identifiable as a doublet by western analyses), the mechanism behind production of the second, more slowly migrating isofom has remained elusive. We have found that this second dfMR1 isofom is generated through an alternative translational start site in the dfmr1 5' UTR. Translation of the predominant, smaller form of dfMR1 (dfMR1-S) begins at a canonical start codon (AUG) and translation of the minor, larger form (dfMR1-L) begins upstream at a non-canonical start codon (CUG). The 5'UTR sequence is well conserved in several Drosophila species suggesting that it is likely important for dfMR1 function. To assess the contribution of dfMR1-L to dfMR1 activity, we generated transgenic flies that express only dfMR1-L or only dfMR1-S. We are currently investigating whether the N-terminal extension contributes to dfMR1 function by using our transgenic lines to evaluate several processes known to require dfMR1 activity (including dendritic pruning, axon guidance, circadian behavior, short term memory, and oogenesis). Our latest results will be presented.

877B A Genome-wide RNAi Screen for Modifiers of Aggregates Formation by Mutant Huntingtin in Drosophila. Sheng Zhang1, Richard Binari2, Rui Zhou1, Norbert Perrimon1. 1) Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Houston, TX; 2) Department of Genetics and Howard Hughes Medical Institute, Harvard Medical School, Boston, MA. Protein aggregates are a common pathological feature of most neurodegenerative diseases (NDs). Understanding their formation and regulation will help clarify their controversial roles in disease pathogenesis. To date there has been few systematic study of aggregates formation in Drosophila, a model organism that has been applied extensively in modelling NDs and screening for toxicity modifiers. We generated transgenic flies lines that express eGFP-tagged mutant Huntingtin (Htt) fragments with different length of polyglutamine (polyQ) tract, and showed that these Htt mutants develop protein aggregates in a polyQ-length and age-dependent manner in Drosophila. To identify central regulators of protein aggregation, we further generated stable Drosophila cell lines expressing these Htt mutants and also established a cell-based quantitative assay that allows automated measurement of aggregates within cells. We then performed a genome-wide RNA interference (RNAi) screen for regulators of mutant Htt aggregation and isolated 126 genes involved in diverse cellular processes. Interestingly, although our screen focused only on mutant Htt aggregation, several of the identified candidates were known previously as toxicity modifiers of NDs. Moreover, modulating the in vivo activity of hsp10 (CG6603) or trl1, two hits from the screen, affect neurodegeneration in a dose-dependent manner in a Drosophila model of Huntington’s disease (HD). Thus, other aggregates regulators isolated in our screen may identify additional genes involved in protein folding pathway and neurotoxicity.

879C Morphological evolution in Nasonia species through multiple noncoding changes. David W. Loehlin, John H. Werren. Biology, University of Rochester, Rochester, NY. The importance of noncoding cis-regulatory changes to the evolution of complex traits has been the subject of recent controversy. The wasp Nasonia is an emerging model for complex trait evolution, owing to cross-fertile species with sequenced genomes and male haploidy. We positional cloned large-effect QTL for sex-specific species differences in wing size. These loci alter wing size through a combination of cell size and cell number changes. Three of four genomic loci affecting wing size between species contain only noncoding, presumably cis-regulatory
sequence. One locus lies between prospero and doublesex and is associated with sex-specific, wing-specific changes in doublesex expression level. The other three loci are clustered in a region containing genes of unknown function. These results support the claim that cis-regulatory changes are important for morphological evolution above the species level.

880A
**Reduced and Dispersed HOX cluster in the two spotted spider mite, Tetranychus urticae.** Ryan M. Pace1, Miodrag Gribić2, Lisa Nagy1. 1) Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721; 2) Department of Biology, University of Western Ontario, London, ONtario, N6A 5B7, Canada.

HOX genes are clustered in genomes across the Metazoa. HOX genes also share a common DNA binding motif and are typically expressed in discrete domains along the A/P body axis. They primarily function to specify cell fate along the A/P body axis. HOX genes have frequently been shown to have a co-linear arrangement: their physical order on the chromosome parallels the position of their expression domains along the A/P axis during development. We have analyzed the genomic organization of the HOX cluster in the spider mite Tetranychus urticae. With a genome 3/5 the size of the fruit fly Drosophila melanogaster, an embryonic development of 39 hours and an egg to adult transition of less than 7 days, T. urticae, serves as an ideal model organism. We identified six of the eight canonical HOX genes within the recently sequenced T. urticae genome. Confirmation of the orthology assignments was done by constructing a comprehensive phylogenetic tree. Notable for their absence are the HOX genes Antp and abdA. We also identified a duplication of the rogue HOX gene fushi tarazu. HOX genes are typically found in single clusters within the genome, although in some cases they have split into two clusters (e.g. the D. melanogaster ANT-C and BX-C clusters). Interestingly, the T. urticae HOX genes are found in three distinct clusters and the region typically found between Deformed to fushi tarazu is characterized by dramatic intergenic loss. In addition, transcript length, relative to HOX genes in other arthropods, is reduced for the most 5′ genes and expanded for the most 3′. Exon number also varies from gene to gene in comparison to species sharing ancestral lineages. The discovery of a dispersed and reduced spider mite HOX cluster is unexpected given that other chelicerates show an increased number of HOX genes but is consistent with the reduced size of the T. urticae genome. Experiments are in progress to test the expression and function of the T. urticae HOX genes.

881B
**Insertion of fruitfly TEs in the genomes of parasitic wasps.** Neil Milan1,2, Todd Schlenke1,2. 1) Dept of Biology, Emory University, Atlanta, GA; 2) PBEE Program, Emory University, Atlanta, GA.

Parasitic wasps require hosts to complete their development into reproducing adults. In wasps that infect fruitflies, females oviposit into suitable fruitfly larvae or pupae; once a wasp larva emerges from its egg, it continues to develop and consume its host until emergence as an adult from the host’s pupal case. Given such an intimate interaction between host and parasitoid, we have been investigating the possibility that transposable elements (TEs), which are mobile within host cells, can move between host and parasitoid cells. Previously, we used data from PCR assays and various control experiments to propose that horizontal transfer is common in this system. Here, we present the results of Southern blot hybridization and inverse PCR to confirm that fruitfly TEs are inserted in wasp genomes, and to estimate the copy number of fruitfly TEs in wasp genomes.

882C
**Evolution of CTCF binding site in Drosophila species.** Xiaochun Ni1,2, Yong Zhang1, Kevin White1,2. 1) Ecology & Evolution, Univ Chicago, Chicago, IL; 2) Institute for genomics and systems biology, Univ Chicago, Chicago, IL.

Transcription factor (TF) binding sites are important regulatory elements in determining spatial temporal gene expression pattern. Characterization of binding site change in different species is crucial toward better understanding of gene regulation and its evolution. By the means of chromatin immuno-precipitation and next generation high-through-put sequencing (ChIP-seq), binding sites of the insulator protein CTCF at the white-pre-pupa stage were determined in four Drosophila species: D. melanogaster, D. simulans, D. yakuba and D. pseudoobscura. Further comparative analysis revealed that though with a similar enriched binding motif and genome position distribution, the genome-wide binding profile of CTCF is actually fast evolving in a linear like manner. More experiments and analysis are in progress toward finding the functional relevance of binding site evolution.

883A
**Various measures of fitness associated with male-killing Wolbachia in Drosophila innubila.** Robert Uncick, Lisa Boeio, John Jaenike. Department of Biology, University of Rochester, Rochester, NY.

Male-killing bacteria are quite common in arthropods. In most cases mothers whose sons are killed as embryos are though to have daughters who benefit from the killing. Theoretical benefits for daughters include decreased sibling competition for limited resources, decreased costly inbreeding and cannibalism of dead embryos. In no system has an actual benefit been determined. We use a variety of techniques to investigate the benefit of a male-killing Wolbachia in Drosophila innubila. Our results suggest that no single factor can explain the maintenance of infection, but perhaps several factors play a role.

884B
**Quantitative genetic analysis suggests causal association between cuticular hydrocarbon composition and desiccation survival in Drosophila melanogaster.** Brad R. Foley1, Marina Telonis-Scott2. 1) Molec Cell Biol, Univ Southern California, Los Angeles, CA; 2) Department of Genetics, University of Melbourne, VIC AU.

Survival to low relative humidity is a complex adaptation, and many repeated instances of evolution to desiccation have been observed among Drosophila populations and species. One general mechanism for desiccation resistance is Cuticular Hydrocarbon (CHC) melting point. We performed the first Quantitative Trait Locus (QTL) map of population level genetic variation in desiccation resistance in D. melanogaster. Using a panel of Recombinant Inbred Lines (RILs) derived from a single natural population, we mapped QTL in both sexes throughout the genome. We found that in both sexes, CHCs correlated strongly with desiccation resistance. At most desiccation resistance loci there was a significant association between CHCs and desiccation resistance of the sort predicted from clinal patterns of CHC variation and biochemical properties of lipids. This association was stronger in females than males, perhaps due to correlations between CHCs used for waterproofing and sexual signalling in males. CHC evolution may be a common mechanism for desiccation resistance in D. melanogaster. It will be interesting to compare patterns of CHC variation and desiccation resistance in species which adapt to desiccation, and rainforest restricted species which cannot.

885C
**Computational and experimental network analysis to find loci “missed” by Genome-Wide Association Studies (GWAS): leveraging the

There is growing concern that standard Genome-Wide Association Studies (GWAS) will reach a limit in their ability to find loci contributing to “dark heritability”, the heritability underlying the majority of disease and physiology phenotypes that cannot be explained by the loci discovered in GWAS. It is clear that many loci contributing to dark heritability will have effects that are too small to be discovered by GWAS, when mapping directly from genetic polymorphism to phenotype, and this will be true even as study sample sizes and genominc marker coverage increase. With this research, we demonstrate the power of network GWAS analysis for finding small effect loci. Intuitively, our approach is to find important intermediate phenotypes that produce variation in downstream complex traits, and map loci with weak associations with the downstream traits but strong associations with these intermediate phenotypes. Using wing size and other complex wing phenotypes measured in the Drosophila Genetic Reference Panel (DGRP) as a model system, we make use of bioinformatics approaches and computational network analysis methods developed by our group to find appropriate intermediate phenotypes among larval wing gene expression phenotypes. We use locus associations with these phenotypes as natural perturbations to drive this network discovery, and in the process identify loci with weak effects on the wing that would be missed by a GWAS mapping directly to the wing phenotype. The broad objective of this research is to develop integrative methods for discovering loci with small effects by intermediate GWAS mapping that can be adapted to the analysis of complex human disease.

886A

Habitat selection of body color polymorphism in Drosophila jambulina. SEEMA RAMNIWAS, SHAMA SINGH, RAVI PARIKASH. GENETICS, M. D.UNIVERSITY, ROHTAK.

Drosophila jambulina exhibits color dimorphism controlled by a single locus but its ecological significance is not clear. Dark and light morphs differ significantly in body melanisation, desiccation resistance, rate of water loss, mating activity and fecundity. Interestingly, this species lacks clinal variation for body size, desiccation resistance and life history traits. For body melanisation, lack of geographical variation as well as plastic effects is not in agreement with a thermal melanism hypothesis. However, based on field data, there are seasonal changes in phenotypic frequency of dark and light body color morphs which correlate significantly with variation in humidity levels. Under short-term (8 hours) desiccation stress, we observed higher number of assortative matings, longer copulation period and increased fecundity for dark strains as compared with light strains. By contrast, both the morphs when exposed to high humid conditions exhibited higher assortative matings and fecundity for light strains as compared with dark strains. In tropical populations of D. jambulina, body color polymorphism seems to be maintained through humidity changes as opposed to thermal melanism. Thus, seasonal changes in the frequency of body color morphs in this tropical species supports melanism-desiccation hypothesis.

887B


Sexual differences between fly males and females are regulated at several levels. Sex specific programs include: i) X chromosome dosage compensation; ii) somatic sexual differences and iii) male courtship behavior. These processes are regulated by a large set of genes that involve both protein coding and non-coding RNAs and operate by both transcriptional and post-transcriptional processes.

Dosage compensation equals the levels of X-linked transcripts between heterogametic males (XY) and homogametic females (XX). In males, at least 5 proteins (MSL-1, MSL-2, MSL-3, MLE, MOF) and two long non-coding RNAs roX1 and roX2 form a ribonucleoprotein complex whose association at hundreds of sites along the X chromosome operate by controlling the levels of transcription. These complexes enable the doubling of the expression levels of X-linked genes. In females regulation, Male-specific-lethal-2 (msl-2) mRNA translation is repressed through Sex-lethal (Sxl) binding to its message UTRs, therefore preventing dosage compensation.

MicroRNAs comprise an extensive class of regulatory RNAs playing a major role in the regulation of gene expression throughout animal development and likely influence the output of many protein-coding genes. We will present work investigating the role of miRNAs in regulating sex-specific gene expression in Drosophila. Using high throughput sequencing methodology, the coordination between the expression of miRNAs and several sex determination genes in various male and female tissues has been identified. Mechanisms involving networks of miRNAs influencing the diverse programs of sex determination and contributing in particular to the transcriptional regulation of an entire chromosome during dosage compensation will be proposed.

888C

Identification of a role for DWnt4 in Drosophila heart development. Petra Pandur, Helen Tauc, Kathrin Werner, Tabea Mann. University of Ulm, Institute for Biochemistry and Molecular Biology, Ulm, Germany.

Different Wnt signaling pathways play critical roles in various steps of cardiogenesis in vertebrates. In Drosophila, it has been demonstrated that the Wg signaling pathway mediated by armadillo is essential for normal cardiogenesis. In contrast, DWnt4 has been shown to act through an armadillo-independent signaling pathway. Since a function of DWnt4 in fly heart development has not been described yet, we analyzed DWnt4 mutant embryos for possible heart defects. First we re-evaluated the expression pattern of DWnt4 with respect to a possible role in heart development. Our results show the presence of DWnt4 transcripts in the ectoderm as well as in the mesoderm in the dorsal region of the embryo where heart cells become specified. Moreover, we detected DWnt4 mRNA expression in the myocardial cells at stage 16. This novel finding suggests a function for DWnt4 at late stages when the heart tube forms. The heart phenotype in DWnt4 mutant embryos is characterized by various degrees of disrupted expression of all investigated cardiac markers. In summary, our data introduce DWnt4 as a novel secreted growth factor involved in fly heart development. The integration of DWnt4 with other known signaling pathways that function in heart development awaits further investigation.

889A


Arginine kinase (AK) is the primary phosphagen kinase in the fruitfly, Drosophila melanogaster, and is especially abundant in indirect flight muscle. However, significant activity has also been found in other tissues, such as the digestive tract, brain and ganglia, and the reproductive tract. Arginine kinase is the product of a single gene, Argk which produces six alternative transcripts, RA, RB, RC, RD, RE and RF. A unique protein isoform appears in mature ovaries and persists in the early embryo, but it is not known which transcript was responsible for this protein isoform. The developmental profile of this form in developing ovaries, fluorescent microscopy of ovarioles labeled with antibody to AK, fluorescent in situ hybridization (FISH) of labeled RNA probes specific for the RA, RB, RC and RD transcripts, and fluorescent microscopy of
green fluorescent protein (GFP) protein-trap mutations strongly suggest that the RB transcript is responsible for the ovarian form of arginine kinase.

890B

The hybrid lethality gene Lhr is required for female fertility. Rajavasireddy P Satyaki, Daniel Barbash. Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Crosses of Drosophila melanogaster females and D. simulans males produce sterile female and lethal male progeny. Such post-zygotic reproductive isolation can be explained using the framework of the Dobzhansky-Muller model. This model posits that negative epistasis between divergent genes of incipient species causes hybrid incompatibility. The Lethal hybrid rescue (Lhr) gene was discovered as a mutation in D. simulans that suppresses hybrid male lethality. Lethal hybrid rescue (Lhr) encodes a fast evolving protein that localizes to pericentric heterochromatin. The mechanism by which Lhr causes hybrid lethality is unknown, as is its normal function within D. melanogaster and D. simulans. The only known Lhr loss of function alleles exist in D. simulans and have no apparent phenotypes, but they may not be null mutations. To enable the test of considerable wealth of genetic tools available in D. melanogaster, we have disrupted the Lhr gene in D. melanogaster through homologous recombination to make a null allele. Preliminary data shows that the Lhr mutant line has reduced female fertility. Furthermore, we observe that the fertility defect is due to the lower fecundity of the mutant females as well as a low hatch rate of the eggs laid by the Lhr mutant mothers. Experiments are underway to examine the mechanistic basis for this phenotype.

891C


A fundamental question in developmental biology is how cell rearrangements are initiated and controlled during morphogenesis and tissue remodeling. In Drosophila oogenesis, somatic follicle cells surround each developing germ cell cyst, and develop coordinately with the oocyte to create the mature egg. Precursor follicle cells initially form a simple cuboidal epithelium, which becomes subdivided into domains of distinct cell fates. As the domains progressively differentiate, it undergoes a stereotypical reorganization to create a specific structure of the eggshell. Each eggshell structure has features that promote survival of the developing embryo. Our lab investigates the role of TGF-β superfamily signals in follicle cell morphogenesis. Dpp, a homolog of mammalian BMP2 and BMP4, is expressed in the anterior-most follicle cells. BMP signaling during mid-oogenesis regulates gene expression in the domain of cells that will migrate into the germ cell complex to cover the anterior oocyte. These cells will later form the operculum and ventral collar of the eggshell, which are sensitive to levels of Dpp. However, the use of fixed tissues for these studies precluded a definitive test of the requirement for BMP to induce or maintain the movement of follicle cells to separate the nurse cells and the oocyte. To pursue this question, we have adopted the methodology to culture living egg chamber from Prasad and Montell. We will report our progress in developing this system to study BMP signaling in a living epithelium.

892A


The Drosophila tracheal system is composed of a network of tubes that sprout from 10 pairs of epithelial sacs. The network forms by a series of successive branching and fusion events. During this process, specialized tip cells lead the migration of new branches and mediate their interconnection. During the branch fusion process, some tip cells become specialized as “fusion cells” and convert themselves into seamless tubes that span the branch fusion joint. Other tip cells become specialized as “terminal cells” that go on to form branched, blind-ended seamless tubes that ramify extensively on target tissues, like muscle, and act as the site of gas exchange. Similar to tracheal terminal and fusion cells, endothelial tip cells that connect new sprouts to other tubes in the vascular network during angiogenesis have been shown to form seamless tubes. So-called “seamless endothelial cells” are believed to create seamless tubes by a cell hollowing process in which vesicles and vacuoles are trafficked to the center of the cell and coalesce and fuse to form an intracellular lumen bounded by apical membrane. I will exploit factors identified in a genetic mosaic screen, such as whacked, to determine the genetic and molecular mechanisms controlling seamless tube formation. The primary defect in whacked mutants is the overgrowth of tubes at the distal tip of terminal branches. whacked encodes a RabGAP protein. Interestingly, overexpression of Whacked results in a striking phenotype, causing the overgrowth of tubes proximal to the terminal cell of the tube. Additional work has revealed that Rab35 is the key target of Whacked RabGAP activity: expression of a constitutively active isoform of Rab35 phenocopies whacked loss of function and expression of a dominant negative isoform of Rab35 (Rab35DN) phenocopies the whacked overexpression defect. Moreover, expression of Rab35DN in the tracheal system can suppress whacked terminal cell morphogenesis defects and rescue whacked mutant animals to viability. Thus, we predict that Rab35, and its regulator Whacked, direct polarized tube growth.

893B

The Role of Tudor in Germline Development. Ju-Yu Wang1, Ying Huang2, Rui-Ming Xu3,3, Ruth Lehmann1. 1) Developmental Genetics Program, The Skirball Institute, Howard Hughes Medical Institute, Department of Cell Biology, NYU School of Medicine, New York; 2) Structural Biology Program, The Skirball Institute, NYU School of Medicine, New York; 3) 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 and germ cell formation. Previously, we have shown that a small number of non-overlapping tudor domains or a substantial reduction in overall Tudor protein is sufficient for abdomen development. In contrast, we found there is a requirement for specific tudor domains in germ cell formation, Tudor localization and polar granule architecture. The tudor domain is a protein motif that binds efficiently to other proteins that contain methylated arginine and lysine residues. To shed light on the function of Tudor in germ cell formation we have used a number of different approaches to identify molecules that interact with Tudor. Tudor domains consist of four β-strands that fold into a barrel-like structure. Structural analysis and sequence alignment of an individual Drosophila tudor domain confirms the presence of aromatic cages, which have been implicated in recognition and binding of methylation targets. Recent studies show that Aubergine, a germline-specific Argonaute protein contains symmetric dimethyl-arginines (sDMAs), associates with Tudor through its sDMA modification. To test if individual tudor domains play specific roles in germ cell formation and in Aubergine association, we mutated aromatic residues in individual tudor domains. Results from these studies will be presented.

894C

Male fertility depends on proper differentiation of accessory gland in Drosophila. Ryunosuke Minami1, Kiichiro Taniguchi2, Akihiko

The Drosophila homeobox gene defective proventriculus (dve) is expressed in various tissues including male reproductive organs such as accessory gland (AG). AG has homologous functions to mammalian prostate gland, and is composed of two types of cell: main cell and secondary cell. These precursor cells are mononuclear and differentiate into binucleated mature cells that secrete accessory gland proteins (Acps) into seminal fluid. These Acps induce female postmating behavior and thus increasing progeny production. Dve expression starts at around 24 hours after puparium formation, and continues through adult stages in secondary cells. Although the dve gene is transcriptionally active, Dve protein is undetectable in mature main cells. It is repressed by Pair-rule, which is essential for AG development, together with a proteasome system. Temporally-regulated dve repression in main cells seems to be crucial for binucleation, because forced dve expression strongly inhibited it. Furthermore, loss of dve activity also resulted in inhibition of binucleation and failure of secondary cell differentiation. dve mutant males showed greatly reduced fertility with reduced size of AG, although their courtship activity, spermatogenesis, and sperm motility were normal. To examine the mechanism of reduced fertility, we checked female post-mating behavior. Females mated with a dve mutant male laid the small number of eggs, and the hatching rate of these eggs was significantly lower than that of control. These results suggest that proper differentiation of AG is crucial for male fertility.

895A
Racquetball is required for meiotic division and differentiation in the male germline. Catherine C. Baker, Lucineh Parsanian, Margaret Fuller. Developmental Biology, Stanford University School of Medicine, Stanford, CA.

In Drosophila spermatogenesis, both the timing of the meiotic divisions and the extensive post-meiotic morphological changes required for formation of mature sperm are heavily dependent on translational control. The majority of the RNAs whose gene products are required for both divisions and differentiation are transcribed in spermatocytes during meiotic G2 prophase; in contrast, post-meiotic spermatids are largely transcriptionally quiescent. We have identified a protein that may act to stabilize specific RNAs during spermatogenesis. The CG17838 gene (which we named racquetball, or rq) encodes the human hnRNPF/RQ family, whose best-known member hnRNP Q has been shown to act in complexes to protect specific RNAs from endonuclease or deadenylase attack. RNAi knockdown of rq in testis leads to a meiotic arrest phenotype. Consistent with the failure of rq RNAi spermatocytes to complete meiosis, normal upregulation of CycB protein in late spermatocytes, just prior to meiotic division, is not detectable. In situ hybridization with a cycB probe showed while cycB is expressed across all spermatocytes in wild-type testes, it is detected only in early spermatocytes in rq RNAi testes. A similar result was obtained for fzo, a message which is transcribed in spermatocytes but whose protein product is not required until after meiotic division. In contrast to their expression in rq RNAi, both cycB and fzo are expressed throughout arrested spermatocytes in testes mutant for eIF4G2 (Baker and Fuller, 2007, and unpublished data). The cycB and fzo expression pattern in rq RNAi is not the result of a non-specific downregulation of all RNAs in late rq spermatocytes, as boule transcript is detectable throughout rq spermatocytes. We are currently employing a candidate gene approach to identify potential cofactors and antagonists of Rq function.

896B
Differentially expressed profiles in the larval testes of Wolbachia infected and uninfected Drosophila. Yu-Feng Wang, Ya Zheng, Jia-Lin Wang. Hubei Key Laboratory of Genetic Regulation and Integrative Biology, College of Life Science, Huazhong Normal University. 430079 Wuhan, P. R. China.

Wolbachia bacteria are endosymbionts found frequently in arthropods and nematodes. They may manipulate the reproduction of their hosts by several mechanisms, including cytoplasmic incompatibility (CI), parthenogenesis, feminization and male killing. CI is the most commonly phenotype induced by Wolbachia infection and causes the developmental arrest of embryos derived from the crosses of infected males with uninfected females. In order to know the differences of sperms between Wolbachia infected and uninfected males, total RNA was isolated from the testes of Drosophila melanogaster larvae with and without (tetracycline treated) Wolbachia (wMel) infection. Transcription profiles were compared between these two testes by microarray. 178 genes were identified to have at least 2 fold changes of transcripts, including 49 genes with descriptions of functions and 129 genes with unknown functions. The expression levels of 80 genes were up-regulated, while 98 genes were found to be down-regulated. Differentially expressed patterns related to immune response, metabolism and regulators were identified. Interestingly, the genes putatively involved in spermatogenesis were found to be down-regulated, perhaps reflecting a disruption in the normal process of sperm production. The current data provide a set of genes that changed expressions in larval testes after infection with Wolbachia, which may be the candidates for further studies on the Wolbachia/host interaction and spermatogenesis. The possible mechanisms by which Wolbachia infection impacts the sperm fertility of their hosts needs to be explored in detail. Acknowledgements This work was supported by the National Nature Science Foundation of China (30970405) and the key project of Chinese ministry of education (109118).

897C

Gut homeostasis is tightly regulated and is achieved through a complex balance between immune and developmental mechanisms. However, little is known about the effects of bacteria on gut epithelium renewal and to a larger extent on gut morphology, as well as the pathways involved in this balance. We previously demonstrated that ingestion of a Gram-negative bacterium, Erwinia carotovora 15 (Ecc15), dramatically impacts the physiology of the gut, inducing a stress response and an increased epithelial renewal through intestinal stem cell proliferation. We further demonstrated that the JAK/STAT pathway is required for bacteria-induced stem cell proliferation. Interestingly, indigenous gut microbiota activate the same, albeit reduced, program at basal levels. In the present work, we analyze the role of the EGF pathway in bacteria-induced epithelium renewal. We first observe that the EGF pathway synergizes with the JAK-STAT pathway to promote stem cell proliferation and differentiation in response to damage caused by ingested bacteria. Additionally, flies lacking a functional EGF pathway are highly susceptible to enteric infections. Altogether, these results indicate that bacteria influence both gut homeostasis and morphology through the complex regulation of immune (Imd) and developmental (JAK/STAT, EGF) pathways.

898A

Metabolism and immunity are two evolutionarily relevant phenotypes that have a large and direct impact on survival and reproduction.
Understanding intersections between the two provides a valuable insight into metabolic disease, longevity, and the evolution of complex traits. Here we show that the immune response changes in response to diet and discuss the relationships between pathogen, type of immune response, diet, and metabolic state. A subset of lines from the Drosophila Genomic Reference Panel were evaluated for bacterial load and metabolic state on two diets known to differ in generation time. We found significant variation among the lines for bacterial load; however, bacterial loads were strongly correlated across diet with surprising little genotype by environment interaction. Congruent with previous studies, flies reared on the less nutritious media showed greater amounts of variability and lower resistance. Comparisons between these treatments reveal the relative importance of variation in metabolic state to the effectiveness of the immune response. These experiments provide a foundation for future studies to define the genes and pathways responsible for co-regulation of immune response and metabolism.

899B

A role for Big-Bang in defense against natural infections. Nicolas Matt, Eva Berros, François Bonnay, Marie Husson, Jean-Marc Reichhart. Institut de Biologie Moléculaire et Cellulaire, UPR 9022 CNRS, Université de Strasbourg, STRASBOURG, France.

Drosophila’s innate immunity relies on a multilayered response. The epithelia constitute a first and efficient barrier against microorganism invasions. However, if microorganisms enter the general cavity, humoral and cellular responses intervene to fight the infection. The most documented aspect of this immune system is the expression, under the control of the Toll and IMD pathways, of a battery of antimicrobial peptides in the fat body and their secretion in the hemolymph. Most of the studies performed so far rely on artificial infections imposed with a constant concentration of pathogen (by hypodermic injection of the pathogen in the digestive tract), this experimental system being only an approximation of the important defense mechanisms that take place in the epithelia. Aiming at better understand these epithelia-related defense mechanisms, it was of major interest to develop natural infection procedures, either by contact or feeding with pathogenic microorganisms that can naturally infect and kill the flies. Here we analyse a new drosophila mutant, big-bang, that was obtained by mobilizing a P-element inserted in the genome of the fly. Big-bang encodes a PDZ domain protein of unknown function, that appear to be specifically involved in epithelial defense mechanisms.

900C

FOXO-dependent regulation of innate immune homeostasis. Gerrit Loch, Thomas Becker, Marc Beyer, Ingo Zinke, Anna C. Aschbrenner, Pilar Carerra, Therese Inhester, Joachim L. Schultzze, Michael Hoch. LIMES - Life and Medical Sciences Institute, University of Bonn, Bonn, Germany.

The innate immune system represents an ancient host defence mechanism that protects against invading microorganisms. An important class of immune effector molecules to fight pathogen infections are antimicrobial peptides (AMPs) that are produced in plants and animals. In Drosophila, the induction of AMPs in response to infection is regulated through the activation of the evolutionarily conserved Toll and immune deficiency (IMD) pathways. We show that AMP activation can be achieved independently of these immunoregulatory pathways by the transcription factor FOXO, a key regulator of stress resistance, metabolism and ageing. In non-infected animals, AMP genes are activated in response to nuclear FOXO activity when induced by starvation, using insulin signalling mutants, or by applying small molecule inhibitors. AMP induction is lost in foxo null mutants but enhanced when FOXO is overexpressed. Expression of AMP genes in response to FOXO activity can also be triggered in animals unable to respond to immune challenges due to defects in the Toll and IMD pathways. Molecular experiments at the Drosomycin promoter indicate that FOXO directly binds to its regulatory region, thereby inducing its transcription. In vivo studies in Drosophila, but also studies in human cells indicate that a FOXO dependent regulation of AMPs is evolutionarily conserved. Our results indicate a new mechanism of cross-regulation of metabolism and innate immunity by which AMP genes can be activated under normal physiological conditions in response to the energy status of cells and tissues. The sparse production of AMPs in epithelial tissues in response to FOXO may help modulating the defence reaction without harming the host tissues, in particular when animals are suffering from energy shortage or stress.

901A

Proteolytic genes have distinct roles in regulating environmental influence on aggressive behavior. Lance French1, Ronald Dorenbos2, Sarah Cerel1,2, Michael Zito2, Edward Kravitz2. 1) Center for Structural & Functional Neuroscience, University of Montana, Missoula, MT; 2) Dept of Neurobiology, Harvard Medical School, Boston, MA.

Aggressive behavior is critical for survival and reproduction yet can be strongly modified by environmental and genetic factors. In vertebrates and invertebrates, social experience with conspecifics in a group-housed setting suppressed male aggressiveness. We took a candidate gene approach to identify molecular mechanisms that regulate environmental impacts on aggression and examined a series of extracellular proteases. Results from qPCR experiments indicate that yippee7, Jon65Aiii, and Jon65Av expression dynamically changes in response to isolated vs. group housing. After determining a start value in newly eclosed males, we found that expression of each gene is significantly upregulated in isolated males and decreased in group-housed males. Further PCR experiments determined that yippee7, Jon65Av, and Jon65Av transcripts are found in dissected male brains. To test if regulated proteolysis is an essential mechanism for translating social experience effects, we eliminated individual gene expression in the male nervous system using RNA-mediated interference transgenes. Our results indicate that changes in territorial behavior are observed in group-housed males without Yippee7 function. Group-housed elav-Gal4;UAS-dicer2;UAS-yippee7th experimental males share the food resource for a significantly longer period of time than controls during pairing in a fight chamber. Studies are ongoing to determine whether aggression is suppressed in these specific proteolytic gene mutants with a goal of understanding how context-specific aggression emerges from past experience. Supported by NIH grants to EAK (GM-074675 and 067645) and to SJC (NIH COBRE grant P20RR015583, from the National Center for Research Resources).

902B

A New Conserved Neural Signaling System for Hunger Regulation of Food Intake in Drosophila. Ting Zhang1, Ping Shen1,2. 1) Neuroscience Program, University of Georgia, Athens, GA; 2) Cellular Biology Department, University of Georgia, Athens, GA.

Hunger-elicited feeding rate increase is one main component of hunger response. However, the molecular basis and neural circuits for this feeding rate control remain largely uncharacterized. To understand the underlying mechanisms, we have performed transgenic analyses and behavior assays to identify and characterize genes and molecular pathways in the Drosophila larva model.

Here, we demonstrate that a gene conserved between flies and humans, named moderate eater-1 (me-1), regulates feeding rate in Drosophila larvae. Functional knockdown of me-1 in the nervous system attenuates hunger-induced increase of feeding rate, while neural overexpression of me-1 increases the feeding rate of fed larvae but does not elicit general hunger response. We also provide evidence that the me-1 activity in octopaminergic neurons plays a prominent role in hunger regulation of ingestion rate. Larvae expressing me-1 RNAi or a dominant-negative form of dynamin [shi(st1)], driven by Tdc2-gal4, both showed attenuated hunger-driven increase of feeding rate, suggesting that me-1 promotes
hunger-driven feeding rate increase by positively modulating the activities of octopaminergic neurons. Our findings have revealed a novel regulatory mechanism for fly food intake, and raised the possibility that regulation of food intake in mammals might also involve a homologous mechanism.

903C

**Forgetting is regulated through Rac activity in Drosophila.** Yiechun Shuai1, Binyan Lu1, Lianzhang Wang1, Kan Sun1, Ying Hu1, Yi Zhong1,2, 1) School of Life Sciences, Tsinghua University, Beijing 100084, China; 2) Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA.

Initially acquired memory dissipates rapidly if not consolidated. Such memory decay is thought to result either from the inherently labile nature of newly-acquired memories or from interference by subsequently attained information. Here we report that a small G-protein Rac-dependent forgetting mechanism contributes to both passive memory decay and interference-induced forgetting in Drosophila. Inhibition of Rac activity leads to slower decay of early memory, extending it from a few hours to more than one day, and to blockade of interference-induced forgetting. Conversely, elevated Rac activity in mushroom body neurons accelerates memory decay. This forgetting mechanism does not affect memory acquisition and is independent of Rutabaga adenyl cyclase-mediated memory formation mechanisms. Endogenous Rac activation is evoked during passive memory decay and also during reversal training (wherein counterclockwise memories are lost), but on different time scales. We suggest that Rac’s role in actin cytoskeleton remodeling may contribute to memory erasure.

904A

**Stathmin is Required for Axonal Transport in Drosophila.** Jason Duncan1, Nikki Lytle1, Louise Parker2, Lawrence Goldstein1. 1) Department of Biology, Willamette University, Salem, OR; 2) University of California Berkeley, Berkeley, CA; 3) University of California San Diego, La Jolla, CA.

Neurons utilize a microtubule-based transport system to bidirectionally transport vesicles, organelles between the cell body and the synaptic terminal through the axon. We have identified the protein stathmin (stai), which regulates the dynamics of the microtubule cytoskeleton, as a component necessary for axonal transport in Drosophila. Several mutations in stai have been isolated that exhibit phenotypes consistent with severe defects in axonal transport. In mutant third instar larvae, the posterior body segments sharply flip up after each peristaltic contraction during the crawling cycle, indicating paralysis of the posterior musculature. In addition, axons of the longitudinal segmental nerves that emerge from the brain and bilaterally innervate the body wall musculature of each larval segment contain focal swellings and accumulations of transported components. Western blot analyses of these larvae indicate dramatic reductions in the levels of tubulin protein, yet we observed only minor alterations in the microtubule architecture of both segmental nerve axons and cells of the body wall musculature. Mutant larvae also have reduced levels of the conventional kinesin heavy and light chain motor components. A small percentage of stai mutants survive to the adult stage but have significantly reduced life spans. These adults have severe movement defects, often dragging their hind limbs as they walk. Unexpectedly, they also exhibit a progressive, age-dependent seizure phenotype, characteristic of the ‘bang sensitive’ mutants that have altered neuronal excitability. Electrophysiological analysis indicates these animals have a lower evoked seizure threshold than wildtype animals. Collectively, our data demonstrate that stai is essential for microtubule-based axonal transport, and uncover a novel role in the regulation of neuronal excitability.

905B

**Mushroom Body Neurons Promote Wakefulness.** Gang Liu, Ravi Allada. Department of Neurobiology and Physiology, Northwestern University, Evanston, IL.

Drosophila exhibits nearly all of the behavioral characteristics of mammalian sleep. Our lab has previously shown a sleep-promoting role for the mushroom bodies (MB) [Pitman et al, 2006]. Nonetheless, coincident work suggested the possibility that subpopulations of mushroom body neurons might be wake promoting [Joiner et al, 2006]. The MBs consist of three main classes of neurons, with distinct projection into the alpha/beta lobes, alpha’/beta’ lobes and gamma lobes. These neurons also have specialized functions in learning and memory. In order to investigate those functional subsets within the MB, we have been using GAL80 and GAL4/UAS combinations to localize neural subsets that regulate sleep. By using bacterial depolarization-activated sodium channel NaChBac to increase neuronal excitability, we identified a number of GAL4 lines with shared expression in MB gamma lobe neurons, which demonstrated reduced and fragmented sleep. Gal80 lines that suppress MB GAL4 expression including in the gamma neurons could suppress these sleep phenotypes. We observed similar results using the temperature-gated cation channel dITpA1. Evidence also suggests that activating the CAMP pathway also reduce sleep. These studies define specific MB circuits involved in both memory and sleep.

906C

**Disabled is a bona fide component of the Abl signaling network.** Jeong K Song, Edward Giniger. NINDS/NIH, Bethesda, MD.

Abl is an essential regulator of cell migration and morphogenesis in both vertebrates and invertebrates. It has long been speculated that the adaptor protein, Disabled (Dab), which is itself a key regulator of neuronal migration in the vertebrate brain, might be a component of this signaling pathway but this has been controversial. We now demonstrate that null mutations of Drosophila dab display phenotypes that mimic abl mutant phenotypes, both in axon guidance and in epithelial morphogenesis. The dab mutant interacts genetically with mutations in abl, and in the Abl accessory factors, trio and enabled (ena). Genetic epistasis tests show that dab functions upstream of abl and ena, and consistent with this, we show that Dab is required for subcellular localization of these two proteins. We therefore infer that Dab is indeed a bona fide component of the core Abl signaling pathway in Drosophila.

907A

**Serotonergic neurons regulate growth by controlling insulin-like peptide secretion.** Tom Hartl, Jian Cao, Julie Ni, Dan Kaplan, Kaye Suyama, Matthew Scott. Department of Developmental Biology, Stanford University, Stanford, CA.

Cell proliferation, growth, and energy metabolism are tightly regulated processes in the course of animal development and are primary facets of an organism’s physiological and evolutionary fitness. IGFs and insulin are central mediators of these processes. IGFs control tissue growth by initiating signaling cascades that stimulate cell growth and proliferation rates, whereas insulin is essential in balancing energy storage or utilization based on an organism’s metabolic needs. A fascinating biological problem is how IGFs and insulin are regulated by the brain in response to an organism’s ever-changing environmental and physiological states. For example, allocation of energy for growth can be suppressed during times of hibernation, migration, starvation, or danger, and growth can be accelerated to cope with environmental factors, such as the need to reach a size sufficient to survive a winter. In mammals, it is clear that the neural signals dopamine, serotonin, acetylcholine, and adrenalin are
critical factors in a cascade of regulatory events that culminates in decisions about when to release and/or synthesize IGF/insulin and thus control growth and metabolism. Similarly, in Drosophila, neural control of IGF/insulin release governs growth and blood sugar levels. Our lab has traced this neural control to the #4 serotonergic neurons. This provides a unique genetically tractable system to (1) learn how the serotonergic neurons regulate IGF/insulin release and (2) as a starting point for defining the regulatory circuitry that integrates sensory information upstream of homeostatic and growth controls. Our long-term goal is to learn how neural regulation alters subconscious (e.g., digestive enzymes) and conscious (e.g., hunting) behaviors to control an organism’s growth and energy balance.

908B
RNAi screen for genes involved in the asymmetric division of Drosophila neural stem cell. Huaishan Wang¹, Xiaohang Yang², ³. 1) Institute of Molecular and Cell Biology, 61 Biopolis Drive, Proteus Building, Singapore 138673; 2) Department of Anatomy, Block MD10, Yong Loo Lin School of Medicine, 4 Medical Drive, Singapore 117597.

Drosophila neural stem cells, also known as neuroblasts, provide an excellent model system for investigating asymmetric cell division mechanisms. Neuroblasts divide asymmetrically during development to produce a self-renewing neuroblast and a ganglion mother cell which divides terminally to generate two neurons or glia. To better understand the molecular network controlling the asymmetric cell division, we conducted an RNA interference screen on 200 candidate genes whose protein products were suggested to interact with known players involved in asymmetric division, such as aPKC and Par-6. We specifically expressed RNAi constructs in larval brain neuroblasts, looking for phenotypes of delocalization of Miranda or abnormal cell size. Knockdown of one of the genes, cmin, led to cytoplasmic Miranda phenotype in neuroblast MARCM clones in the larval brain. Anti-CMIM staining indicates that CMIN is asymmetrically localized to the apical side of the dividing neuroblasts. We are currently in the process of functional analyses of CMIN role in asymmetric cell division.

909C
Dendritic and Axonal Targeting along Medial to Lateral Axis in the Antennal Lobe Are Regulated by Meigo, a Putative UDP-Sugar Transporter. Sayaka Sekine¹, Laqun Luo¹, Masayuki Murai², Takahiro Chihara³,⁴. 1) Genetics, Pharm Sci, Univ Tokyo, Japan; 2) PRESTO, JST, Japan; 3) Research EST, JST, Japan; 4) Department of Developmental Neurosciences, KIMI and Dept Riel, Singapore Univ, SIngapore.

The wiring of functional neural network results from numerous synaptic connections among neurons. The proper “targeting” of both axons and dendrites actively contribute to the synaptic match making, however the molecular mechanism is largely unknown. During the development of Drosophila olfactory system, the axon of primary neuron (olfactory receptor neuron; ORN) target one of ~50 glomeruli in the antennal lobe (AL), and the dendrites of secondary neuron (projection neuron; PN) also target single glomerulus independently of ORN axon, offering us a good model system to study and compare the targeting mechanisms of synaptic partners. To obtain mutants that exhibit dendritic misrouting in PN, we performed MARCM-based screen and isolated a mutant, meigoB3-37. The dendritic targeting of PNs homozygous for meigoB3-37 (meigoB3-37/PN) shifted to the medial side of the AL, but the axonal trajectory and branching were largely normal, indicating that meigoB3-37 mutation causes the targeting defects specifically in dendrite but not axon of PN. Interestingly, however, meigoB3-37 ORN exhibited the defects in axonal targeting. The axon of meigoB3-37 ORN mistargeted to the medial side of AL, which was similar to those seen in meigoB3-37 PN dendrites. The responsible gene, meigo, encodes a putative UDP-sugar transporter, which is conserved from yeast to human. Meigo was localized at ER and Golgi, and the null mutants for the other UDP-sugar transporters (slalom, fringe connection) did not exhibit defects in PN dendritic targeting. Together with the mosaic rescue experiments, our results indicate that Meigo is cell-autonomously required for neuronal targeting along the medial to lateral (M-L) axis in the AL, probably by regulating the glycosylation and/or folding of cell surface proteins. Further analyses may offer new insights into the molecular mechanism for forming and/or recognizing the positional information along M-L axis in the neural wiring.

910A
HB9-mediated Activation of the Forkhead Domain Containing Protein, FD59a, in the CNS. Haluc Lakin, Yi Zhu, James Skeath. Dept Genetics, Washington Univ, St Louis, St Louis, MO.

The central nervous system (CNS) is composed of an enormous number of different cell types. Distinct transcriptional regulatory networks play a central role in directing different subsets of cells down distinct paths of differentiation. In the fly and vertebrate CNS, the HB9 and Nkx6 homeodomain-containing transcription factors promote motoneuron differentiation, however the downstream genes through which these two factors direct motoneuron differentiation remain largely unknown. Through a microarray-based approach, we found that other transcription factors were significantly over-represented in the set of genes repressed by HB9 and Nkx6 in the CNS, identifying transcriptional repression of the expression of other transcription factors as a primary mechanism through which HB9 and Nkx6 regulate neuronal differentiation. Additional gene expression and genetic tests on HB9/Nkx6-regulated genes focused our attention on the forkhead domain-containing protein FD59a, one of the few transcription factors up-regulated by HB9 and Nkx6 in the CNS. FD59a is expressed in a distinct cluster of lineally-related HB9+ neurons in the lateral region of the CNS, with HB9 and Nkx6 function being necessary and sufficient for FD59a expression in this cell lineage. We have generated antibodies specific to FD59a and are in the process of generating single-gene mutations in Fd59a. Fd59a is expressed exclusively in the CNS during embryonic development in two distinct clusters of cells: the lateral cluster of three-five HB9+ cells and a medial set of four octopaminergic neurons. In the larval nerve cord, essentially all octopaminergic neurons express Fd59a, while in the brain only a subset of octopaminergic neurons expresses Fd59a. Of note, while Fd59a localizes to the nucleus in most Fd59a+ neurons, Fd59a localizes to the cytoplasm in a small subset of neurons. Present studies focus on identifying the signaling pathways that regulate the subcellular localization of Fd59a.

911B
Deciphering the mechanisms underlying the refinement of synaptic axonal branches. Marlen Schlieder¹,²,³, Marion Langen¹,²,³, Bassem Hassan¹,²,³. 1) Laboratory of Neurogenetics, Department of Molecular and Developmental Genetics, VIB, Leuven, Belgium; 2) Department of Human Genetics, K.U. Leuven School of Medicine, Leuven, Belgium; 3) Doctoral Program in Molecular and Developmental Genetics, K.U. Leuven Group Biomedicine, Leuven, Belgium.

Early phases of nervous system development are characterized by excessive axon outgrowth and exuberant branch formation. To refine synaptic connections and establish mature neuronal networks, redundant or inappropriate synaptic axon branches are pruned via mechanisms like degeneration, retraction and axosome shedding. Whether the majority of these mechanisms are genetically programmed or dependent on synaptic activity, or both remains controversial. The Dorsal Cluster Neurons (DCN) are a valuable model to investigate axon branch pruning in the central nervous system of Drosophila melanogaster. The DCN extend their axons towards the lobula and medulla of the optic lobe, whereby medulla innervating axons establish a highly stereotyped pattern of synaptic axon branches. We find that this pattern is achieved by regulated axon pruning after an excessive axon growth phase during pupal development. Silencing of DCN neuronal activity fails to alter the normal branch
Moreover, we show that this enhancer is activated by Drosophila Myocyte enhancing factor 2 (MEF2), Scalloped (SD) and VG but repressed by vestigial (vg) regulation during indirect flight muscle development. We show here that vg is controlled by the Notch anti-Notch signaling pathway.

The gene vestigial (vg) plays a key role in indirect flight muscle (IFM) development. We show that vg is controlled by the Notch anti-Notch signaling pathway. Our results demonstrate that dCIP4 acts downstream of Cdc42 to activate the postsynaptic Wsp-Arp2/3 pathway. We also show that BMP signaling is necessary for synaptic overgrowth in larvae lacking postsynaptic dCIP4 or wsp. Finally, dCIP4 and Wsp inhibit Gbb secretion. Thus, we propose that dCIP4 restrains synaptic growth by inhibiting postsynaptic Gbb secretion through the Wsp-Arp2/3 pathway.

**Ommatidial rotation is driven by sequential recruitment and nuclear migration and confined by the specific spacing arrangement in Drosophila eye development.**

Chia-hsin Hsu1,2,3, Cheng-Ting Chien1. 1) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan; 2) Bioinformatics Program, Taiwan International Graduate Program, Academia Sinica; 3) Institute of Biomedical Informatics, National Yang-Ming University.

Ommatidial rotation, an essential process for the formation of the hexagonal array of ommatidia in adult eyes, involves dynamic cell-cell rearrangement during Drosophila eye development. In this study, we identify a series of nuclei configurations by tracing the 3D position of individual cells within a rotating ommatidial cluster. We found that the ommatidial cluster does not rotate as a unit but involves a stepwise sequence that is highly correlated with cell recruitment and nuclear migration events when observing the paired photoreceptors. The layer of positional changing cell is often correlated with the recruited target layer (larval nuclear migration target layer). In the first 45° rotation, the rotation event is correlated with the R1 recruitment when R1 nuclei rise to join the cluster. The nuclei of the cluster cells are pushed clockwise by R1. The angle change of paired R2-R3 is correlated with the z-axis position of up-rising R1. Moreover, the space between ommatidia is the limiting factor for the pushing phenomena. When providing more space between ommatidia by delaying R8 formation, the ommatidia anterior to the space stop the initial state, which do not have the rotation. In the second 90° rotation, the rotation event is correlated with the recruitment of polar cone cell (PLC) and the R6, R7 descending events. We observe the cell positions from the ommatidia in different ages that implies the recruitment of PLC causing the anterior cone cell (AC), R7 and posterior cone cell (PC) changing their positions sequentially in clockwise. When we analyze the nuclear position in two rotation mutants nemo and LanA and found that the z-axial position of R7 is abnormal in these mutants. Furthermore, the positions of these four cells on the top layer of the ommatidia corresponded to the proposed model in different perturbations.

**Ey-Cut Antagonism Controls Eye-Antenna Division.** Cheng-wei Wang1,2, Y. Henry Sun1,2. 1) Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan, Republic of China; 2) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, Republic of China.

In *Drosophila melanogaster*, the eye and antennae originate from a cluster of 20–30 cells set aside during embryonic development. Theses cells uniformly express ey gene. The eye or antennal identity of these cells is not determined until mid or late second instar with the restricted expression of Cut, a homeodomain transcriptional repressor in the antennal field and ey in the eye field. The mechanisms responsible for subdividing this epithelium into distinct eye and antennal fields are poorly understood. We tested whether Ey and Cut could antagonize each other for the restriction of cell identity. We generated ectopic Cut expressing clones and found that Cut autonomously inhibited ey. Using the same method and mutant analysis we found Ey did not inhibit Cut directly but through activating SO (sine ocellus) to repress Cut in the second larval eye field. These results suggest that the mutual antagonism between Ey and Cut plays a role in the segregation of the eye and antennal fields.

**Functional Analysis of the Mitf gene in Drosophila.** Tianyi Zhang, Francesca Pignoni. Dept Ophthalmology, Upstate Medical University, Syracuse, NY.

Microphthalmia-related transcription factor (Mitf) is a basic Helix-Loop-Helix Zip (bHLH-Zip) protein that controls the mammalian development of many cell types, such as melanocytes, osteoblasts, mast cells and the retinal pigment epithelium. Mammalian Mitf gene encodes multiple isoforms, and some of them perform different functions. Furthermore, there are three genes, TFEB, TFE3 and TFEC, with very similar bHLH-Zip domains to Mitf in mammalian genome. The gene redundancy makes it difficult to analyze the functions of Mitf family genes in mammals. Here we use the Drosophila model to study Mitf functions based on two reasons: 1) there is only one Mitf gene in Drosophila, which makes the functional analysis simpler; 2) The powerful Drosophila genetic tools can help us to dissect Mitf functions in details. Our results show that Drosophila Mitf has important functions in cell proliferation, differentiation, and cell shape determination.

**vestigial regulation during indirect flight muscle development.** Alexis Lalouette1, Petar Kasherov1, Frédéric Bernard2, Annie Dutriaux1, Sabrina Grenetier1, Alain Zider1, Joel Silber1. 1) Equipe Génétique Moléculaire de la Différenciation INSTITUT JACQUES-MONOD CNRS et université Paris Diderot Bât.Buffon - 15 rue Hélène Brion 75205 PARIS CEDEX 13 - FRANCE; 2) Department of Physiology Development and Neuroscience, University of Cambridge, Cambridge CB2 3DY, United Kingdom.

The gene vestigial (vg) plays a key role in indirect flight muscle (IFM) development. We show here that vg is controlled by the Notch anti-Notch signaling pathway in myoblasts and is regulated by a novel 822 bp enhancer during IFM differentiation. Interestingly, this muscle enhancer is activated in developing fibers and in a small number of myoblasts before the fusion of myoblasts with the developing muscle fibers. Moreover, we show that this enhancer is activated by Drosophila Myocyte enhancing factor 2 (MEF2), Scalloped (SD) and VG but repressed by...
Twist, demonstrating a sensitivity to differentiation in vivo. In vitro experiments reveal that SD can directly bind this enhancer and MEF2 can physically interact with both SD and TWI. Cumulatively, our data reveal the interplay between different myogenic factors responsible for the expression of a vg enhancer activated during muscle differentiation.

917B Modulation of locomotor activity, rather than sleep, contributes to metabolic homeostasis in flies. Paul S Hartley¹, James Catterson¹, Katherine James², Margarete Hecker³, Anthony Harmar². 1) Circadian Physiology, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, Midlothian, United Kingdom; 2) Cell Biology, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, Midlothian, United Kingdom.

Modulation of locomotor activity, rather than sleep, contributes to metabolic homeostasis in Drosophila. Drosophila is an important model for metabolism and sleep research, however little or no data exist regarding the effect of diet upon fly sleep-wake behaviour. We therefore examined these effects. Sleep-wake behaviour was monitored for several days by infrared beam crossing and food intake was measured using capillary feeding assays. ATP levels and the activity of metabolic signaling pathways were also determined. Dietary protein shortened daytime rest and nocturnal sleep bouts without affecting the length or intensity of locomotor bouts. Although flies decreased their food intake, they ingested more sugar as the sucrose content of their diet increased. The increase in sucrose ingestion correlated with increased locomotor activity and a decrease in sleep. These changes were due to increased length and intensity of locomotor bouts but not modification of sleepbout length. Basal ATP was unchanged by increased ingestion of sucrose, whereas insulin signaling was decreased. Metformin (an activator of the AMP activated kinase), reversed the increase in daytime (but not nocturnal) locomotor activity, whilst day and night time sleep were unaffected. These findings indicate that changes to total sleep and sleep architecture can be regulated by dietary carbohydrate and protein, respectively. However, the effect of carbohydrates on sleep is indirect and due to changes in locomotor behaviour. Therefore, modulation of locomotor activity, rather than sleep, contributes to metabolic homeostasis in Drosophila.


One of the theories of aging is the free radicals theory that links reactive oxygen species (ROS) to cellular damages altering metabolic processes thus giving rise to the aging phenotype. Since the main intracellular ROS production site is the mitochondrion, it is believed that this organelle plays a central role in aging. Consistent with this, in several organisms, mutations of mitochondrial components have been shown to modulate lifespan and/or oxidative stress resistance. Surprisingly, mitochondrial genes inactivation leads to life shortening diseases in humans whereas lengthened lifespan can be observed in C. elegans. Interestingly, in this latter organism, inactivation of some genes encoding mitochondrial proteins (Mit genes) could increase or decrease longevity in a manner dependant on both gene inactivation level and on the stage (developmental or adulthood) at which inactivation occurs. We recently investigated whether inactivation of D. melanogaster orthologs of genes encoding components from the electron transport chain (ETC) and mitochondrial ribosomal protein could modulate lifespan and/or oxidative stress resistance. We tested different levels of inactivation during development or adulthood using the GAL4GS (gene switch)/UAS system to ubiquitously express RNAi contracts targeting the considered genes. We observed complex effects depending on sex of individuals and inactivation levels and period, that we will discuss in comparison with data from other organisms.

919A Drosophila Salt inducible kinase (SIK) suppresses lipolysis through inhibiting FOXO during feeding. Biao Wang¹, John Thomas², Marc Montminy³. 1) PBL-M, Salk Inst Biological, La Jolla, CA; 2) MNL-T, Salk Inst Biological, La Jolla, CA.

Under feeding conditions, increases in circulating pancreatic insulin have been shown to inhibit hepatic glucose production through the phosphorylation of the CREB coactivator CRT2 by Salt Inducible Kinases (SIK2), a member of the stress and energy sensing AMPK family of Sfr/Thr kinases. Although SIK2 is expressed most abundantly in adipose tissue, its role in this tissue remains unclear. Here we show that a Drosophila SIK2 homolog is expressed in fat body, the fly equivalent of mammalian liver and white adipose tissue, where it undergoes AKT-mediated phosphorylation and activation in response to feeding. Activated SIK, in turn, appears critical for triglyceride accumulation; SIK mutant flies have reduced lipid storage and they are sensitive to starvation relative to wild-type flies. Loss of SIK expression leads to increased rates of lipolysis in fat body via increases in mrNA amounts for the triglyceride lipase Brummer, the Drosophila homolog of mammalian Adipose Triglyceride Lipase (ATGL). SIK suppresses Brummer gene expression through inhibition of the forkhead transcription factor FOXO in response to insulin signaling. As disruption of Brummer or FOXO expression rescues fat storage in SIK mutant flies, our results illustrate a novel pathway by which SIK and perhaps other members of the AMPK family maintain energy balance in response to dietary signals.


Peroxisome proliferator activated receptor gamma (PPAR-γ) coactivator-1 alpha (PGC-1α) is a transcriptional co-activator that regulates oxidative metabolism in kidney, heart, brain and skeletal muscle. PGC-1α can cause an increase in mitochondrial biogenesis in skeletal muscle and has been implicated in obesity, diabetes and gluconeogenesis. Extensive database searches revealed a single homolog for mammalian PGC-1 in Drosophila called spargel. Spargel is described as a transcription co-factor with an ability to regulate mRNA transcription. Earlier observations reported spargel expression was significantly increased after the introduction of food following a long period of starvation. This nutrient induced enhancement of spargel expression is mediated through FOXO transcription factor (Gershman et al., Physiol Genomics 29:24-34, 2007). A preexisting EP insertion P{EPgy2}spargel located in the 5’UTR of spargel enabled us to overexpress this gene in Drosophila to about 4.4 fold. Lifespan measurements showed a 33% increase in median lifespan and up to a 20% increase in maximum life span. When we assessed the rate of mortality we found an overproduction of spargel delayed the mortality rate and hence the rate of aging. Interestingly, the median lifespan of a spargel hypomorphic line was severely reduced; furthermore, a spargel loss of function mutant was embryonic lethal. With respect to spargel’s involvement in life span extension, we also determined that spargel is regulated through the insulin signaling pathway. We found spargel expression was suppressed in InR (Insulin Receptor) heteroallelic mutants, while spargel expression was increased when InR was overexpressed. These results suggest spargel is essential for development and prolonging adult life span.
SOD2, the principal scavenger of mitochondrial superoxide, is essential for adult survival but dispensable for pre-adult development. Atanu Duttaroy, Subhas Mukherjee, Forde Renee. Dept Biol, Howard Univ, Washington, DC.

Production of superoxides and its reduced derivatives during oxygen metabolism are expected to cause cellular damage starting from development all the way up to the adult stage of life. Inside mitochondria, superoxides are scavenged by Manganese Superoxide dismutase or SOD2. Since, absence of SOD2 confers early postnatal lethality spanning the taxa from mammal to Drosophila it suggests the broad-spectrum importance of the enzyme in cellular physiology. We wanted to inquire how important SOD2 action is during development and during adult life. We first established that SOD2 protein is maternally contributed expectedly because embryonic development requires protection from oxidative stress. But this hypothesis is nullified from our observation that depletion of SOD2 from the oocytes does not affect fertilization and subsequent development of the egg up to the adult hood. To further prove this point we checked for cell size differences, cell cycle progression, induction of cell death and autophagy in tissues developing under elevated oxidative condition. Our analysis failed to demonstrate the influence of elevated ROS on cell size, cell growth and apoptosis. Interestingly however we can demonstrate that unsacenged superoxides can promote autophagy in SOD2 null fat bodies. In contrast to development, knocking down SOD2 in the adult life resulted in a significant decline in lifespan and activation of SOD2 in the Sod2 null mutant adults can rescue the life span of the mutant adults significantly. Moreover, under hypoxic situation (5% Oxygen), Sod2 null adults with activated SOD2 have an extended post-eclosion life from less than 24 hours to 21 days. This observation demonstrates that decreased ROS production is essentially correlated with adult survival, which could be indicative of the importance of SOD2 in aging biology.

922A

Over-expression of a diacylglycerol lipase gene extends lifespan in Drosophila and C. elegans. Hong-Dar Wang¹, Lin-Kwei Yu¹, Tzu-Yu Kao¹, Yi-Chun Wu², Yi-Chun Chen³, Yi-Chun Lin¹, Tzu-En Hsu¹. 1) Department of Life Science and Institute of Biotechnology, National Tsing Hua University, Hsinchu, Taiwan; 2) Institute of Molecular and Cellular Biology, National Taiwan University, Taipei, Taiwan.

Longevity is often associated with increased resistance to different environmental stresses. The screening for mutants that exhibit better resistance to stresses allows us to identify longevity mutants. Here, we reported the isolation of an EP-inserted longevity mutant with up-regulation of a diacylglycerol lipase (DAGL) gene. DAGL decreased mitochondrial superoxide dismutase, and possessed enhanced lifespan. The phosphorylated levels of eIF4E-binding protein (4EBP) and S6 kinase (S6K), two key components of Target of Rapamycin (TOR) pathway, were decreased in the longevity mutant compared to the control, suggesting that DAGL over-expression enhances longevity in Drosophila via reduced TOR activity. In addition, we also demonstrated that the transgenic worm over-expressing DAGL orthologous gene increased lifespan and RNA-interfered expression of DAGL decreased lifespan in C. elegans. On the other hand, the DAGL mutant worms showed decreased lifespan and lowered resistance to oxidative stress. The knockdown of either DAG kinase or oligopeptide-transporter-2, both involved in TOR pathway, rescue the short-lived defect of the DAGL mutants. Together, it is suggested that over-expression of DAGL increases lifespan and stress resistance via decreased TOR activity in Drosophila and C. elegans.

923B

The D. melanogaster male germline expresses a tissue-specific homolog of the small subunit of the General Transcription Factor, TFIIA. Mark A. Hiller, Elizabeth Balraj, Erin McDowell, Margaret Wood, Cynthia Cain. Dept of Biology, Goucher College, Baltimore, MD.

Eukaryotic General Transcription Factors are necessary to position RNA polymerase at promoters and initiate transcription. Isoforms of several General Transcription Factors subunits are known to be important for tissue-specific gene expression. The General Transcription Factor TFIIID is comprised of TBP (TATA-binding protein) and up to fourteen TAFs (TBP-associated factors). In D. melanogaster, testis-specific homologs of several TFIIID subunits (testis-TAFs) are present in the testis. Mutations in the testis-TAF encoding genes are known to be important for tissue-specific gene expression. The General Transcription Factor TFIID is comprised of TBP (TATA-binding protein) and up to fourteen TAFs (TBP-associated factors). In D. melanogaster, testis-specific homologs of several TFIIID subunits (testis-TAFs) are necessary for the transcription of some genes in the testes. We show that the gene tfiia-s-2, called TFIIA-S-2, encodes the small subunit, a 14 kD protein. We show that the gene tfiia-s-2 encodes the small subunit, a 14 kD protein. We show that the gene tfiia-s-2 (CG11639) encodes a male germline-specific homolog of the 14kD subunit. In situ hybridization indicates that tfiia-s-2 is expressed in gonial cells and primary spermatocytes of the testes. Reverse transcriptase PCR experiments demonstrate that two different messages are encoded by tfiia-s-2 due to alternative splicing. We propose that there are different forms of TFIIA, each containing the TFIIA-L gene product and one of the gamma subunits, are present in D. melanogaster testes. These forms of TFIIA may interact with either TFIIID or the testis-specific TFID-like complex to regulate gene expression in the testis.

We are characterizing the ability of TFIIA-S-2 containing complexes to physically associate with subunits of TFIIID, including TBP and the TBP-associated factors (TAFs).

924C

Computational discovery of cis-regulatory elements in multiple Drosophila species. Manonmani Arunachalam¹, Karthik Jayasurya¹, Pavel Tomanack², Uwe Ohler¹. 1) Duke University, Durham, NC., USA; 2) Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

Gene regulation lies at the heart of most biological processes and transcription factors are the key molecules that control tissues specific gene expression. In higher eukaryotes transcription factors control gene expression by binding regulatory DNA segments called cis-regulatory modules (CRMs). We here present two approaches that utilize co-expressed genes data to identify cis-regulatory elements and modules. In the first 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. 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. This method discovers over-represented motifs in a set of co-regulated genes using the exhaustive motif enumeration technique. Clustering the predicted motifs identifies the CRMs, which assist in translating a combinatorial code of TF inputs into a specific gene expression output. After searching the whole genome, predicted CRMs were verified experimentally by establishing expression patterns of the genes that are associated with these CRMs. To identify orthogonal CRMs in distantly related species we developed a non-alignment based method based on word frequencies, where the given sequences are compared using Poisson based metric. When starting with a set of CRMs involved in Drosophila development, we show here that our non-alignment method has better discriminative power than conservation scores based on alignments, and successfully detects similar CRMs in distantly related species (D. ananassae, D. pseudoobscura, D. willistoni, D. mojavensis, D. virilis, D. grimshawi). This method proved efficient in discriminating functional CRMs from known non-functional candidates with similar binding site sequence occurrences.

925A

Chromatin insulator and the promoter targeting module the timing of long-range enhancer-promoter interactions in the

The homeotic genes are essential to the patterning of the anterior-posterior axis along the developing Drosophila embryo. The expression timing and levels of these genes are crucial for the correct specification of segmental identity. The Abdominal-B (Abd-B) gene is first detected in the most posterior abdominal segments at high levels and gradually appears in progressively anterior abdominal segments in lower amounts. Regulatory mutations affecting this expression pattern produce homeotic transformations in the abdomen. The promoter targeting sequences (PTS) from Abd-B locus overcome the enhancer blocking effect of insulators and facilitate long-range enhancer-promoter interactions in transgenic flies (1, 2). In this study, we found that transgene activation by the IAB5 enhancer can be delayed by inserting a 9.5 kb 3' Abd-B regulatory region containing the Frontabdominal-8 (Fab-8) insulator and the PTS element. We found that the delay is caused by the PTS and an insulator, and it is not specific to the enhancer or the promoter tested. Based on these findings, we hypothesize that the delay of remote enhancers is responsible for the Abd-B expression pattern, which is at least in part due to the regulatory activities of the PTS elements and chromatin boundaries.

926B

Analysis of the transcriptional regulation of atonal, the proneural gene for photoreceptor neurons in the fruit fly visual system. Qingxiang Zhou, Tianyi Zhang, Francesca Pignoni. Department of Ophthalmology, SUNY Upstate Medical University, Syracuse, NY.

In Drosophila, there are three different types of photoreceptor organs: the compound eye, the ocelli and the Bolwig’s organ (or larval eye). Expression of ato in both early eye and ocelli. We also confirm that ato expression in the larval eye is dependent on a separate control region. The regulatory mechanisms at work in controlling ato expression in different types of photosensitive organs raise novel questions about their evolutionary relationship.

927C


Abdominal B (Abd-B) is a homeotic gene from the BX-C that gives identity to the posterior segments of the fly (A5-A8). Abd-B expression is controlled by a large cis-regulatory region of over 80kb. We have engineered by recombineering a 111 kb-long BAC covering the whole cis-regulatory regions and in which we inserted the Gal4 coding sequences within the Abd-B 5'UTR. The BAC was then introduced into fly genome by phiC31 mediated integration in the 51C platform. When coupled with a UAS GFP, the reporter mimics endogenous Abd-B expression as we know of it during embryogenesis. This Abd-b-Gal4 BAC enables us to follow Abd-B expression during developmental stages difficult to reach with antibodies i.e larvae, pupae and adults. Very surprisingly we detect strong GFP expression in adult salivary glands. Expression in the salivary glands depends on the presence of the BAC in the background. We believe that the BAC and the endogenous Abd-B are coming together in a complex, interacting with each other, in a process analogous to transvection. Moreover, this seems to give a salivary gland phenotype where expression of ato in both early eye and ocelli. We also confirm that ato expression in the larval eye is dependent on a separate control region. The regulatory mechanisms at work in controlling ato expression in different types of photosensitive organs raise novel questions about their evolutionary relationship.

928A


The majority of genes encoded in eukaryotic genomes contain a single open reading frame (ORF) per mRNA transcript, where translation is facilitated by the presence of a modified 5’ cap. In the fruitfly, D. melanogaster, over 100 dicistronic genes have been annotated, in which two non-overlapping ORFs are encoded in a single mRNA transcript. The mechanisms of translation for the downstream gene in dicistronic cassettes differ significantly from monocistronic transcripts since the downstream gene does not contain a 5’ cap. We hypothesize that embedded within the inter-cistronic region (ICR) between the two ORFs are RNA secondary structures that enable downstream translation in a cap-independent manner. We are examining this hypothesis in the twelve sequenced Drosophilid genomes. Using annotation and bioinformatic tools such as Apollo and BLAST, I have created gene models for the dicistronic gene pair Tim9b/CG12788 for all twelve species and designed primers to confirm whether these two genes are linked in dicistronic transcripts. I have generated cDNA samples from whole animals for ten of the Drosophilid species and used RT-PCR to confirm that the orthologous genes are also dicistronic. To test whether the ICR region for each species can support translation of the downstream gene, I have cloned each species’ ICR into a dual fluorescent reporter construct to measure the relative expression of the upstream and downstream genes in transfected Drosophila S2 cells. These studies significantly improve the genomic annotation of this dicistronic gene in several Drosophilid species and provide evidence that the dicistronic structure of this gene is conserved over evolutionary time. Furthermore, we provide the first practical test of putative functional regions that may control internal ribosomal entry for this gene.

929B

Role of multi sex combs in histone synthesis during differentiation. Severine Landais, Leanne Jones. Lab Gen, Salk Inst, La Jolla, CA.

Mutations occurring in the Drosophila multi sex combs (mxc) gene give rise to developmental defects that mimic loss of Polycomb function, which led to its classification as a polycomb gene more than 20 years ago. Strong loss-of-function mutations result in larval lethality; however, flies carrying a viable, hypomorphic mutation in mxc (mxc G46) exhibit defects in hematopoiesis, as well as sterility of both males and females. Gonads from hemizygous mxc G46 males or mxc G46/mxc22-A-6 females contain fewer germ cells that are unable to differentiate properly. Furthermore, germline stem cells (GSCs) are lost over time. Expression of an mxc G46 rescue transgene led to suppression of the germline phenotypes. In germ cells, Mxc protein localizes specifically to the Histone Locus Body (HLB) on chromosome 2, as determined by co-localization with LSM10 and LSM11, known components of the histone mRNA processing machinery. In addition, poly-adenylated histone mRNAs are detected in testes from mxc mutant flies, indicating that histone mRNA processing is impaired. These results provide a possible link
between histone production and the onset of differentiation during development.

930C  
**Cross regulatory interactions between abd-A and Abd-B.** Maheshwar Gummalla1, Sandrine Galetti1, Robert Maeda1, Henrik Gyurkovics2, François Karch1. 1) Zoology and animal biology, University of Geneva, Geneva, Switzerland; 2) Institute of Genetics, Biological Research Centre Szeged, Hungary.

In Drosophila embryos posterior hox genes usually repress the directly more anteriorly expressed hox gene. These negative cross-regulatory interactions are thought to be at the origin of the overall complementary domain of expression of the different hox genes along the AP axis. For example Abd-B is expressed in a gradient from PS10-14 where the intensity and number of cells expressing Abd-B is higher towards the posterior. abd-A is expressed in a uniform pattern from PS 7-12. Because loss of Abd-B leads to the expansion of abd-A expression in PS13, it is usually thought that abd-A is down regulated by Abd-B in a concentration dependent manner. In order to verify this hypothesis we have performed two-color confocal analysis on embryonic tissues using antibodies directed against Abd-A and Abd-B proteins. While the 2 proteins are overall expressed in complementary patterns in the epidermis, we find that many cells in the CNS co-express both Abd-A and Abd-B and that, quite often, the cells with the highest levels of Abd-B also express the highest levels of abd-A. Using various mutations we find that the Abd-B protein does not seem to work as a transcriptional repressor of the abd-A gene in the CNS.

931A  
**Confirmation of dicistronic gene structures in several Drosophilid species.** Henry C. Hunter, Christopher D. Smith. Cell and Molecular Biology, San Francisco State University, San Francisco, CA.

Encoding multiple genes in a single messenger RNA (mRNA) is a strategy common among prokaryotes and viruses, but not eukaryotic cells. While di- or multi-cistronic genes were once thought to be non-existent in eukaryotes, they have now been observed in the genomes of a number of species, including humans. High-quality genomic annotation of *Drosophila melanogaster* (fruit fly) has revealed over 100 dicistronic genes, where two non-overlapping genes are expressed from a single mRNA transcript. The mechanisms that mediate dicistronic expression in eukaryotes are not yet clearly described, but recent studies have shown that RNAs play a role in dicistronic gene expression. Recent results show that U bodies have been proposed to be specific sites for snRNP assembly because they contain U snRNPs and SMN. U bodies invariably associate with P bodies, which are involved in mRNA decay and translational control. However, it remains unknown whether other SMN complex proteins also localise in U bodies. In *Drosophila* there are four SMN complex proteins, namely SMN, Gemin2, Gemin3 and Gemin5. Here we use the *Drosophila* egg chamber as a model system to study the subcellular distribution of two SMN complex proteins, Gemin3 and Gemin5. We found that both Gemin3 and Gemin5 localise to U bodies but not P bodies. In addition, we compare Gemin3 and Me31B, two DEAD-box RNA helicases through bioinformatic and cytological approaches. Gemin3 and Me31B belong to two different DEAD-box RNA helicase subfamilies and reside in U bodies and P bodies, respectively. Our results show that U bodies contain multiple SMN complex proteins, supporting the idea that U bodies are involved in snRNP biogenesis.

932B  
**Drosophila Survival Motor Neuron (SMN) complex proteins Gemin3 and Gemin5 are components of U bodies.** Ruben Cauchi1,2, Luis Sanchez-Pulido1, Ji-Long Liu1. 1) MRC Functional Genomics Unit, Department of Physiology, Anatomy & Genetics, University of Oxford, Oxford, UK; 2) Department of Physiology & Biochemistry, University of Malta, Msida, Malta.

Uridine-rich small nuclear ribonucleoproteins (U snRNPs) play key roles in pre-mRNA processing in the nucleus, the assembly of most U snRNPs takes place in the cytoplasm and is facilitated by the survival motor neuron (SMN) complex. Discrete cytoplasmic RNA granules called U bodies have been proposed to be specific sites for snRNP assembly because they contain U snRNPs and SMN. U bodies invariably associate with P bodies, which are involved in mRNA decay and translational control. However, it remains unknown whether other SMN complex proteins also localise in U bodies. In *Drosophila* there are four SMN complex proteins, namely SMN, Gemin2, Gemin3 and Gemin5. Here we use the *Drosophila* egg chamber as a model system to study the subcellular distribution of two SMN complex proteins, Gemin3 and Gemin5. We found that both Gemin3 and Gemin5 localise to U bodies but not P bodies. In addition, we compare Gemin3 and Me31B, two DEAD-box RNA helicases through bioinformatic and cytological approaches. Gemin3 and Me31B belong to two different DEAD-box RNA helicase subfamilies and reside in U bodies and P bodies, respectively. Our results show that U bodies contain multiple SMN complex proteins, supporting the idea that U bodies are involved in snRNP biogenesis.

933C  
**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 germ 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 indicates 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. Interestingly, these same genes often appear as standard monocistronic transcripts in some distantly related species, suggesting that there exist mechanisms that allow genes to merge and become expressed as dicistronic products. Recently, 11 other Drosophila genes have been completed, providing an excellent system to study the structure of dicistronic genes over evolutionary time. While full-length cDNA evidence supports the existence of these genes in *D. melanogaster*, to date there has been no direct evidence that these genes are also dicistronic in other species. We identified and annotated orthologs of several *D. melanogaster* dicistronic genes and their putative gene structures in multiple Drosophilid species. We used these annotations to develop PCR primers to confirm the existence of each dicistronic transcript in cDNA samples. RT-PCR cDNAs were sequenced and compared to verified dicistronic genes to identify gene structural changes over evolution. These results are useful to refine existing dicistronic gene annotations, aid in discovering how dicistronic genes function in eukaryotes, and provide a detailed comparative system to study the forces that shape the evolution of gene structures over evolutionary time.

934A  
**A Transient Niche Regulates the Specification of Drosophila Intestinal Stem Cells.** Divya Mathur, Alyssa Bost, Ian Driver, Benjamin Ohlstein. COLUMBIA UNIVERSITY MEDICAL CENTER, NEW YORK, NY.

Stem cell niches are locations where stem cells reside and self-renew. Although studies have shown how niches maintain stem cell fate during tissue homeostasis, less is known about their roles in establishing stem cells. The adult Drosophila midgut is maintained by intestinal stem cells (ISCs); however, where they are established is unknown. Here, we show that an ISC progenitor generates a niche cell via Notch signaling. This niche uses the bone morphogenetic protein 2/4 homolog, decapentaplegic, to allow progenitors to divide in an undifferentiated state and
subsequently breaks down and dies, resulting in the specification of ISCs in the adult midgut. Our results demonstrate a paradigm for stem cell-niche biology, where progenitors generate transient niches that determine stem cell fate and may give insights into stem cell specification in other tissues.

935B
The role of headcase in regulating stem cell behavior in the Drosophila testis. Pedro Resende1,2, Darrell Tran1, Monica Boyle1, Leanne Jones1. 1) Salk Institute for Biological Studies, San Diego, CA, 10010 North Torrey Pines R; 2) GABBA PhD programe, Faculty of Medicine University of Porto, A. Prof. Hernãní Monteiro 4200 - 319 Porto.

Stem cells are characterized by their ability to divide and generate both new stem cells (self-renew) as well as different specialized cell types (differentiation). This peculiarity gives adult stem cells a central role in the maintenance and regeneration of body tissues, such as, blood, skin and sperm, throughout the life of an individual. The process of spermatogenesis in Drosophila provides an excellent system for analyzing the relation between stem cells and their microenvironment (the “niche”). It allows the study of stem cell behavior in vivo, using readily available markers for both the stem cells themselves and other components of the niche during different phases of development. Germline stem cells (GSCs) and somatic stem cells (SSCs) surround and are in direct contact with hub cells, a cluster of post-mitotic somatic cells that are a key component of the stem cell niche in the testis. Whereas GSCs sustain spermatogenesis, SSCs produce cyst cells that encapsulate the maturing germ cells and ensure differentiation. We aim to explore the role of the headcase (hdc) gene in the Drosophila testis stem cell niche. Hdc protein is expressed in hub cells, GSCs and SSCs and their progeny. We will examine whether hdc functions in hub cells and/or stem cells regulating stem cell behavior.

In addition, we propose to determine whether hdc interacts genetically with other pathways known to be important in regulating stem cell behavior. Understanding the regulation of signals acting in the niche that shape stem cell fate will allow us to design better strategies for isolation and cultivation of stem cells in vitro and transplantation of stem cells in the course of regenerative medicine.

936C
The use of ChIP-SEQ and BAC recombinerring to study how cis-regulatory sequences function. Angelike M. Statopoulos1, Anil Gruber1, Katherine Fisher1, Shirley Pepke2, Manor Samanta1, Barbara Wold1. 1) Division of Biology, Caltech, Pasadena, CA; 2) Caltech Center for Advanced Computing Research, Caltech, Pasadena, CA; 3) Systemix Institute, Los Altos, CA.

Whole-genome methods have greatly improved our understanding of how cis-regulatory sequences (CRMs) contribute to animal development. Identifying cis-regulatory sequences in Drosophila using approaches such as ChIP-chip has made it possible to identify/predict a large number of CRMs with relative ease. Yet once the CRM sequences are obtained, understanding the logic of how transcription factor sites are organized to support gene expression that is both spatial and temporally regulated is still a challenge. To understand the logic of CRMs, analysis at the level of individual binding events must be undertaken - entailing experiments that are both time-consuming and laborious.

Here we discuss experimental approaches that provide unique insights into the function of CRMs relatively quickly: CHIP-seq can identify in vivo binding of transcription factors to DNA with resolution of ±25 bp and transgenic reporters constructed using BAC recombinerring can assay more rigorously the function of these sequences, in a context that is similar to the native one. Each of these technical advances has the potential to elucidate important new insights, in general, into the cis-regulatory mechanisms that control gene expression. To demonstrate, we show how ChIP-seq can define the in vivo binding site consensus for transcription factors and how recombinerring methods can be utilized to demonstrate how particular enhancers function autonomously within the early embryo to support expression that is both spatially and temporally distinct.

937A
Transcriptomes without genomes: De novo transcriptome assembly and transcriptional profiling in a drosophilid parasite of Arabidopsis thaliana. Noah K Whiteman1, Timothy B Sackton2. 1) Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ; 2) Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

Flowering plants and the insects that consume them comprise most species of multi-cellular life on Earth. Understanding the molecular and evolutionary basis of the plant-herbivore interface requires genetic and genomic model plants and insects. The model plant Arabidopsis thaliana has yielded key insight into plant defenses against insects, but there is currently no chewing herbivore model of Arabidopsis. Fortunately, a fly named S, flavus is nested phylogenetically within the subgenus Drosophila and feed on Arabidopsis in nature as a herbivore. Unlike more distantly related Drosophila species, S. flavus is a true herbivore; we have established a culture of S. flavus on Arabidopsis in the laboratory with the long-term goal of combining the tools of Arabidopsis and Drosophila to develop a model pathosystem for studying defense signaling pathways in Arabidopsis, Drosophila xenobiotic metabolism, and the evolution of host specificity and herbivory. Here we use Arabidopsis and Drosophila genetic and genomic tools to demonstrate the potential of this system for a more integrative understanding the plant-herbivore interface. To provide a genomic platform for exploring the herbivore response to host defenses, we used next-generation massively parallel (454 and Illumina) sequencing to assemble and characterize the transcriptome of S. flavus, using the 12 sequenced Drosophila genomes as references. We then conducted a proof of principle experiment in which leaffamers were reared on Arabidopsis loss of function mutants that are deficient in canonical anti-herbivore defense pathways or on wildtype plants. Short-read Illumina sequencing was performed on the leaffminer RNA pools to identify pathways mediated by specific Arabidopsis defense compounds and these were mapped back to the transcriptome. By leveraging the tools of two genomic model systems we show that this pathosystem provides a powerful platform for understanding plant-insect interactions.

938B
Targeted mutagenesis outside the Drosophila species. Michal Zurovec1, Yoko Takasu1,2, Isao Kobayashi Isao Kobayashi2, Kelly Beumer2, Keiho Uchina2, Hideki Sezutsu2, Suresh Sajwan1, Dana Carroll1, Toshiki Tamura1. 1) Dept Physiology, Inst Entomology, Ceske Budejovic, Czech Republic; 2) National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan; 3) Department of Biochemistry, University of Utah School of Medicine, 15 N.Medical Drive East, Room 4100, Salt Lake City, Utah 84112, USA.

Targeted mutagenesis is a crucial method for gene function analysis. A simplified procedure of gene targeting employs direct microinjection of custom-designed Zinc Finger Nuclease (ZFN) mRNAs into fruit fly embryos. To assess the use of this method to gene targeting in other insects, we choose the silkworm (Bombyx mori) and mutagenized its epidermal color marker gene BmBLO2S2, which regulates the formation of uric acid granules in the larval skin. Our results showed that ZFN mRNA microinjection is effective to induce somatic, as well as germline, mutations in a targeted gene by non-homologous end joining (NHEJ). We received a large number (up to 72%) of somatic mosaics in G0 and at least 9 types of germline mutants in G1, which included 7 bp or longer deletions, as well as single nucleotide insertions. Our observations suggest that the silkworm experimental system employing transient expression of ZFN and repair machinery is comparable with that of Drosophila. Moreover, the frequency of germline mutants in G1 was sufficient to be used for gene targeting relying on a screen without previous knowledge of the genotype.