

45th Annual Drosophila Research Conference

PROGRAM ADDENDUM

• **Platform Presenter Changes:**

1 st Author	Program #	Session Title	Substitute Presenter
Y. Ding	20	Meiosis, Mitosis and Cell Division	K. Johansen
R. Udan	71	Signal Transduction II	M. Kango-Singh

• **Platform Abstract Correction:**

130 The Drosophila histone acetyltransferase of dTip60 is a component of a multiprotein complex with functions in cell cycle and epigenetic control of gene expression. **Authors corrected:** T. Kusch¹, W. H. McDonald², L. Florens¹, J. Haug¹, M. Washburn¹, J. R. Yates III², S. M. Abmayr¹, J. L. Workman¹.

1) Stowers Institute for Medical Research, Kansas City, MO and 2) Scripps Research Institute, La Jolla, CA.

• **Poster Presenter Changes:**

1 st Author	Poster #	Substitute Presenter
R. El Bejjani	201C	G. Bosco
A. Gortchakov	273C	A. Yurlova
H. Y. Chan	712A	N. Y. Huen

• **Poster Presentations Cancelled:**

Poster Author	Poster #
C. Brawley	247A
J. Wu	415A
N. Haines	591C
D. Grifoni	651C
G. Carney	764B
R. Wilson	827B
L. Reed	833B

• **FlyBase Index Correction:**

bcd 81, 139, 334A, 336C, 341B, 509B, 546C, 810C

• **Additional Exhibitors:**

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The NASA Ames Research Center Space Station Biological Research Project Insect Habitat science team will study *Drosophila melanogaster* on board

the Station to learn how microgravity affects development, nervous system function, growth, reproduction, aging, gene expression and mutagenesis.

• **Exhibitor Changes:**

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• **Exhibitor Cancellation:**

■ **VARIAN, INC. 103**

• **Late Poster Abstracts (see complete text of abstracts beginning on page 7):**

First Author/Presenter	Poster #	Abstract Title and Co-Authors
MEIOSIS, MITOSIS AND CELL DIVISION		
Sinka, R.	911B	A genome-wide RNAi screen to identify cell cycle genes in <i>Drosophila melanogaster</i>. R. Sinka, M. Cooper, L. Frenz, D. Jones, F. Scaerou, C. Midgley, G. Bell, M. Bettencourt-Dias, X. H. Zhang, L. Capalbo, D. M. Glover.
Sinka, R.	912C	A functional genomic screen to identify protein kinases involved in the regulation of mitosis. R. Sinka, M. Bettencourt-Dias, R. Giet, A. Mazumdar, W. Lock, F. Balloux, P. Zafiroopoulos, S. Yamaguchi, S. Winter, R. Carthew, D. M. Glover.
Wainman, A.	913A	MOPB1-like genes in <i>D. melanogaster</i>. A. Wainman, C. M. Domingues, A. Tavares, D. M. Glover.
Wei, H.-C.	914B	Study of the effects of phosphoinositides in <i>Drosophila</i> male meiotic cytokinesis. H.-C. Wei, G. Plevoy, J. A. Brill.
CYTOSKELETON AND CELLULAR BIOLOGY		
Andres, A.	915C	Dissecting salivary gland secretion using a gene-trap strategy. A. Andres, J. Shreck, R. Boyles, N. Bond, D. Hoshizaki, J. Merriam.
Hoshizaki, D. K.	916A	Fat body dissociation during metamorphosis. D. K. Hoshizaki, N. Bond, J. Utz, A. Nelliott.
Burgess, J.	917B	Characterization of PI 4-kinase homologs in <i>D. melanogaster</i>. J. Burgess, R. Vakilian, R. McBride, G. Plevoy, J. Brill.

First Author/Presenter	Poster #	Abstract Title and Co-Authors
GENOME AND CHROMOSOME STRUCTURE		
O'Dor, E.	918C	A link between mitosis and silencing: The role of the Pollycomb Group in cell cycle progression. E. O'Dor, H. Brock.
REGULATION OF GENE EXPRESSION		
Brown, R. P.	919A	Do polymorphisms in the proximal promoter cause variation in gene expression between Drosophila strains? R. P. Brown, M. E. Feder.
Garcia-Zaragoza, E.	920B	Two functionally identical modular enhancers in Drosophila Troponin T gene establish the correct protein levels in different muscle types. E. Garcia-Zaragoza, J. A. Mas, J. Vivar, M. Cervera.
Zinzen, R. P.	921C	A regulatory code for neurogenic gene expression in the Drosophila embryo. R. P. Zinzen, M. Markstein, P. Markstein, K. Yee, A. Erives, A. Stathopoulos, M. Levine.
Fernandez-Moreno, M. A.	922A	Drosophila as an animal model of mitochondrial pathophysiology. M. A. Fernandez-Moreno, C. Adan, B. Bornstein, P. de la Pena, C. Garcia-Valleho, MC. Gil, E. Gonzalez, R. Hernandez, F. Martinez-Azorin, P. Ochoa, A. Sanchez, A. Seguido, R. Garesse, C. L. Farr, N. Luo, A. T. Lagina III, Y. Matsushima, L. Kaguni.
Rasheva, V. I.	923B	dMBD-R2 is essential for Drosophila development. V. I. Rasheva, M.-J. Lin, N. M. Reddy, T.-Y. Tzeng, M.-S. Hung, L.-Y. Tang, P.-G. Lee, H.-J. Xie, C.-K. J. Shen.
Lehmann, Michael	924C	The role of Pipsqueak in GAGA-binding chromatin complexes. M. Lehmann, A. Schwendemann, T. Siegmund. R. Greenaway.
Soares, L. D.	925A	"Auto"-regulation of BMP signaling. L. D. Soares, S. Ballard, K. Wharton.
Pilyugin, M.	926B	Interaction of Drosophila PSC protein with transcription factors. M. Pilyugin, J. Larsson, V. Pirrotta.
SIGNAL TRANSDUCTION		
Betson, M.	927C	Genetic analysis of the Rho effector kinase, PKN, in Drosophila. M. Betson, J. Settleman.
Mills, I.	928A	A proteomic screen to identify substrates of the protein phosphatase Eyes Absent. I. Mills, I. Rebay.
Ruhf, M. L.	929B	A Drosophila EMS-mutagenesis screen to isolate novel effectors in the TOR signaling pathway. M. L. Ruhf, J. Montagne, G. Thomas.
Han, C.	930C	Distinct and collaborative roles of Drosophila EXT family tumor suppressors in morphogen signaling and gradient formation. C. Han, T. Y. Belenkaya, X. Lin.
Gryzik, T.	931A	Identification of two novel FGF encoding genes required for mesoderm migration. T. Gryzik, H. A. J. Müller.
Yan, D.	932B	Role of proteoglycan in FGF receptor signaling during tracheal and heart development in Drosophila. D. Yan, D. Metzger, X. Lin.
Harrison, S. M. W.	933C	Genetic interactions of the small GTPase, RIC, with multiple signaling pathways in Drosophila. S. M. W. Harrison, M. L. Spencer, P. D. Wes, C. Montell, D. A. Andres, D. A. Harrison.
Wang, T.	934A	Calx functions as a sodium calcium exchanger in phototransduction. T. Wang, R. Hardie, C. Montell.

First Author/Presenter	Poster #	Abstract Title and Co-Authors
PATTERN FORMATION		
Sexton, T. R.	935B	The role of Upd as a potential morphogen in follicular fate specification. T. R. Sexton, R. Xi, S. M. W. Harrison, D. A. Harrison.
Berger, S. M.	936C	A role for Stardust in light-dependent retinal degeneration? S. M. Berger, F. Grawe, B. Busse, K. Johnson, E. Knust.
Gödde, W.	937A	Maternal control of epithelial development in Drosophila. W. Gödde, E. Knust.
Wasserscheid, I.	938B	The role of the Drosophila gene <i>bazooka</i> in morphogenetic processes. I. Wasserscheid, E. Knust.
Walters, Z. S.	939C	Role of <i>skinhead</i> in growth and patterning at the wing margin. Z. S. Walters, R. G. Phillips.
Monteiro, A.	940A	Using germ line transformations to test the function of genes implicated in the development of butterfly wing patterns. A. Monteiro, J. M. Marcus, D. M. Ramos.
Cordero, J. B.	941B	Early pupal cell death contributes to Drosophila retinal patterning. J. B. Cordero, O. Jassim, S. Bao, R. Cagan.
Pichler, S.	942C	Strategy for cloning a gene on the X chromosome required for nuclear spacing and investigation of the mechanisms underlying the establishment of nuclear density domains. S. Pichler, T. Gregor, D. Glover.
GAMETOGENESIS AND SEX DETERMINATION		
Kugler, J. M.	943A	Development of the telotrophic meroistic ovary of the bug <i>Dysdercus intermedius</i> (Heteroptera, Pyrrhocoridae). J. M. Kugler, J. Trauner, R. Rübsam, J. Büning.
Lin, M.-D.	944B	Drosophila decapping protein 1, dDcp1, with Exu-Yps RNP complex directs the posterior deposition of oskar mRNA in oocyte. M.-D. Lin, S.-R. Fang, X. Jiao, M. Kiledjianand, T.-B. Chou.
Avila, F. W.	945C	STAT function is needed to maintain, but not initiate Sx/ expression during sex determination. F. W. Avila, J. W. Erickson.
Yamamoto, M.-T.	946A	Genetic characterization of paternal effect genes on sperm storage and fertilization. M.-T. Yamamoto, M. Tomaru, M. Hara, T. Ohsako.
ORGANOGENESIS		
Ghabrial, A. S.	947B	Identification of mutants with defects in tracheole outgrowth and function. A. S. Ghabrial, B. P. Levi, M. A. Krasnow.
Martinek, N. N.	948C	Drosophila SPARC (dsparc), a basal lamina component, is required for embryonic development. N. N. Martinek, M. J. Ringuette.
NEUROGENETICS AND NEURAL DEVELOPMENT		
Garces, A.	949A	Specification of Drosophila pioneer motor neuron identity by a genetic pathway involving even-skipped, grain and zfh-1. A. Garces, S. Thor.
Mende, M.	950B	Drosophila Paxillin interacts with the Spectraplakins Shot and is required for neuromuscular junction formation. M. Mende, T. Böckers, H. Sabe, A. Subramanian, R. Ueda, T. Volk, R. Yagi, A. Prokop.

First Author/Presenter	Poster #	Abstract Title and Co-Authors
Mettler, U.	951C	Investigations on the temporal regulation of <i>hunchback</i> within neuroblast lineages of <i>Drosophila</i> . U. Mettler, J. Urban.
Vogler, G.	952A	Analysis of <i>zfh-1</i> and <i>zfh-2</i> function in the developing CNS of <i>D. melanogaster</i> . G. Vogler, J. Urban.
Yuasa, Y.	953B	Combinational expression of three transcriptional factors is essential for the PROS expression in the longitudinal glia. Y. Yuasa, Y. Hiromi.
Garrity, P. A.	954C	Slit, Robo and Robo3 control optic lobe morphogenesis. P. A. Garrity, T. D. Tayler, M. Robichaux.
Toriya, M.	955A	Regulation of notch signaling during proliferation and differentiation of postembryonic neuroblasts. M. Toriya, K. Nakao, H. Okano.
Olofsson, B. M.	956B	Condensation of the central nervous system in embryonic <i>Drosophila</i> depends on hemocyte-deposited extracellular matrix and neural activity. B. M. Olofsson, D. T. Page.
NEURAL PHYSIOLOGY AND BEHAVIOR		
Burns, R.	957C	Carbon dioxide, high-frequency light pulses, and extreme temperature as triggers of seizure and paralysis in the bang-sensitive paralytic mutants. R. Burns, C. Boyek, D. Kuebler.
Jaramillo, A. M.	958A	Characterizing the alternative splice variants of SLOB in <i>Drosophila</i> heads. A. M. Jaramillo, H. Fei, T. Weiger, I. B. Levitan.
Bray, S.	959B	The male-specific pheromone receptor GR68A is required for normal <i>Drosophila</i> courtship. S. Bray, H. Amrein.
Preuss, F.	960C	<i>Drosophila</i> doubletime mutations which either shorten or lengthen the period of circadian rhythms decrease the protein kinase activity of casein kinase I. F. Preuss, J. Fan, E. Bjes, S. Bao, M. Kalive, E. Schuenemann, M. Muskus, J. Price.
Suh, J.	961A	A role for EBONY-containing glia in the circadian regulation of locomotor activity. J. Suh, F. R. Jackson.
Chan, Y.	962B	Feminization of cholinergic neurons enhances aggressive behavior in male <i>D. melanogaster</i> . Y. Chan, S. Certel, E. Kravitz.
Pokrzywa, M. A.	963C	<i>D. melanogaster</i> as an animal disease-model for familial amyloidotic polyneuropathy. M. Pokrzywa, I. Dacklin, M. Oliveberg, D. Hultmark, E. Lundgren.
Caldwell, J. C.	964A	The <i>beethoven-reduced ocelli</i> genetic interval (polytene region 36D). J. C. Caldwell, Y. Sharma, J. S. Jacobs, D. F. Ebert.
EVOLUTION AND QUANTITATIVE GENETICS		
Bryan, B.	965B	Nuclear-mitochondrial coadaptation: Functional consequences of mitochondrial genome introgression from <i>D. simulans</i> into <i>D. melanogaster</i> . B. Bryan, R. Wagaman, E. Goldstein, T. Sackton, L. Sheldahl, D. Rand.
Montano, D. A.	966C	Population genetics in the American tropics. Comparative genetic structures of <i>Drosophila pseudoobscura</i> from Arizona, USA and from Columbia. D. A. Montano, G. Forero, H. Correa.
Takano-Shimizu, T.	967A	Variations and nonrandom associations at <i>Drosophila</i> chemoreceptor genes. T. Takano-Shimizu, A. Kawabe, N. Inomata, N. Nanba, R. Kondo, Y. Inoue, M. Itoh.
Goltsev, Y.	968B	Evolution of gene expression in Diptera. Distinct features of patterning gene expression in <i>Anopheles gambiae</i> early embryo. Y. Goltsev, B. Peterson, W. Hsiung, G. Lanzarro, M. Levine.

First Author/Presenter	Poster #	Abstract Title and Co-Authors
Herranz, R.	969C	An Evo-Devo approach leading to a structural-functional model of Troponin genes in insects. R. Herranz, J. Mateos, J. A. Mas, E. Garcia-Zaragoza, M. Cervera, R. Marco.
Herranz, R.	970A	Diversification and independent evolution of TpnC genes in insects. R. Herranz, J. Mateos, C. Diaz, T. P. Nguyen, T. L. Lovato, R. M. Cripps, R. Marco.
Rajpurohit, S.	971B	Reproductive fitness related variations among Indian <i>Drosophila</i> species. S. Rajpurohit, P. Tyagi, A. Bhardwaj, R. Parkash.
Mezey, J. G.	972C	Naturally segregating QTL affecting wing shape of <i>Drosophila melanogaster</i>. J. G. Mezey.
Wagaman, R.	973A	Mitochondrial genotype interacts with insulin signaling and dietary restriction to determine <i>Drosophila</i> longevity. R. Wagaman, E. Goldstein, D. Rand.
Herreman, T. W.	974B	Misregulation of the transcriptome in <i>Drosophila</i> species hybrids is mediated through the nucleolar protein Hmr. T. W. Herreman, K. P. White.
IMMUNE SYSTEM AND CELL DEATH		
Cox, C. R.	975C	<i>Enterococcus faecalis</i> gastrointestinal tract colonization of <i>D. melanogaster</i> by commensal strains and strains of known virulence. C. R. Cox, M. S. Gilmore.
TECHNIQUES AND GENOMICS		
Buch, S.	976A	Microarray analysis of dTOR–target of rapamycin in <i>D. melanogaster</i>. S. Buch, J. D. Katzenberger, M. Bauer, I. Zinke, M. Bonaus, M. J. Pankratz.
Perusse, J. R.	977B	Expression profile of <i>Anopheles gambiae</i> in response to infection by <i>Plasmodium falciparum</i>. J. R. Perusse, S. M. Kanzok, L. Zheng, K. P. White.
Armknecht, S. L.	978C	Genome-wide screening at the <i>Drosophila</i> RNAi Screening Center. S. L. Armknecht, I. T. Flockhart, M. Booker, J. Murphy, S. Talala, N. Ramadan.
Sousa-Neves, R. S.	979A	Generation of large site-directed terminal deficiencies on the Fourth Chromosome. R. S. Sousa-Neves, T. Lukacsovich, J. L. Marsh.
Papatsenko, D.	980B	Compositional features of transcriptional code and discovery of cis-regulatory modules (CRMs) in the genome of <i>Drosophila</i>. D. Papatsenko, S. Small.
Mendez Lago, M.	981C	Transposon-based innovative method for sequencing highly repetitive heterochromatic DNA in BAC/oriV clones. M. Mendez Lago, J. Wild, J. P. Abad, A. Martin-Gallardo, A. Villasante, W. Szybalski.
Walther, S.	982A	Nutrinomics: A combined genomic and proteomic approach to identify nutrition controlled components of metabolic pathways in <i>Drosophila</i>. S. Walther, I. Zinke, M. J. Pankratz.

MEIOSIS, MITOSIS AND CELL DIVISION**911B**

A genome-wide RNAi screen to identify cell cycle genes in *Drosophila melanogaster*. R. Sinka¹, M. Cooper², L. Frenz², D. Jones², F. Scaerou², C. Midgley², G. Bell², M. Bettencourt-Dias¹, X.H. Zhang¹, L. Capalbo¹, D.M. Glover^{1,2}. 1) Department of Genetics, University of Cambridge; 2) Cyclacel Ltd, Polgen Division, Babraham Research Campus, Babraham, Cambridge.

The availability of the fully sequenced and annotated *Drosophila melanogaster* genome, combined with highly effective double stranded RNAi in tissue culture cells, has enabled the function of selected genes to be examined in different biological contexts. We are using these approaches to identify novel genes with cell cycle related functions. We designed and synthesized 13600 gene specific primer pairs, reflecting Flybase release 2 of the whole *Drosophila* genome, for production of gene specific dsRNAs. We used a robotic platform to introduce dsRNA for individual genes into the D.Mel-2 cell line and the effect on cell cycle progression was analysed. The effect on cell cycle progression was analysed in a primary screen by estimating the mitotic index from the proportion of cells displaying staining to a phosphorylated Histone-H3 specific antibody. We found that knockdown of approximately 4% of the genes examined resulted in a significant change of mitotic index. We are currently investigating the cell cycle specific function of these genes using immunofluorescence microscopy.

912C

A functional genomic screen to identify protein kinases involved in the regulation of mitosis. R. Sinka¹, M. Bettencourt-Dias¹, R. Giet², A. Mazumdar¹, W. Lock¹, F. Balloux¹, P. Zafiroopoulos¹, S. Yamaguchi³, S. Winter³, R. Carthew³, D.M. Glover¹. 1) Department of Genetics, University of Cambridge, Cambridge, England; 2) CNRS-UMR-6061 Universit de Rennes 1-Facult de Mdecine 2, RENNES FRANCE; 3) Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Illinois 60208, USA.

Mitosis is a highly dynamic process that depends on networks of protein phosphorylation and dephosphorylation. The availability of a fully sequenced and annotated genome, combined with the ease of use of double stranded RNA mediated interference (RNAi) in *D. melanogaster* tissue culture cells, made possible the systematic exploration of the protein kinome for functions in the regulation of mitosis. Here we describe a quantitative screen to test the entire set of *Drosophila* protein kinases for a function in mitosis. We have used FACS analysis to identify changes in the progression through the cell cycle, and to check for aneuploidy, polyploidy and cell death. Visualization of centrosomes, microtubules and DNA has enabled the quantitation of multiple cell cycle parameters such as mitotic index, percentages of cells in different phases of mitosis, defects in duplication, maturation and separation of centrosomes, abnormalities of condensation and segregation of chromosomes, and defects in spindle assembly and cytokinesis. The disruption of the expression of approximately a third of the protein kinases led to a dysfunction in mitosis. Our methodological approach has permitted the grouping of the protein kinases into phenotypic classes. We have identified novel enzymes with a role in specific processes such as spindle assembly and chromosome segregation. Interestingly, we have found that the depletion of several known signaling kinases leads to very specific problems in mitosis. This opens the exciting possibility that old pathways play novel roles and/or there are new forms of regulation of mitosis.

913A

MOB1-like genes in *Drosophila melanogaster*. A. Wainman¹, C.M. Domingues², A. Tavares^{2,3}, D.M. Glover¹. 1) Department of Genetics, University of Cambridge, Cambridge, United Kingdom; 2) Cell Division Group, Instituto Gulbenkian de Ciência, Oeiras, Portugal; 3) Departamento de Engenharia Química, Instituto Superior Técnico, Lisboa, Portugal.

S. cerevisiae MOB1 is an essential gene required for completion of mitosis and maintenance of cell ploidy. Although deletion of MOB1 in yeast is lethal, conditional mutants arrest in late mitosis as large budded cells with separated chromatin and long bipolar spindles. In both *S. cerevisiae* and *S. pombe* Mob1p physically interacts with the Dbf2p/Sid2p kinase being essential for cytokinesis and mitotic exit.

In order to gain a better insight into cell division mechanisms in higher eukaryotes, we have cloned the orthologues of yeast MOB1 in *Drosophila melanogaster*. We have found a family of four MOB1-like genes in the *Drosophila* genome, coding for proteins sharing significant homology with the yeast protein, hereafter named *dmob1*, 2, 3 and 4. Real-time imaging in syncytial embryos using YFP-fused proteins showed a highly dynamic behaviour for these MOB1-like proteins.

Depletion of the Dmob proteins from S2 cells by RNAi, gives no visible phenotype, however depletion of Dmob4 by RNAi in the fly is lethal, whilst mutation of *dmob1* results in reduced male fertility. A search for the underlying cellular cause of these phenotypes is now under way.

914B

Study of the effects of phosphoinositides in *Drosophila* male meiotic cytokinesis. H.-C. Wei, G. Polevoy, J.A. Brill. Dept Developmental Biol, Hosp Sick Children, Toronto, ON, CANADA.

Drosophila male meiosis provides an excellent model to study cytokinesis as the unique spermatid morphology offers a clear landmark to identify genes causing cytokinesis defects. Mutations in *fwd*, which encodes a *Drosophila* type III β phosphatidylinositol (PI) 4-kinase, cause defects in meiotic cytokinesis, demonstrating that phosphoinositol lipids are important for this process (Brill *et al.*, 2000). Possible targets of regulation by PI polyphosphates in cytokinesis include vesicle transportation, membrane addition and actin organization. For example, a number of actin regulatory proteins, including capping protein, profilin, cofilin and septins, are regulated by direct binding to PI lipids. To further examine the role of PI lipids in male germ cell development, we expressed the *Salmonella* inositol phospholipid phosphatase SigD in *Drosophila* testes and found that the resulting flies were dominant male sterile. Examination of SigD-expressing testes revealed defects in meiotic cytokinesis and spermatid elongation. In particular, we found that septin organization was quite aberrant in SigD testes. Using GFP fused to a PH domain that specifically binds PI(4,5)P₂ (PIP₂), we found that plasma membrane PIP₂ was diminished in the SigD testes. Consistent with a depletion of PIP₂ by SigD, expression of a transgene containing YFP fused to the *Drosophila* phosphoinositol 4-phosphate 5-kinase Skittles (Sktl) partially rescued the defect in spermatid elongation. Taken together, our results show that PIP₂ plays an important role in male meiotic cytokinesis and later stages of sperm development.

CYTOSKELETON AND CELLULAR BIOLOGY**915C**

Dissecting salivary gland secretion using a gene-trap strategy. A. Andres¹, J. Shreck¹, R. Boyles¹, N. Bond¹, D. Hoshizaki¹, J. Merriam². 1) Dept Biol Sci, Univ Nevada-Las Vegas, Las Vegas, NV; 2) Dept Biol, Univ California-Los Angeles, Los Angeles, CA.

The larval salivary gland of the third instar is an excellent model system for dissecting the molecular components that are utilized during a regulated secretion in a non-excitabile cell type. The gland produces a massive amount of glue during the mid-third instar. These glue (Sgs) proteins are loaded into secretory granules that are exocytosed into the lumen of the gland in response to the steroid hormone, ecdysone, approximately 4-6 hours prior to puparium formation. We have developed some very effective assay tools that can be used in conjugation with temporally and spatially specific Gal4 drivers to manipulate candidate molecules *in vivo*. Here we present an analysis of some "Gene-Trap" lines in which a GFP exon has been incorporated into endogenous proteins. The candidate molecules were tested from a collection of stocks generated in the Cooley Lab. Those polypeptides that are expressed in the salivary gland have been noted. Examples of proteins that display altered patterns of expression and/or localization after the increase in ecdysone titer are presented, along with a strategy for testing their function using RNAi.

916A

Fat body dissociation during metamorphosis. D.K. Hoshizaki, N. Bond, J. Utz, A. Nelliott. Biological Sciences, University of Nevada, Las Vegas, NV.

In *Drosophila* metamorphosis is characterized by the loss and transformation of larval tissues in preparation for adult life. The majority of larval tissues undergo programmed cell death, but the fat body escapes destruction and instead dissociates into individual cells. These cells are metabolically active and serve as a reservoir of storage proteins that fuels the development of adult tissues.

The phenomenon of fat-body dissociation during metamorphosis represents a unique and novel system to study the genetic and cell biology of cell-cell detachment. Here, we describe in detail the morphological changes associated with tissue dissociation in live *Drosophila melanogaster* using fluorescent and confocal imaging. We also present a detailed study of the role of ecdysone signaling in fat-body dissociation through the use of a dominant-negative ecdysone-receptor mutant.

917B

Characterization of PI 4-kinase homologs in *Drosophila melanogaster*. J. Burgess^{1,2}, R. Vakilian^{1,2}, R. Mc Bride¹, G. Polevoy¹, J. Brill^{1,2}. 1) Developmental Biology, Hospital for Sick Children, Toronto, ON, Canada; 2) Department of Medical Genetics and Microbiology, University of Toronto, Toronto, ON, Canada.

Phosphatidylinositol (PI) is a fatty molecule that makes up a small (5-7%) but significant percentage of membrane lipids. The PI molecule is modified by lipid kinases that phosphorylate the inositol ring at various positions, generating a number of related PI lipids. PI derivatives are involved in regulating the actin cytoskeleton, trafficking

through the secretory pathway, plasma membrane endocytosis, as well as cell proliferation. More recently, it was shown in mammalian cells that phosphatidylinositol 4-phosphate (PI4P), which is enriched at the Golgi, plays a key role in recruiting components of the clathrin adaptor complex AP-1 (Wang *et al.*, 2003). Previously, it was found that mutations in the *Drosophila* gene *fwd* (PI 4-kinase III β) cause defects in cytokinesis during male meiosis (Brill *et al.*, 2000). As *fwd* appears to be required only for male fertility, we reasoned that other PI 4-kinases must carry out any essential functions in PI 4-phosphate biosynthesis. Using BLAST, we identified two other PI 4-kinase homologs in the *Drosophila* genome, which we have termed PI4KII α and PI4KIII α . We are currently undertaking a genetic screen to generate small deletions in these genes by imprecise excision using transposable P elements. In addition, we have constructed transgenic RNAi snap-back constructs composed of fused genomic and cDNA sequences to knock down the function of the two PI 4-kinase genes *in vivo*. In parallel, we are using RNAi to determine the function of these PI 4-kinases in S2 and Kc167 cells. We have demonstrated by RT-PCR that all three PI 4-kinases are expressed in these cells and have designed GFP reporter constructs to monitor the localization of phosphoinositides after gene knockdown. We have also performed *in situ* hybridization experiments to determine the expression pattern of these genes in embryos and adult testes.

GENOME AND CHROMOSOME STRUCTURE

918C

A link between mitosis and silencing: the role of the Polycomb Group in cell cycle progression. E. O'Dor, H. Brock. Dept Zoology, Univ British Columbia, Vancouver, BC, Canada.

The products of the Polycomb Group (PcG) of genes are members of multimeric complexes that pattern the anteroposterior axis during development by maintaining the silenced state of Hox genes. This epigenetic silencing requires a mechanism to transmit a cell's determined state from one cell cycle to the next in order to maintain fidelity of the developmental program. Maintenance must involve co-ordination between the PcG with components of the cell cycle machinery. We show that most early *Drosophila melanogaster* PcG mutant embryos tested show a variety of mitotic phenotypes including chromatin bridges at anaphase, nuclear fallout, asynchronous nuclei, and polyploid nuclei at frequencies significantly greater than wild type. In addition, we show that the presence of severe chromatin bridges delays the normal relocalization of Polyhomeotic-proximal (PH-P) protein to chromosomes at telophase. This delay in relocalization may disrupt the targeting or formation of PH-P-containing silencing complexes in daughter nuclei, leading to derepression of PcG targets and homeotic transformations. This model also predicts that mutants for cell cycle proteins which delay chromosome segregation but do not interact with PcG proteins, may disrupt relocalization of epigenetic silencing factors and appear to have, or enhance, homeotic phenotypes. We propose that PcG proteins have a secondary role in mitotic progression to ensure they are faithfully relocalized to their targets in the subsequent cell cycle.

REGULATION OF GENE EXPRESSION

919A

Do polymorphisms in the proximal promoter cause variation in gene expression between *Drosophila* strains? R.P. Brown, M.E. Feder. Organismal Biology & Anatomy, The University of Chicago, Chicago, IL.

We asked to what extent transcriptomic variation among populations has its basis in nucleotide polymorphisms in the proximal promoters of the corresponding genes. We began by choosing two sets of genes from pre-existing microarray data whose transcription levels vary or do not vary between two strains of *Drosophila melanogaster*. One kB of the proximal promoters of 10-15 genes in each set was sequenced and compared between strains. Promoter polymorphisms, including single-nucleotide, microsatellites, indels, and tandem repeats, were identified. The sequence was analyzed with pattern recognition programs to ascertain whether promoter sequence differences correlated with mRNA expression levels fell within regions such as consensus binding sites for known transcription factors. Candidate promoter differences will be analyzed for differences in transcriptional activity via luciferase reporter constructs. Positive findings will support the importance of *cis*-regulatory polymorphism for transcriptomic variation among populations, negative findings will implicate *trans*-regulatory or downstream mechanisms, and mixed findings will implicate all mechanisms. This research is significant because it will help elucidate the nucleotide basis for variation in the transcriptome.

920B

Two functionally identical modular enhancers in *Drosophila* Troponin T gene establish the correct protein levels in different muscle types. E. Garcia-Zaragoza, J.A. Mas, J. Vivar, M. Cervera. Dept. Biochemistry, Instituto de Investigaciones Biomédicas UAM-CSIC.

The control of muscle specific expression is one of the principal mechanisms by which diversity is generated amongst muscle types. In an attempt to elucidate the regulatory mechanisms that control fibre diversity in any given muscle, we have focused our attention on the transcriptional regulation of the *Drosophila* Troponin T gene. Two, non-redundant, functionally identical, enhancer-like elements activate Troponin T transcription independently in all major muscles of the embryo and larvae, as well as in adult somatic and visceral muscles. Here, we propose that the differential but concerted interaction of these two elements underlies the mechanism by which a particular muscle-type establish the correct levels of Troponin T expression, adapting these levels to their specific needs. This mechanism is not exclusive to the Troponin T gene, but is also relevant to the muscle specific Troponin I and Tropomyosin genes. In conjunction with *in vivo* transgenic studies, an *in silico* analysis of the Troponin T enhancer-like sequences revealed that both these elements are organised in a modular fashion. Extending this analysis to the Troponin I and Tropomyosin regulatory elements, the two other components of the muscle-regulatory complex, we have discovered a similar modular organisation of phylogenetically conserved domains. Reference: J.A. Mas, E. Garcia-Zaragoza and M. Cervera. Mol. Cell. Biol. April 2004; 15(4) (In press).

921C

A regulatory code for neurogenic gene expression in the *Drosophila* embryo. R.P. Zinzen, M. Markstein, P. Markstein, K. Yee, A. Erives, A. Stathopoulos, M. Levine. UC Berkeley, MCB, Division of Genetics, Berkeley, CA.

Bioinformatics methods have identified novel enhancers that mediate restricted expression in the *Drosophila* embryo. However, only a small fraction of the predicted enhancers actually work when tested *in vivo*. In the present study, co-regulated neurogenic enhancers that are activated by intermediate levels of the Dorsal regulatory gradient are shown to contain several shared sequence motifs. These motifs permitted the identification of new neurogenic enhancers with high precision: five of seven predicted enhancers direct restricted expression within ventral regions of the neurogenic ectoderm. Mutations in some of the shared motifs disrupt enhancer function, and evidence is presented that the Twist and Su(H) regulatory proteins are essential for the specification of the ventral neurogenic ectoderm prior to gastrulation. The regulatory model of neurogenic gene expression defined in this study permitted the identification of a neurogenic enhancer in the distant *Anopheles* genome. We discuss the prospects for deciphering regulatory codes that link primary DNA sequence information with predicted patterns of gene expression.

922A

***Drosophila* as an animal model of mitochondrial pathophysiology.** M. A. Fernandez-Moreno¹, C. Adan¹, B. Bornstein¹, P. de la Peña¹, C. Garcia-Vallejo¹, M. C. Gil¹, E. Gonzalez¹, R. Hernandez¹, F. Martinez-Azorin¹, P. Ochoa¹, A. Sanchez¹, A. Seguido¹, R. Garesse¹, C; L. Farr², N. Luo², A. T. Lagina III², Y. Matsushima², L. Kaguni². 1) D. de Bioquímica/IIB, CSIC/UAM, Madrid, Spain; 2) D. of Biochemistry. Michigan State University. Michigan. USA.

mtDNA mutations are in the origin of some devastating diseases. Mitochondrial biogenesis involves proliferation and maturation and requires the coordinated expression of two genomes, nuclear and mitochondrial. During development, a precise and poorly understood genetic program regulates mitochondrial biogenesis in the different tissues to afford their variable energetic demands. Although a large number of mtDNA mutations have been associated with mitochondrial diseases, the relationship between genotype and phenotype is unknown. The generation of animal models provides an invaluable tool to understand this group of complex diseases. Our objective is to characterize mechanisms controlling mitochondrial biogenesis in *Drosophila melanogaster* and to establish *Drosophila* as an animal model of mitochondrial diseases. To this end, we have characterized the promoter region of nine nuclear genes involved in mtDNA metabolism. We have demonstrated that DREF, a transcription factor which regulates genes for nuclear DNA replication, also controls the expression of genes involved in mtDNA replication. We have determined the spatio-temporal pattern of expression of some of these genes during development. Finally, we have engineered *in vitro* the catalytic subunit of the mitochondrial DNA polymerase to abolish its proofreading activity, and to generate transgenic flies that overexpress the enzyme. Depending on the level and the spatio-temporal pattern of expression, we have induced a variety of phenotypes ranging from a decrease in life span to pupal lethality due to a severe mtDNA depletion. Our next aim is to use homologous recombination to generate mutants carrying the genes with the mutations of their human orthologous.

923B

***dMBD-R2* is essential for *Drosophila* development.** V.I. Rasheva, M.-J. Lin, N.M. Reddy, T.-Y. Tzeng, M.-S. Hung, L.-Y. Tang, P.-G. Lee, H.-J. Xie, C.-K. J. Shen. Inst Molecular Biol, Academia Sinica, Taipei, Taiwan.

The family of methyl-DNA binding (MBD) proteins is widely presented in life organisms and is found to participate in the transcriptional regulation, gene silencing and carcinogenesis. *dMBD-R2* is one of five *Drosophila* proteins identified to possess MBD-like domain. It consists a PHD finger, a Tudor domain, a zinc finger and DNA-binding domain. To identify the function of these domains we have characterized molecularly the gene *dMBD-R2* and studied its P-element insertion mutant and ectopic expressing phenotypes. Using RT PCR we find that the gene *dMBD-R2* is expressed ubiquitously during development, with lower level of expression during first, second and third larva stages, and higher expression level in females comparing to the males. P-element insertion was excised using the transposase and 40 excision lines were analyzed for lethality and fertility. Germline clones of P-element insertion mutant were generated and the phenotypes of somatic clones were observed. Our observations indicate that *dMBD-R2* plays role in apoptosis pathway and is essential for *Drosophila* sterility. Our results show that the *dMBD-R2* is involved in regulation of *Drosophila* immunity and is a melanotic tumour gene. The heterozygous double excision mutants began to develop melanotic masses in the stage at second instar larva, earlier than most of known melanotic tumour genes and this masses appeared in 30-70% of larva. We suggest that *dMBD-R2* gene possesses pleiotropic effects and influences *Drosophila* development via its involvement in multiple pathways.

924C

The role of Pipsqueak in GAGA-binding chromatin complexes. M. Lehmann¹, A. Schwendemann², Th. Siegmund³, R. Greenaway¹. 1) Biological Sciences, University of Arkansas, Fayetteville, AR, USA; 2) Institut für Biologie der Freien Universität Berlin - Genetik - , Berlin, Germany; 3) DeveloGen AG, Göttingen, Germany.

Although described for plants, sea urchins and mammals including humans, regulatory GAGA-DNA elements and GAGA-binding proteins have been most extensively studied in *Drosophila melanogaster*. Complexes formed by these proteins disrupt the normal nucleosome pattern and seem to create an alternative chromatin architecture. This architecture in turn is believed to provide a platform on which other proteins can execute a variety of different regulatory functions including gene silencing and activation. Two *Drosophila* proteins, the GAGA factor and Pipsqueak can directly bind to GAGA-sequence DNA and seem to be the core components of GAGA-binding protein complexes. We are studying the specific function of Psq within the core complex as well as the interaction of the complex with other proteins in the context of homeotic gene regulation.

925A

"Auto"-regulation of BMP signaling. L. Soares, S. Ballard, K. Wharton. Molecular Biology, Cell Biology & Biochemistry Department, Brown University, Providence, RI.

Bone morphogenetic proteins (BMPs) have been identified as candidate morphogens. Given that the efficacy of a morphogen gradient depends on specific concentration or activity thresholds, we expect the level of morphogens or their activities to be tightly regulated in developing tissues. Currently, the means by which this is accomplished is poorly understood. Our studies on the relative contributions of two BMPs, *decapentaplegic* (*dpp*) and *glass-bottom boat* (*gbb*), to the development of the *Drosophila* wing have uncovered a previously unappreciated aspect of transcriptional regulation that is critical to ensuring the integrity of the BMP activity gradient necessary for wing patterning. Our data show that BMP signaling plays a role in modulating levels of *dpp* expression in the stripe of cells along the A/P boundary of the wing disc and is, therefore, critical for maintaining appropriate levels of the Dpp ligand for proper patterning of the wing imaginal disc. These findings indicate that *dpp* transcription is regulated in at least two ways: It is spatially restricted to the cells along the A/P boundary of the *Drosophila* imaginal wing disc by Hh signaling and maintained at a certain level by BMP signaling dependent repression of *dpp* expression levels. The tight regulation of *dpp* transcription is critical for maintaining the proper balance of BMP ligands necessary for patterning the wing imaginal disc.

926B

Interaction of *Drosophila* PSC protein with transcription factors. M. Pilyugin¹, J. Larsson², V. Pirrotta¹. 1) University of Geneva, Switzerland; 2) University of Umeå, Sweden.

Posterior sex combs (PSC) protein is one of the major players in Polycomb-mediated silencing in *Drosophila*. It has been shown that PSC and Polycomb (PC) proteins interact in the nucleus with TBP and other transcription factors. Here we demonstrate that PSC can interact with TBP protein *in vivo*, independently of PC protein, suggesting a role for PSC as a link between PC complexes and transcription factors. We also found a novel highly conserved transcription factor, Limpet, interacting with RING finger domain of PSC. We demonstrated that Limpet protein may represent a physical link between PSC and Zeste and can be found in a complex with PC protein.

SIGNAL TRANSDUCTION**927C**

Genetic analysis of the Rho effector kinase, PKN, in Drosophila. M. Betson, J. Settleman. MGH Cancer Center and Harvard Medical School, Charlestown, MA.

Rho family GTPases play an important role in diverse cellular processes and are essential for embryonic development in many organisms including *Drosophila*. Several putative effector proteins have been identified which interact directly with Rho family members and mediate their functions. These include kinases such as Rho-kinase, Citron kinase and Pkn.

Drosophila Pkn, like its three mammalian orthologs, has N-terminal leucine-zipper repeats and a C-terminal kinase domain which is closely related to those of protein kinase Cs. Pkn interacts with Rho1 and Rac via its leucine zipper repeats. Flies homozygous for a null mutation in the Pkn gene die between late embryonic and pupal stages, and homozygous mutants never eclose. Germ-line clone mutants of Pkn exhibit dorsal closure defects. In vitro studies have implicated mammalian PKNs in the regulation of gene expression and the actin cytoskeleton. In addition, PKN has been implicated in prostate cancer, where it has been observed that expression of PKN is upregulated in prostate cancer tissue. PKN was found to interact with the androgen receptor (AR), a key regulator of prostate cancer progression, and to promote AR-dependent transcription.

Several proteins have been shown to bind to and be phosphorylated by mammalian PKN in vitro, however, the in vivo significance of these interactions remains unclear. Therefore, we are undertaking a screen to identify physiologically relevant components of the Rho-Pkn signaling pathway. We are screening for dominant modifiers of a wing phenotype generated by overexpression of the kinase domain of Pkn using the GAL4/UAS system.

In addition, we are exploiting a rescue approach to determine whether Rho or Rac binding is important for Pkn function, and if the kinase domain of PKC can functionally substitute for that of Pkn.

928A

A proteomic screen to identify substrates of the protein phosphatase Eyes Absent. I. Mills, I. Rebay. Whitehead Institute, Department of Biology, MIT, Cambridge, MA.

Eyes Absent (eya), a member of the retinal determination network, encodes an evolutionarily conserved nuclear protein necessary for proper organ development, including the eye in *Drosophila*, and the eye, kidney, muscle, lung, and ear in vertebrates. Although Eya does not have any DNA capabilities, it has been shown to act as a transactivator when bound to the homeodomain protein, Sine Oculis (So). Recently, a novel function for Eya as a magnesium dependent protein phosphatase has been demonstrated. Also, Eya's functions as a transcription factor and a phosphatase have been shown to be essential for eye development. To investigate further the phosphatase function of Eya, we have employed a proteomics approach, *in vitro* expression cloning (IVEC), to determine potential Eya substrates. Utilizing this technique, we screened ~6,000 cDNA clones of the *Drosophila* Gene Collection (DGC) represented in 260 *in vitro* transcribed and translated protein pools. We have used this technique as a binding assay to isolate proteins that interact with Eya. Out of 260 protein pools, 36 pools contain clones that interact with Eya. We will present the results of this screen and discuss further studies we will utilize to understand the relevance of these substrates. These studies will contribute to the understanding of Eya's phosphatase activity in transcriptional regulation.

929B

A Drosophila EMS-mutagenesis screen to isolate novel effectors in the TOR signaling pathway. M. L. Ruhf, J. Montagne, G. Thomas. FMI, Maulbeerstrasse 66, CH 4058, Basel, Switzerland.

Cell growth is a fundamental biological process, whereby cells accumulate increased mass. Since it is an important determinant of the size of cells, organs and organisms, this process needs to be properly regulated. Mitogenic and nutritional signals must be integrated for cell growth. The protein kinase Target of Rapamycin (TOR) in response to available nutrient has been shown to regulate major anabolic and catabolic responses, including protein synthesis and autophagy, respectively. However, despite a great deal of effort, we still know little concerning the identity of upstream sensors, which act on TOR, nor the identity of many of its potential downstream effectors. TOR's ability to regulate ribosome biogenesis and protein synthesis is achieved in part, by two of its best-described targets, S6K and 4EBP. However in *Drosophila*, the phenotypes of dTOR, dS6K and d4EBP mutants clearly reveal that dTOR has additional targets, which have yet to be described. The function of dTOR can be specifically blocked by rapamycin or its orally viable derivative, RAD001 (Novartis), which is presently in clinical trials in a number of therapeutic indications. When RAD001 is administered to *Drosophila* via their food, the development of the larvae is dramatically delayed. These observations were the basis of an EMS based genetic screen that will be presented.

930C

Distinct and collaborative roles of Drosophila EXT family tumor suppressors in morphogen signalling and gradient formation. C. Han^{1,2}, T.Y. Belenkaya², X. Lin^{1,2}. 1) Dept Developmental Biol, Univ Cincinnati, Cincinnati, OH; 2) Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Heparan sulfate proteoglycans (HSPG) have been implicated in regulating the signalling activities of secreted morphogen molecules including Wingless (Wg), Hedgehog (Hh) and Decapentaplegic (Dpp). HSPG consists of a protein core to which heparan sulfate (HS) glycosaminoglycan (GAG) chains are attached. The formation of HS GAG chains is catalyzed by glycosyltransferases encoded by members of the *EXT* family of putative tumor suppressors linked to hereditary multiple exostoses (HME). Previous studies in *Drosophila* demonstrated that *tout-velu* (*ttv*), the Drosophila EXT1 is required for Hh movement. However, the functions of other EXT family members are unknown. Here we have identified and isolated the other two members of *Drosophila* EXT family genes, which are named as *sister of tout-velu* (*sotv*) and *brother of tout-velu* (*botv*) encoding *Drosophila* homologues of vertebrate EXT2 and EXT-like 3 (EXTL3), respectively. We show that both Hh and Dpp signalling activities as well as their morphogen distributions are defective in cells mutant for *ttv*, *sotv* or *botv* in the wing disc. Surprisingly, while Wg morphogen distribution is abnormal in *ttv*, *sotv* and *botv*, Wg signalling is only defective in *botv* or *ttv-sotv* double mutant, but neither in *ttv* nor in *sotv* alone, suggesting that Ttv and Sotv are redundant in Wg signaling. We demonstrate further that Ttv and Sotv form a complex and are co-localized *in vivo*. Our results along with previous studies on Ttv provide evidence that all three *Drosophila* EXT proteins are required for the biosynthesis of HSPGs and for the gradient formation of the Wg, Hh and Dpp morphogens. Our results also suggest that HSPGs have two distinct roles in Wg morphogen distribution and its signalling.

931A

Identification of two novel FGF encoding genes required for mesoderm migration. T. Gryzik, H.A.J. Müller. Inst Genetik, Heinrich-Heine Univ, Duesseldorf, Germany.

During gastrulation, mesodermal cells invaginate through the ventral furrow, flatten and begin to migrate in a dorso-lateral direction on the underlying neuroectoderm. The activities of a fibroblast growth factor (FGF) receptor, called Heartless (Htl), and another component, called Downstream of FGF receptor (Dof) are necessary for the proper directional migration of mesodermal cells. To identify additional genes required for mesoderm migration we have performed a genome-wide screen and identified seven genomic regions essential for mesoderm migration. Here we present the identification of two closely linked genes that are required to promote mesoderm migration. We have cloned these two genes and found that they encode two novel FGFs, which were only partially annotated in the genome. The primary sequence of the predicted proteins features a conserved FGF core domain, which shares 36% to 33% identical amino acids to the FGF domain of the vertebrate FGF8/17/18 subgroup. We therefore called these genes *DFGF8-1* and *DFGF8-2*. dsRNAi experiments revealed that both genes are required for mesoderm migration. We further show that Htl signaling is blocked in embryos deficient for these genes. Both genes are highly expressed in the neuroectoderm, the substrate for mesoderm migration. Together our results suggest that these novel FGFs represent the ligands for the FGF receptor Htl.

932B

Role of proteoglycan in FGF receptor signaling during tracheal and heart development in Drosophila. D. Yan^{1,2}, D. Metzger^{1,2}, X. Lin^{1,2}. 1) Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) Department of Developmental Biology, University of Cincinnati, Cincinnati, OH.

Heparan sulfate proteoglycans (HSPGs) are cell surface and extracellular matrix molecules composed of a protein core to which heparan sulfate (HS) glycosaminoglycan (GAG) chains are attached. Previous genetic studies have shown that HSPGs are required for the signaling activities of two *Drosophila* FGF receptors *Heartless* (*Htl*) and *Breathless* (*Btl*), which are essential for mesoderm migration and tracheal branching, respectively. Embryos mutant for *sugarless* and *sulfateless*, two genes encoding the homologs of UDP-D-glucose dehydrogenase and heparin/heparan sulfate N-deacetylase/N-sulfotransferase, are defective in both *Htl* and *Btl* signaling. However, currently it is unknown which HSPGs are involved and whether other HS GAG biosynthesis enzymes have specificity in FGF signaling. Genetic screens in our lab have identified and isolated null mutations of *Dally* and *Dally-like* (*Dly*), two *Drosophila* glypican members of HSPG, as well as mutations in members of EXT family genes, *brother of tout-velu* (*botv*) and *sister of tout-velu* (*sotv*). We have analyzed the roles of these genes in FGF signaling in embryonic mesoderm migration and tracheal branching. Our data demonstrate that both HSPG cores and HS GAG biosynthesis enzymes can contribute to the specificity of FGF signaling.

933C**Genetic interactions of the small GTPase, RIC, with multiple signaling pathways in Drosophila.** S.M.W.

Harrison¹, M.L. Spencer², P.D. Wes³, C. Montell³, D.A. Andres², D.A. Harrison¹. 1) Dept. of Biology, University of Kentucky, Lexington, KY; 2) Dept. of Molecular and Cellular Biochemistry, University of Kentucky College of Medicine, Lexington, KY; 3) Depts. of Biological Chemistry and Neuroscience, The Johns Hopkins University, School of Medicine, Baltimore, MD.

Drosophila RIC is a member of a subfamily of Ras-related small GTPases that includes mammalian Rit and Rin. These GTPases share several features including the absence of prenylation signal, a distinct effector domain, and in the case of Rin and RIC, the presence of a calmodulin-binding domain. To address the physiological role of this Ras subfamily in vivo, activated forms of the Drosophila Ric gene were introduced into flies. Expression of activated RIC proteins altered the development of well-characterized adult structures, including wing veins and photoreceptors of the compound eye. The activated RIC phenotype could be moderated by the reduction of dosage of several genes in the Ras signaling cascade, and exacerbated by the reduction of several genes in the DPP pathway. The phenotype was also enhanced by a reduction in calmodulin suggesting that RIC signaling is negatively regulated by this molecule. To further identify molecules that interact with RIC, we have scanned the Drosophila genome using a series of deficiencies to uncover regions that either enhance or suppress the activated RIC phenotype. Candidate genes within these regions are currently being tested.

934A**Calx functions as a sodium calcium exchanger in phototransduction.** T. Wang¹, R. Hardie³, C. Montell^{1,2}.

1) Biological Chemistry, Johns Hopkins University, Baltimore, MD; 2) Department of Neuroscience, Johns Hopkins University, Baltimore, MD; 3) Department of Anatomy, Cambridge University, Cambridge CB2 3DY UK.

The light response of Drosophila photoreceptor is mediated by a G protein coupling pathway. Upon the light stimulation the Transient Receptor Potential (TRP), Transient Receptor Potential-like (TRPL) and Transient Receptor Potential (TRP) channels are activated which promotes calcium and sodium influx and depolarization. After the light signal stops, the TRP, TRPL and TRP channel close and the cells repolarize. However, the mechanism which permits the photoreceptor to maintain the depolarization state and calcium homeostasis is still unknown. In this work we isolated a new phototransduction mutant through 3-rd chromosome forward genetic screen. The mutant not only display an inactivation no afterpotential (ina) ERG phenotype but is also characterized by light-dependent retinal degeneration. We found that a sodium calcium exchanger: Calx is disrupted in the ina mutant. Transgenic heat shock calx can rescue the ina mutant and overexpress of calx also causes an abnormal ERG phenotype. In addition, Calx is localized in the rhabdomere region in photoreceptor cells. The calcium sodium exchanger current is dramatically reduced in the calx mutant and restored in calx heat shock rescued flies. Calx is the only exchanger corresponding to the calcium sodium exchanger current in the photoreceptor cells and functions to maintain the depolarization state and calcium homeostasis in the phototransduction pathway.

PATTERN FORMATION**935B****The role of Upd as a potential morphogen in follicular fate specification.** T.R. Sexton, R. Xi, S.M.W. Harrison, D.A. Harrison. Dept Biol, Univ Kentucky, Lexington, KY.

Janus Kinase (JAK) activity is involved in follicular epithelial development in Drosophila oogenesis. Upd, the ligand for the pathway, is expressed and secreted from the polar cells of each developing egg chamber. JAK activity is graded on the follicular epithelium with the highest activity at the poles and lowest activity in the central region of each egg chamber. In anterior development, follicular cells that are most polar will develop into border cells, whereas those that are least polar will adopt a stretch cell or a centripetal cell fate. Alteration of JAK activity has shifted distribution of the cell types. With higher levels of JAK activity there is an increase in number of border cells, whereas in lower levels of JAK activity there is a decrease in number of border cells. This has led to proposal that Upd is acting as a morphogen during follicular cell development during oogenesis. Although Upd is known to be expressed in the polar cells, no distribution of the Upd protein has ever been detected. We seek to find the extracellular distribution, possible gradient formation, and mechanism of movement of Upd during Drosophila oogenesis. Current strategies being utilized are the development of specific antibodies against Upd and the construction of a chimeric version of Upd with a GFP fusion on the C terminus. The Drosophila genome sequence has also revealed two Upd-like genes. These genes, Upd2 and Upd3, are expressed in patterns that partially overlap the expression of Upd. Upd2 expression overlaps the expression of Upd during embryogenesis whereas Upd3 expression overlaps the expression of Upd during oogenesis. Interestingly, results from ectopic expression of Upd2 in oogenesis are similar to the ectopic expression of Upd. We seek to investigate the role of the Upd-like proteins in JAK activity during oogenesis.

936C

A role for Stardust in light-dependent retinal degeneration? S. M. Berger¹, F. Grawe¹, B. Busse¹, K. Johnson², E. Knust¹. 1) Institut fuer Genetik, Heinrich-Heine-Universitaet, Universitaetsstr.1, 40225 Duesseldorf, Germany; 2) Institut fuer Genetik, Universitaet zu Koeln, Weyertal 121, 50931 Koeln, Germany.

Mutations in the *Drosophila* gene *crumbs* lead to morphological defects in the eye and to light-dependent retinal degeneration (LRD). In order to identify additional proteins involved in this process we focussed on Stardust (Sdt). Sdt belongs to the MAGUK family of scaffolding proteins and is one of the known interaction partners of the transmembrane protein Crumbs (Crb) in the subapical region of embryonic epithelia.

In photoreceptor cells Sdt colocalizes with Crb at the stalk membrane which connects the rhabdomere with the Zonula adherens. The localisation of Sdt depends on Crb and vice versa. One of three previously described *sdt* alleles displays a morphological *crb*-like eye phenotype with shorter and thicker rhabdomeres and a strongly reduced stalk membrane. However, none of these alleles shows LRD. We are currently investigating novel *sdt* alleles with respect to their morphological phenotype and LRD.

937A

Maternal control of epithelial development in *Drosophila*. W. Gödde, E. Knust. Universität Düsseldorf, Institut für Genetik, Düsseldorf, NRW, Germany.

In the *Drosophila* embryo establishment of epithelial polarity begins at cellularisation and has to be maintained during further morphogenetic processes. While zygotically expressed genes contributing to the maintenance of epithelial polarity are known (e.g. *crumbs* and *stardust*), there is evidence that major aspects of this processes are maternally controlled. In order to identify additional genes, a collection of P-element induced mutations were analysed in embryos derived from germ line clones. Three of them with severe defects in cuticle formation were selected for a more detailed characterisation. The phenotypic analysis performed so far suggests their involvement in cellularisation, maintenance of epithelial polarity and morphogenesis. A detailed characterisation will be presented.

938B

The role of the *Drosophila* gene *bazooka* in morphogenetic processes. I. Wasserscheid, E. Knust. Heinrich Heine Univ. Düsseldorf, Institut für Genetik, 40225 Düsseldorf, Germany.

The *Drosophila* protein Bazooka, a scaffolding protein with three PDZ domains, is known for its role in the establishment and maintenance of apico-basal cell polarity in epithelia. It localizes apically in a complex with Par-6 and aPKC to define the apical membrane. *bazooka*-mutant embryos die during embryogenesis with highly affected epithelia. In the epidermis cell polarity and tissue integrity are affected. In other epithelia morphogenic defects and cell shape abnormalities can be observed. We are investigating the role of *baz* in facilitating cell shape changes and morphogenesis. An analysis of phenotypes generated by overexpression of *baz* in the wing will be presented. Our data suggest overexpression causes cell shape abnormalities and morphogenic defects as a consequence of an abnormal actin cytoskeleton.

939C

Role of *skinhead* in growth and patterning at the wing margin. Z.S. Walters, R.G. Phillips. School of Life Sciences, University of Sussex, Brighton, United Kingdom.

Growth and patterning in the *Drosophila* wing imaginal disc is initiated in response to a self-regulating signal from the DV boundary. The secreted peptide, Wingless, forms at least part of this signal, regulating expression of *achaete*, *distalless*, *ventral veins lacking* and *vestigial* in the presumptive wing blade. We will present genetic and molecular analyses of *skinhead* (*skn*), a novel gene identified in a screen for wing growth and pattern defects. *skn* exhibits *wingless*-like phenotypes including loss of wing and duplication of notum and loss of sensory bristles. Homozygous clones (*skn/skn*) in wing imaginal discs exhibit epistasis over loss of function of *shaggy*, a Wingless-signal antagonist. These clones show ectopic activation of the DV boundary enhancer of *vestigial* (*vg[BE]*) and loss of *Distalless* expression indicative of failure of transduction of the signal and of the self-regulatory mechanism. Investigation into the location of *skinhead*, through studies using deficiency strains and PCR methods, has narrowed the search down to a small region on chromosome 2R. By creating P-element jumps around the region and also by making rescue constructs we hope to identify the product of the *skinhead* gene.

940A**Using germ line transformations to test the function of genes implicated in the development of butterfly wing patterns.** A. Monteiro, J.M. Marcus, D.M. Ramos. Biological Sciences, University at Buffalo, Buffalo, NY.

We have developed germ line transformations for the nymphalid butterfly *Bicyclus anynana* in order to be able to test the function of three candidate genes implicated in eyespot development by their suggestive expression patterns. Our efforts have led to the successful transformation of *B. anynana* with piggyBac and hermes constructs carrying the marker gene GFP driven by an eye specific promoter. Ongoing efforts involve another screen with a piggyBac construct carrying DsRed under the control of the eye promoter as well as GFP under the control of a heat-shock promoter from *Drosophila*. We will determine whether we can drive the expression of the reporter gene GFP in animals carrying this construct after subjecting them to localized heat shock.

941B**Early pupal cell death contributes to Drosophila retinal patterning.** J.B. Cordero, O. Jassim, S. Bao, R. Cagan. Molecular Biology and Pharmacology Department, Washington University in St. Louis, Saint Louis, MO 63110.

Programmed cell death (PCD) is utilized to create and especially to refine structure in developing tissues and organs. However, little is understood about the mechanisms that, within a developing epithelium, combine signals to selectively remove some cells while sparing essential neighbors. One popular system for studying this question is the developing *Drosophila* pupal retina, where excess interommatidial support cells are removed to refine the patterned ommatidial array. We present data indicating that PCD occurs earlier within the pupal retina than previously demonstrated. As with later PCD, this death is dependent on Notch activity. Surprisingly, altering *Drosophila* Epidermal Growth Factor Receptor or Ras pathway activity had no effect on this death. Instead, our evidence indicates a role for Wingless signaling to provoke this cell death. Together, these signals regulate an intermediate step in the selective removal of unneeded interommatidial cells that is necessary for a precise retinal pattern.

942C**Strategy for cloning a gene on the X chromosome required for nuclear spacing and investigation of the mechanisms underlying the establishment of nuclear density domains.** S. Pichler^{1,2}, T. Gregor², D. Glover¹.

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In *D. melanogaster*, the earliest morphogenetic changes are visible in cycle 11 of the syncytial blastoderm embryo as inhomogeneities in the spacing of nuclei along the A-P axis (Blankenship and Wieschaus, 2001). In the anterior domain, spaces between nuclei are expanded, in the pre-cephalic furrow domain, distances are the smallest and in the posterior domain, distances are intermediate. We have developed Matlab programmes which measure nuclear densities and distances between nearest nuclei to identify genes that regulate nuclear spacing before cellularization. We have analysed various chromosomal rearrangements and found that in embryos lacking the X chromosome and expressing *D. virilis nullo*, nuclear spacing is uniform. In *gt* homozygous embryos the anterior domain is formed. We have investigated nuclear spacing in *Df(1) Sxl-ra/Dp(1;Y)ct+y+* embryos and recombinants of *gt* and *Df(1)ct4b1* and *Df(1)HA32* and mapped the gene required for nuclear spacing between 7A-7B breakpoints. 7A-7B uncovers 33 genes with CG numbers and we are currently setting up RNAi experiments in pre-syncytial embryos to identify the gene involved in nuclear spacing. In addition, we are genetically fine-mapping region 7A-7B. Modulation of *bicoid* expression causes abnormalities in nuclear spacing. We are investigating the role of zygotic transcription in nuclear spacing by alpha-amanitin injections of pre-syncytial embryos using the nuclear-neighbour-programme. In *spg* homozygous embryos the formation of nuclear density domains is initiated similar to wild-type embryos suggesting that the organization of the cortical actin cytoskeleton is not required for the initiation of nuclear spacing. We are investigating the role of the microtubule cytoskeleton during the syncytial divisions using immunohistochemistry and time-lapse videomicroscopy of GFP-TAU embryos.

GAMETOGENESIS AND SEX DETERMINATION**943A****Development of the Telotrophic Meroistic Ovary of the Bug *Dysdercus intermedius* (Heteroptera, Pyrrhocoridae).** J. M. Kugler, J. Trauner, R. Rüksam, J. Büning. Inst Zoology, Univ Erlangen-Nuremberg, Erlangen, Germany.

In polytrophic meroistic *Drosophila* ovaries eggs develop from syncytial germ cell clusters containing one oocyte and 15 nurse cells. A cluster arises from a stem cell derived cystoblast in four synchronous rounds of mitosis with incomplete cytokinesis. In the telotrophic meroistic ovary all nurse cells are retained in the apical part of the

ovariole, the tropharium. Oocytes mature in the basal vitellarium and are connected to the syncytial tropharium by nutritive cords. In *Dysdercus intermedius* oocyte differentiation is restricted to larval development. In adults, only nurse cells are replenished by mitotic divisions in the tip of the tropharium. It is not known whether the germ cell syncytium is established by incomplete cluster divisions as observed in polytrophic merostic species and lower hemipterans or by fusion of cells or clusters. We analyzed the development of the *Dysdercus* ovary from the third larval instar (L3) on to the adult. In L3, the ovariole anlage contains a compact sphere of germ cells. In the center of this sphere f-actin and phosphotyrosine-containing proteins begin to accumulate, forming the PY-structure. Neither ring canals nor fusomes were detectable in ovarioles, whereas in testes f-actin positive polyfusomes were observed. In L4, germ cells begin to divide rapidly and asynchronously. Simultaneously, the PY-structure expands throughout the center of the germarium. In L5, the PY-structure retracts to an apical position, where it is finally found in adult females. Germ cells losing contact to the PY-structure cease mitosis. The basal-most germ cells enter meiosis indicating the onset of oocyte differentiation. Those located more apically endopolyploidize and become nurse cells. The asynchrony of germ cell divisions as well as the apparent lack of a fusome in the female germ line corroborate the idea that the syncytial nature of the germ cells in bugs is a result of germ cell fusion rather than of incomplete cluster division.

944B

Drosophila decapping protein 1, dDcp1, with Exu-Yps RNP complex directs the posterior deposition of oskar mRNA in oocyte. M.-D. Lin¹, S.-R. Fang¹, X. Jiao², M. Kiledjianand², T.-B. Chou¹. 1) Institute of Molecular and Cellular Biology, National Taiwan University, No.1, Sec.4, Roosevelt Rd., Taipei, Taiwan; 2) Department of Cell Biology and Neuroscience, Rutgers University, 604 Allison Road, Piscataway, NJ 08854-8082, USA.

The mRNA decapping is important for mRNA degradation. The posterior deposition of oskar (*osk*) mRNA in oocyte is critical for pole cells and abdomen formation during *Drosophila* embryogenesis. Here, we present *Drosophila* dDcp1, a decapping enzyme involved in *osk* mRNA deposition. dDcp1 carries intrinsic decapping activity in vitro. The loss-of-function of dDcp1 displayed the posterior group embryonic phenotype with reduction of pole cells. Several posterior determinants including oskar mRNA, Staufen (*Stau*), Osk and Vasa, but not microtubule polarity and organization, are affected by dDcp1 mutation. Through out early oogenesis, dDcp1 is localized posteriorly and co-localized with Exuperantia (*Exu*) in oocyte. We confirmed that dDcp1 is resided in the oskar-*Stau*-*Exu*-Yps RNP complex and specifically control the transportation of *osk* mRNA by directing its posterior localization.

945C

STAT function is needed to maintain, but not initiate *Sxl* expression during sex determination. F. W. Avila¹, J.W. Erickson². 1) Biological Sciences, Columbia University, New York, NY; 2) Biological Sciences, Texas A&M University, College Station, TX.

In *Drosophila*, sex is determined by the female-specific activation of Sex-lethal (*Sxl*) early in embryogenesis. SXL expression is dependent on X-chromosome dose: XX animals are female and XY animals male. X dose is signaled by four X-linked signaling elements (XSEs) that activate transcription of *Sxl* via the establishment promoter, *SxlPe*. Unlike other XSEs that encode transcription factors directly activating *SxlPe*, *os* (*upd*, *sisC*) encodes a ligand for the *Drosophila* JAK/STAT pathway. Activation of the pathway leads to dimerization of maternally supplied STAT that is believed to act directly on *SxlPe*. We are examining the role of STAT in *Sxl* expression. Germline clones removing the maternal contribution of the JAK kinase, *hopscotch*, prevent STAT dimerization and function. In *hop* mutant clones, *SxlPe* is activated normally in all nuclei during cycle 12. However, expression fails to be maintained and is subsequently lost in the central regions of the embryo by nuclear cycle 14. These results show that *os* differs from the strong XSEs *sisA* and *scute* in that it is not required for the initial threshold response to X dose, but rather helps ensure sufficient early SXL expression to establish the autoregulatory splicing control that ultimately maintains the female state.

946A

Genetic characterization of paternal effect genes on sperm storage and fertilization. M-T. Yamamoto¹, M. Tomaru¹, M. Hara¹, T. Ohsako^{1,2}. 1) Dros. Genet. Res. Ctr., Kyoto Inst. Tech., Kyoto, Japan; 2) Soc. Edu. Found., Nara, Japan.

In many animal species, including *Drosophila melanogaster*, females store sperm that are transferred upon copulation, and release them to fertilize ovulated eggs for a given period. Sperm storage and their proper utilization are, thus, integral parts of the reproductive strategy in these species. Effective transfer, storage, release, and fertilization of sperm in the female reproductive organs are considered to be accomplished by paternal effect genes expressed in the normal spermatogenesis. In order to elucidate the roles and functions of paternal effect genes in

sperm storage, release, and fertilization processes in *D. melanogaster*, we screened from 94 male sterile mutations collected in our laboratory for a specific type of male sterility. Five recessive male sterile mutations, *ms(3)236*, *ms(2)n55*, *wasted*, *misfire*, and *ms(3)A3*, were isolated. Males homozygous for each mutation produce normal amount of motile sperm that are capable to be transferred into female upon copulation, but almost completely fail to produce progeny. We examined whether the motile sperm produced from these mutations are stored in the female sperm storage organs and released from the storage organs at ovulation. Three mutations showed defects in sperm storage. The *ms(3)236* sperm enter the storage organs at a significantly low level. Sperm of the *ms(2)n55* and *wasted* males enter the organs but are lost in a few days after copulation because large numbers of sperm are released at each ovulation. The *ms(2)n55* and *wasted* mutations show a defect in fertilization also: the sperm fail to form male pronuclei in the inseminated eggs. Another mutation, *misfire*, is a fertilization defective, exhibiting a failure in male pronucleus formation (Ohsako et al., 2003).

ORGANOGENESIS

947B

Identification of mutants with defects in tracheole outgrowth and function. A.S. Ghabrial^{1,2}, B.P. Levi¹, M.A. Krasnow^{1,2}. 1) Dept Biochem, Stanford Univ, Stanford, CA; 2) HHMI.

The *Drosophila* tracheal (respiratory) system consists of a series of branched and interconnected tubes. The smallest tubes in the tracheal system are called tracheoles and are generated by a mechanism involving the production of cytoplasmic extensions from tracheal terminal cells. Cytoplasmic extensions bud from the terminal cell soma, or from a pre-existing terminal cell branch, and subsequently make a lumen, perhaps through a process of vesicle fusion, thus forming a subcellular tube. We have carried an EMS mutagenesis screen of the 3rd chromosome out to saturation and have identified a number of mutations that disrupt various aspects of tracheole formation or function. A number of the defects observed have not been described in the published tracheal literature. Progress on the characterization of several complementation groups will be presented.

948C

***Drosophila* SPARC (dsparc), a basal lamina component, is required for embryonic development.** N. N. Martinek, M.J. Ringuette. Zoology, Univ Toronto, Toronto, ON, Canada.

SPARC (Secreted Protein, Acidic, Rich in Cysteine)/osteonectin/BM40 is an ancient calcium-binding glycoprotein associated with basal laminae of all animal phyla. In *Drosophila*, *dsparc* is first detected during cellularization, and by stage 13, its expression is restricted to hemocytes and the resultant protein associates with basal laminae. Downregulation of *dsparc* by mutagenesis leads to neurogenic and basal laminae defects as well as embryonic lethality. In *dsparc* mutants, while the hemocytes expressed high levels of type IV collagen, it was not detected in basal laminae. In contrast, type IV collagen mutations lead to decreased *dsparc* expression by hemocytes. Interestingly, intense laminin immunostaining was detected in the disorganized basal laminae of *dsparc* mutants. The data suggest that the expression of *dsparc* and type IV collagen are coordinated. However, the early expression of *dsparc* raises the possibility that the structural integrity of the laminin-rich basal lamina, which forms before the expression of type IV collagen begins, may also be compromised.

NEUROGENETICS AND NEURAL DEVELOPMENT

949A

Specification of *Drosophila* pioneer motor neuron identity by a genetic pathway involving even-skipped, grain and *zfh-1*. A. Garces, S. Thor. Dept Neurobiology, Harvard Medical School, Boston, MA.

In each hemisegment of the developing ventral nerve cord (VNC), a set of ~40 motor neurons are generated. Based upon their muscle targets and axon fasciculation, these motor neurons can be subdivided into several distinct sub-classes. Studies on the even-skipped (*eve*) gene have shown that *eve* is necessary and sufficient for specifying one sub-class, the dorsally projecting (ISN) motor neurons. We find that *grain* (*grn*), a GATA transcription factor, is specifically expressed in all *eve* expressing ISN motor neurons (aCC, RP2 and U/CQ). In *grn* mutants, ISN motor axons are stalled leading to an almost complete lack of innervation of the dorsal target muscles. We furthermore find that the Zn-finger homeodomain transcription factor *Zfh-1* is expressed in the majority of developing motor neurons including the ISN sub-class. Misexpression of *Zfh-1* in the longitudinally projecting dMP2 neurons is sufficient to cause frequent lateral exit from the VNC, indicating that *Zfh-1* can dictate generic motor neuron identity. By analyzing expression of *Eve*, *Grn* and *Zfh-1* in their respective mutant backgrounds, we are able to place these three genes in an *eve*->*grn*->*Zfh-1* regulatory pathway within the aCC motor neuron. However, this pathway is not identical in all ISN motor neurons. In the RP2 motor neuron for instance, *grn* is not

critical for *Zfh-1* expression. We also find differences between the aCC and other ISN motor neurons with respect to the effect of Notch pathway signaling upon expression of *Eve*, *Grn* and *Zfh-1*. Since the aCC motor neuron is unique among the ISN sub-class and plays a critical pioneering role to establish the ISN nerve, we speculate that a unique program may underlie pioneer neuron specification.

950B

Drosophila Paxillin interacts with the Spectraplaklin Shot and is required for neuromuscular junction

formation. M. Mende¹, T. Böckers², H. Sabe⁴, A. Subramanian³, R. Ueda⁵, T. Volk³, R. Yagi⁴, A. Prokop¹. 1) Inst. Genetics, Univ. Mainz, Germany; 2) Dept. Anat. Cell Biol., Univ. Ulm, Germany; 3) Weizmann Inst., Dept. Mol. Genetics, Rehovot, Israel; 4) Dept. Mol. Biol., Osaka Biosci. Inst., Japan; 5) Genet. Networks Res. Group, Mitsubishi Kagaku Inst. Life Sci., Japan.

Functional neuronal networks require the quantitatively and qualitatively appropriate establishment of synapses between neurons and their target cells. An essential gene in this context is *Shot*, a cytoskeletal interacting factor of the Spectraplaklin family of proteins, which is required for the differentiation of presynaptic neuromuscular terminals and postsynaptic sidebranches of motoneurons in the CNS (Prokop, 1998, *J. Cell Biol.* 143: 1283ff.). We have expressed differently tagged domains of *Shot* (Subramanian, 2003, *Curr. Bio.* 13, 1086ff.) using the Gal4/Uas system and studied their localisation within *Drosophila* neurons. We find that specifically the N-terminus of *Shot* is targeted to synapses potentially interacting with proteins in that location. Based on these results we used N-terminal domains as baits in yeast-two-hybrid screens to uncover interaction partners and, through these, understand the function of *Shot* during synapse differentiation. One of the candidate genes obtained is the small LIM-domain protein Paxillin (DPxn). We have confirmed the interaction of DPxn with *Shot* via co-immunoprecipitation studies and demonstrated co-localisation with *Shot* in vivo at tendon cells and NMJs. In tendon cells, DPxn and *Shot* co-localise exclusively on the basal side, and this localisation of DPxn is affected in the absence of *Shot*. A function of DPxn at the NMJ is revealed upon removal of DPxn function via different genetic deletions or RNA interference. Both strategies consistently cause structural aberrations of motoneuronal terminals. Thus, we introduce DPxn as an interaction partner of *Shot* and as a new player of the genetic network underlying the formation of synaptic terminals in *Drosophila*.

951C

Investigations on the temporal regulation of *hunchback* within neuroblast lineages of *Drosophila*. U. Mettler, J. Urban. Institute of Genetics, Becherweg 32, University of Mainz, D-55099 Mainz, Germany.

The neuroblasts (NBs) within the *Drosophila* ventral nerve cord produce specific cell lineages in which unique cell types are generated in a fixed temporal sequence. This seems to be controlled by a set of genes encoding transcription factors, which are sequentially and transiently expressed within the NBs, and whose expression is inherited by the ganglion mother cell (GMC) born at that time. Thus, the timing of the expression of these genes is essential for correct NB lineage development. To study the mechanism of this dynamic regulation, we have focused on the *hunchback* (*hb*) gene which is involved in specifying the fate of early-born NB progeny (Isshiki et al., 2001; Novotny et al., 2002).

Our data suggest that *hb* regulation during NB-lineage development is a two-step process: firstly there is a mitotic signal most likely mediated by the orphan receptor *Seven Up*, leading to a principal switch-off of *hb* expression in the NB and its GMC sibling cell. In the GMC this switch-off seems to be counteracted by the homeodomain transcription factor *Prospero*, which is asymmetrically distributed into the GMC and leads to maintenance of *hb* expression in this cell. The severe general upregulation of *hb*-expressing cells in the ventral nerve cord of the *seven up* mutants, as well as the reduction of *hb*-expressing cells of the *pros*-mutant embryos, suggest that this is a principal antagonistic mechanism within the GMCs of multiple NB lineages. We are currently investigating whether other genes responsible for the temporal specification of NB progeny might also be regulated this way. In a parallel approach we are characterizing the regulatory region of *hb* to find those sequences which are responsible for the correct spatiotemporal expression within certain neuroblast lineages. This work is supported by the Deutsche Forschungsgemeinschaft (UR42/3-4).

952A

Analysis of *zfh-1* and *zfh-2* function in the developing CNS of *Drosophila melanogaster*. G. Vogler, J. Urban. Dept Genetics, Univ Mainz, Mainz, Germany.

Zfh-1 and *zfh-2* belong to a family of transcription factors that possess both zinc-fingers and homeodomains, and which are conserved among different species. It has been shown that the vertebrate homologues of *Zfh-1* and -2 (*ZEB* and *ATBF1*, respectively) can act as transcriptional repressors. However, the role of these genes during CNS development is not well understood. We analyzed the expression pattern of *Zfh-1* and *Zfh-2* in the embryonic CNS

of *Drosophila* with regard to the temporal expression within neuroblast lineages and their distribution in glia cells. We observed that both genes are expressed in a large number of post-mitotic neurons, but with respect to lineage development in a very small temporal overlap: *zfh-1* expression is confined to early born neurons while later-born cells express *zfh-2*. Additionally we found that all glia cells express at least one of the *zfh* genes: *zfh-1* in peripheral and subperineurial glia, *zfh-2* in subperineurial, cell body and interface glia. In addition, we could show that *zfh-2* is alternatively spliced giving rise to at least six isoforms with different compositions of zinc-fingers and alterations in the number of homeodomains. Using a cell-specific cDNA microarray approach with loss-of-function and gain-of-function *zfh-1* and *-2* we want to identify target genes to examine these qualitative differences between the two genes more closely.

953B

Combinational expression of three transcriptional factors is essential for the PROS expression in the longitudinal glia. Y. Yuasa¹, Y. Hiromi^{1,2}. 1) Dept Developmental Genetics, National Inst Genetics, Mishima, Japan; 2) CREST.

During organogenesis cells in the morphogenetic field integrates multiple developmental signals, leading to the genetic specification of a subset of cells and activation of their cell type-specific differentiation program. For example, among approximately ten longitudinal glial cells in each hemisegment, six cells activate expression of a homeodomain transcription factor Prospero (PROS), which directs glial differentiation to support axonal growth. Likewise in the developing compound eye, five cells are selected in each ommatidial unit to express PROS. Analysis of the regulatory mechanism of *pros* expression in the eye revealed that an enhancer element integrates two signals: Ras/MAPK signal culminating in the activation of ets transcription factor Pointed (PNT), and transcription factor Lozenge that provides a prepattern (Xu et al., 2000). We found that the same enhancer element that controls expression of *pros* in the eye also directs *pros* expression in the subset of longitudinal glial cells. To understand the regulatory mechanism of *pros* expression we analyzed trans-acting factors that are necessary for *pros* expression in glial cells. As in the eye, *pros* expression in longitudinal glia was dependent on PNT. Lozenge is not expressed in the glial cells and thus unlikely to participate in *pros* regulation. Instead, glial expression of PROS was greatly reduced in mutants for genes encoding two transcription factors: homeodomain protein REPO and an AT-rich interaction domain protein Dead Ringer/Retained (DRI). All three factors express longitudinal glia. Co-expression of PNT, REPO and DRI activated both the endogenous *pros* gene and the *pros* enhancer in a foreign environment, the embryonic epidermis. We conclude that this *pros* enhancer element integrates multiple inputs in two contexts, to direct *pros* expression through synergistic action of multiple transcription factors.

954C

Slit, Robo and Robo3 control optic lobe morphogenesis. P. A. Garrity, T. D. Tayler, M. Robichaux. Dept Biol, MIT, Cambridge, MA.

The visual system contains a series of discrete but highly interconnected optic ganglia. The positioning of neuronal cell bodies and the pattern of axonal projections within the optic lobes are highly stereotyped. The photoreceptors of the adult eye project to targets in the outermost optic ganglia: the lamina and medulla. In a genetic screen for regulators of optic lobe development, we identified a loss-of-function mutation in *slit*, which encodes an extracellular guidance cue. Slit appears to be expressed by cells at the periphery of the medulla neuropil, and Slit protein is concentrated within the medulla neuropil. The loss of slit function strongly disrupts neuronal connectivity in the optic lobes. Two known receptors for Slit, Robo and Robo3, are also expressed within the developing medulla. The simultaneous reduction of Robo and Robo3 expression in optic lobe neurons through transgenic RNA interference causes optic lobe defects resembling those observed when Slit expression is reduced. In particular, cells lacking Robo and Robo3 inappropriately cross the region that expresses Slit and invade the developing lamina. This invasion of the lamina is accompanied by the mispositioning of lamina glia and the disruption of photoreceptor axon innervation. Taken together these data suggest that Slit and its receptors, Robo and Robo3, act to prevent inappropriate cell movements in the developing visual system.

955A

Regulation of Notch signaling during proliferation and differentiation of postembryonic neuroblasts. M. Toriya^{1,2,3}, K. Nakao^{1,3}, H. Okano^{1,3}. 1) Department of Physiology, Keio University School of Medicine; 2) Department of Cell Biology of Neuroscience, Osaka University School of Medicine; 3) CREST.JST.

The postembryonic neuroblasts (pNBs) in outer proliferation center proliferate by symmetric division until the early third larval stage. Then asymmetric division of pNBs starts by the late third larval stage, by which a pNB divides into a neural progenitor (GMC) which produces neurons/glia as well as a pNB by asymmetric division. We found that both Notch and its adaptor protein, Numb, are highly expressed in pNBs from the early larval stage. On the other

hand, α -Adaptin, a subunit of AP2 complex(AP2-C), is expressed only after the late larval stage. Thus, coexpression of Numb and α -Adaptin correlates with the timing of differentiation of pNBs. Loss of function(LOF) mutation of *Notch* inhibits proliferation of pNBs, demonstrating that *Notch* is required for pNBs to proliferate. In *numb* and *α -adaptin* LOF mutants, overproliferation of pNBs were observed instead of their differentiation at the late larval stage. Furthermore, we found that, in *α -adaptin* mutants, crescents of Miranda at the basal cortex of pNBs often extend to the apical side at metaphase and that Miranda is distributed into large daughter cells instead of small daughter cells at telophase. The Miranda-minus small daughter cells divide more than twice. These observations indicate that Notch and α -adaptin may regulate localization of proteins to be divided asymmetrically such as Miranda. We have also shown that Notch, Numb and α -Adaptin form a protein complex at the late larval stage, and that the level of Notch is much higher in *α -adaptin* mutants, suggesting that Numb and α -adaptin recruit Notch into AP2-C where to be degraded by the endocytosis. In summary, Notch is required for pNBs to continue proliferating and the interaction of Numb and α -Adaptin at the late larval stage inhibits the Notch function to initiate differentiation, presumably through endocytotic degradation.

956B

Condensation of the central nervous system in embryonic *Drosophila* depends on hemocyte-deposited extracellular matrix and neural activity. B. M. Olofsson, D. T. Page. Dept Cell Biol, MRC-LMB, Cambridge, United Kingdom.

Condensation is a process whereby a tissue undergoes a coordinated decrease in size and increase in cellular density during development. Although it occurs in many developmental contexts, the mechanisms underlying this process are largely unknown. Here we investigate how condensation happens in the embryonic *Drosophila* ventral nerve cord (VNC). Two major events coincide with condensation during embryogenesis: the deposition of extracellular matrix by the hemocytes, and the onset of central nervous system activity. Preventing hemocyte migration by removing the function of Pvr or by disrupting Rac1 function in hemocytes inhibits VNC condensation. In the absence of hemocyte migration extracellular matrix components are not deposited around the VNC. Blocking neural activity by targeted expression of tetanus toxin light chain also inhibits condensation. We also find that interfering with Rac1 function in either glia or neurons causes a similar phenotype. Our data suggest that condensation of the VNC during *Drosophila* embryogenesis depends on both hemocyte-deposited extracellular matrix and neural activity, and allows us to propose a mechanism whereby these processes work together to shape the developing nervous system.

NEURAL PHYSIOLOGY AND BEHAVIOR

957C

Carbon dioxide, high-frequency light pulses, and extreme temperature as triggers of seizure and paralysis in the bang-sensitive paralytic mutants. R. Burns, C. Boyek, D. Kuebler. Department of Biology, Franciscan University of Steubenville, Steubenville, OH.

Many different insults, including physical trauma, temperature, light, electrical shock and drugs, can trigger seizures in humans. In *Drosophila*, it is known that electrical shock and physical trauma can trigger seizure activity in the bang-sensitive (BS) paralytic mutants. This seizure activity is characterized by violent uncoordinated contractions of the legs, wings and abdomen that cause the flies to spin rapidly. This seizure activity is followed by a period of paralysis, which is interspersed with bouts of subsequent seizure activity. A similar, albeit less robust response can be induced in wild-type flies following very high voltage shocks indicating that this pattern may be a general response to nervous system shock. To better understand the mechanisms by which this curious response can be triggered, we examined the ability of other insults to cause seizures and paralysis in the BS mutants. Exposure of the BS mutants *easily shocked (eas)*, *bang senseless (bss)* and *slamdance (sda)* to anaesthetizing concentrations of CO₂ triggered seizure activity immediately before paralysis occurred. The subsequent paralysis lasted from one to five minutes depending upon the mutant. During recovery from paralysis, the mutants exhibited seizure activity that was nearly identical to that induced by mechanical trauma. A similar response was seen in the BS mutants following both 30-sec exposures to a high-frequency strobe light and paralyzing cold temperatures. The kinetics of the response depended upon the type and magnitude of the stimulus. Surprisingly, paralyzing high temperatures failed to induce seizure activity either before paralysis or following recovery in any of the fly strains tested. In the case of high temperature, seizure activity was replaced by hyperactivity, which is characterized by moderate leg and wing shaking.

958A

Characterizing the alternative splice variants of SLOB in *Drosophila* heads. A. M. Jaramillo, H. Fei, T. Weiger, I. B. Levitan. Department of Neuroscience, University of Pennsylvania School of Medicine, Philadelphia, PA.

Release 3.1 of the *Drosophila* Genome predicts an additional *slob* transcript that we have named *giant slob*. RT PCR reveals the presence of two *slob* and two *giant slob* transcripts in *Drosophila* heads. *In situ* hybridization on head sections shows that both *slob* and *giant slob* transcripts are in the photoreceptors, optic lobes and brain cortex. Microarray analyses demonstrated the circadian cycling of *slob*. We show by quantitative PCR that both *slob* and *giant slob* transcripts cycle in fly heads. These transcripts are predicted to encode a 52 kDa SLOB, 58 kDa SLOB, 66 kDa GIANT SLOB and 72 kDa GIANT SLOB. Immunohistochemistry on *Drosophila* heads reveals GIANT SLOB to be enriched in the photoreceptors, optic lobes and the pars intercerebralis neurons. Using a heterologous expression system we show that all SLOBs bind to dSlo and 14-3-3, and that these four SLOBs modulate the channel differently.

959B

The male-specific pheromone receptor GR68A is required for normal *Drosophila* courtship. S. Bray, H. Amrein. Duke University, Durham, NC.

Propagation in higher animals requires efficient and accurate display of innate mating behaviors. In *Drosophila melanogaster*, male courtship consists of a stereotypic sequence of six behaviors involving multiple sensory modalities, such as vision, audition and chemosensation. For example, taste bristles located in the male forelegs and the labial palps are thought to recognize female-specific, non-volatile pheromones. Here, we report the identification of a gustatory receptor gene, Gr68a, expressed in about twenty male-specific gustatory receptor neurons of the forelegs. Gr68a expression is dependent on the sex determination gene doublesex, which controls many aspects of sexual differentiation and is necessary for normal courtship behavior. Tetanus toxin-mediated inactivation of Gr68a-expressing neurons, but not other gustatory receptor neurons, leads to a significant reduction in male courtship performance. Moreover, transgene-mediated RNA interference of Gr68a RNA causes a similar phenotype, suggesting that GR68a protein itself is an essential component of normal mating behavior in *Drosophila*. To further delineate the function of GR68a in male courtship, we quantified each courtship step in males with inactivated Gr68a-expressing neurons and observed that a block in progression beyond the tapping step is the cause of the courtship deficit. Thus, our data suggest that GR68a mediates crucial chemosensory input of pheromones secreted by cells in the females abdomen through neurons of the male forelegs, an event that is essential for efficient progression to the later steps in the male courtship behavior. To our knowledge, GR68a is the first pheromone receptor identified in any animal with a specific role in courtship and mating.

960C

***Drosophila* doubletime mutations which either shorten or lengthen the period of circadian rhythms decrease the protein kinase activity of casein kinase I.** F. Preuss¹, J. Fan¹, E. Bjes¹, S. Bao², M. Kalive², E. Schuenemann¹, M. Muskus¹, J. Price¹. 1) School of Biological Sciences, UMKC, Kansas City, MO; 2) Dept. of Biology, West Virginia University, Morgantown, WV 26506.

In both mammals and fruit flies, casein kinase I (DBT) is necessary for circadian rhythms and regulates the phosphorylation of the clock protein *period* (PER). Our lab has examined the effects of both short-period (*dbtS*) and long-period (*dbtL*) mutations on DBT function. The results suggest that DBT can regulate the time course of PERs nuclear localization or stability. DBT is expressed at constant levels throughout the day, but its subcellular localization changes. GST pull down assays showed that wild type DBT, DBTS and DBTL proteins can bind to PER equivalently, and that these interactions are mediated by the evolutionarily conserved N terminal part of DBT. However, both the *dbtS* and *dbtL* mutations reduce the CKI-7-sensitive protein kinase activity of an orthologous *Xenopus* casein kinase I δ as well as of DBT immunoprecipitated from transfected Schneider 2 cell cultures. This data demonstrates that lowered enzyme activity can be associated with both short-period and long-period phenotypes, thereby raising the possibility that the period changes are produced by some other function of DBT besides its kinase activity. However, experiments with a DBT dominant negative showed that kinase activity is essential for mediating degradation of PER in S2 cell cultures. We are currently investigating the hypothesis that the mutations differentially affect the phosphorylation of specific sites in PER and thereby activate or inactivate selected features of PERs circadian program. The results will be discussed in terms of the role of phosphorylation in the generation of the circadian oscillatory mechanism and the modulation of circadian period.

961A

A role for EBONY-containing glia in the circadian regulation of locomotor activity. J. Suh, F.R. Jackson. Department of Neuroscience, Tufts University, Boston, MA.

ebony mutants exhibit morphological and behavioral phenotypes including abnormally dark body color and defects in the circadian regulation of locomotor activity. *ebony* individuals have altered circadian activity rhythms, but exhibit normal eclosion rhythms, suggesting that *ebony* functions in the clock output pathway regulating locomotor activity. The *ebony* gene encodes N- β -alanyl-biogenic amine synthetase (BAS), which conjugates β -alanine with various kinds of biogenic amines. Consistent with a deficit in BAS activity, *ebony* mutants have elevated levels of β -alanine and dopamine in the central nervous system (CNS). We have examined expression patterns of *ebony* in the CNS to gain insight into how this gene contributes to circadian regulation. We confirm that *ebony* RNA shows circadian changes in abundance peaking near the beginning of the day, a time that correlates with the initiation of locomotor activity. We also show that EBONY protein abundance is higher during the day in brains, indicative of a circadian regulation of BAS activity in the CNS. We have performed immunohistochemical studies to identify EBONY-containing cells in the brain and to determine the relationship of those cells to aminergic neurons. Surprisingly, EBONY protein was localized in a subpopulation of CNS glial cells in larvae and adults. Interestingly, these EBONY-containing glia were found to be in close proximity to dopaminergic and serotonergic neurons of the brains, suggesting that they might modulate aminergic functions. Consistent with a role for EBONY-containing glia in the circadian modulation of activity, flies with defective glial cell development show altered locomotor activity rhythms. Furthermore, the overexpression of EBONY in glial cells perturbs rhythmicity. These results support the hypothesis that biogenic amines have a role in the circadian regulation of locomotor activity, and suggest the novel idea that glial cells participate in the orchestration of rhythmic behavior.

962B

Feminization of cholinergic neurons enhances aggressive behavior in male *Drosophila melanogaster*.

Y. Chan, S. Certel, E. Kravitz. Department of Neurobiology, Harvard Medical School, Boston, MA.

Our previous studies have provided a foundation for the quantitative analysis of aggressive behavior in male Canton-S *Drosophila melanogaster* (PNAS 99:5664, 2002). In more recent studies (see Nilsen, Chan and Kravitz, this meeting) we have performed a similar analysis of female fighting behavior. In extending these studies, we now are examining the effects of expressing gender-related gene products on levels and patterns of aggression in flies. Ectopic expression of the *transformer* (*tra*) gene in cholinergic neurons of male flies using the GAL4 system significantly enhances dominant, aggressive behaviors like lunging and boxing. Using various immunocytochemical procedures we are searching for anatomical and/or molecular changes that might account for the behavioral change. Preliminary results show no gross differences in cholinergic cell number or in axon pathfinding in Cha-GAL4/UAS-*tra* flies. Feminization of part of the central nervous system in *Drosophila* using ectopic expression of *tra* has been reported to affect courtship behavior (Science 267:902, 1995). To our knowledge, this is the first time that *tra* expression has been shown to alter aggressive behavior (supported by NIGMS).

963C

***Drosophila melanogaster* as an animal disease-model for Familial Amyloidotic Polyneuropathy.** M.

Pokrzywa¹, I. Dacklin², M. Oliveberg³, D. Hultmark², E. Lundgren¹. 1) Dept. of Molecular Biology, Umea University, Umea, Sweden; 2) Umea Center for Molecular Pathogenesis, Umea University, Umea, Sweden; 3) Dept. of Chemistry, Umea University, Umea, Sweden.

Familial Amyloidotic Polyneuropathy (FAP) is a neurodegenerative disease caused by aggregation of the human plasma protein transthyretin (TTR) in the form of amyloid fibrils mainly around peripheral nerves, but also kidney and heart can be affected. FAP has dominant inheritance, but with low penetrance and late age of onset, suggesting other pathogenic factors besides mutated TTR.

We have designed several highly amyloidogenic mutants of TTR, which are toxic to cultured human neuroblastoma cells, however the mechanism underlying transthyretin-associated neurotoxicity remains still unknown. Efforts to create an animal model for FAP in mice have met limited success. We have therefore turned to creation of transgenic strains of *Drosophila melanogaster* in order to study the disease process and to develop a test system for drug candidates.

We have generated transgenic strains with wild type and four mutants of TTR. Expression has been achieved in the eyes and in the central nervous system by the use of tissue specific drivers GMR-GAL4 and *elav*^{C155}-GAL in adult flies, respectively. Analysis of the expression levels by immunoblot of lysates demonstrates that wild type TTR

occurs in a soluble fraction, while all TTR mutants aggregate. Studies of the behaviour of flies by climbing assay show an interesting phenotype, which at present is analyzed with respect to movement, coordination and perception.

Thus, we have created transgenic flies that express aggregating mutant TTR and can serve to study further FAP development.

964A

The *beethoven-reduced ocelli* genetic interval (polytene region 36D). J. C. Caldwell, Y. Sharma, J. S. Jacobs, D. F. Eberl. Dept Biological Sci, Univ Iowa, Iowa City, IA.

We are characterizing the molecular and genetic organization of polytene region 36D. Our initial focus in region 36D was the *beethoven* (*btv*) mutation, isolated in an EMS mutagenesis screen for auditory behavior mutants. Overlapping deficiencies in this region also uncover a male sterility phenotype and reduced ocelli (*rdo*). Predicted genes in this interval include Neural-Cadherin2 (N-Cad2), which is 75% identical to the neighboring N-Cad gene, Dynein Heavy Chain 36D (DHC36D), which is the cytoplasmic dynein 1b isoform, an RNI-like protein called *Pray for Elves* (*PFE*) and several small ORFs with no obvious motifs.

P-induced male recombination maps *btv* to DHC36D and CG5674, whose ORFs overlap on complementary strands. We have identified a small deletion in the *btv*^{5P1} EMS allele that removes part of DHC36D but leaves CG5674 intact.

P-induced male recombination and excision of the KG02815, KG03741 and KG05889 insertions suggest that *PFE* underlies the *rdo* defect.

CG5674 and CG15150 are good candidates for the male sterile phenotype as ESTs were isolated from testes libraries. We are currently testing whether either or both of these genes are necessary for male fertility.

Supported by NIH DC04848 to DFE.

EVOLUTION AND QUANTITATIVE GENETICS

965B

Nuclear-mitochondrial coadaptation: Functional consequences of mitochondrial genome introgression from *Drosophila simulans* into *D. melanogaster*. B. Bryan, R. Wagaman, E. Goldstein, T. Sackton, L. Sheldahl, D. Rand. Ecology and Evolutionary Biology, Brown University, Providence, RI.

The functional genome of the mitochondria includes a substantial fraction of the nuclear genome. In yeast, about 12% of the nuclear-encoded proteins are related to mitochondrial function. Most of these genes were originally encoded in the mitochondrial genome, but have been transferred to the nucleus during evolution. These interacting genes should become co-adapted through a history of compensatory mutations. We test the coadaptation hypothesis using *Drosophila* strains carrying divergent mtDNAs. Transfer of mtDNA from *D. simulans* to *D. melanogaster* has been achieved with the female sterility rescue line C167.4. We show that a number of fitness traits are disrupted in these mito-nuclear transplant lines. Activity of cytochrome c oxidase (COX) a joint nuclear-mitochondrial enzyme - is reduced in strains of *D. melanogaster* carrying foreign mtDNAs. This disruption is more pronounced in males, a result predicted from weaker selection on male mitochondrial function due to maternal inheritance of mtDNA. Resting oxygen consumption is elevated in *D. melanogaster* flies carrying *D. simulans* mtDNA, indicating reduced respiratory efficiency from disrupted oxidative phosphorylation. Population cage competition experiments show selection against *D. simulans* mtDNAs in a *D. melanogaster* background, but the selection varies in different nuclear strains. Male fertility is reduced in flies carrying *D. simulans* mtDNA in an OregonR background. These mito-nuclear transfer strains provide an effective means of dissection the complex functional genomics of nuclear-mitochondrial coevolution.

966C

Population Genetics in the American Tropics. Comparative Genetic Structures of *Drosophila pseudoobscura* from Arizona, U.S.A. and from Colombia. D. A. Montano^{1,2}, G. Forero¹, H. Correa¹.

1) Universidad Nacional Abierta, Bogota, Colombia; 2) Universidad Antonio Narino, Bogota Colombia South America.

The classic group of *Drosophila* species *D. Pseudoobscura*, *D. P. Bogotana* constitute an interesting study case for such a multilocus genetic approach. Because these species have played a central role in the development of evolutionary theory and in speciation studies (Dobzhansky 1937), they are particularly appropriate for in depth genetic analysis. This study was conducted using multiple isoenzymatic genes across two different populations. The Population of Bear Canyon showed high variation comparative with Bogota population. It was depaupered with very few alleles and with overall 3.58 percent; of the loci assayed were variable, with an average heterozygosity

among loci and samples equal to 0.23. The effective size of alleles at Bear Canyon was 1.508. The mean polymorphism at Bear Canyon was 53.85 percent; and mean heterozygosity about of 24 percent; and mean observed heterozygosity about of 18 percent. Here, we report results of structure genetic based on isozymes. This analysis provided strong support for the differences genetic of Bear Canyon with Bogota. The data were tested for genetic subdivision among populations using two methods. First, geographic heterogeneity of frequency distribution was estimated through a Monte carlo simulation as described by Roff and Bentzen, (1989); second, analysis was to analyzed the genetic structure within and among populations using variance component estimates in a hierarchical analysis. Two hierarchical levels were recognized, within populations and among populations. This results showed that population subdivision exist, a significant proportion of the variance component would be due to differences among populations.

967A

Variations and nonrandom associations at *Drosophila* chemoreceptor genes. T. Takano-Shimizu^{1,2}, A. Kawabe¹, N. Inomata³, N. Nanba⁴, R. Kondo⁵, Y. Inoue⁶, M. Itoh⁴. 1) Population Genetics, National Institute of Genetics, Mishima, Shizuoka, JAPAN; 2) School of Advanced Sciences, The Graduate University for Advanced Studies, Hayama, Kanagawa, JAPAN; 3) Dept of Biology, Kyushu University, Fukuoka, JAPAN; 4) Dept of Applied Biology, Kyoto Institute of Technology, Kyoto, JAPAN; 5) Dept of Biology, Ochanomizu University, Tokyo, JAPAN; 6) Dept of International Studies, Osaka University of Foreign Studies, Osaka, JAPAN.

Multilocus selection such as truncation selection can effectively reduce mutation load. Many quantitative characters including complex genetic diseases are likely to be under this type of selection, but direct measure of selection in natural populations remains to be done. Multilocus selection with epistasis, besides genetic drift and gene flow, can generate linkage disequilibrium, from which we could infer the pattern and degree of selection. Many previous allozyme-polymorphism analyses of *Drosophila* have failed to detect linkage disequilibrium, but this does not imply a complete lack of linkage disequilibrium at whole genome level. Indeed, previous *Drosophila* studies have demonstrated the existence of recombination load, meaning that some natural variants are not randomly combined in adult individuals, but in linkage disequilibria because of advantageous and disadvantageous combinations of natural variants. We undertook an analysis of linkage disequilibria between polymorphisms at *Drosophila* chemoreceptor genes, finding many significant non-random associations and a significant excess of haplotypes composed of one frequent and one rarer allele in replacement polymorphisms. These results suggest significance of multilocus selection in shaping the within-species variation. We could, in turn, make functional connections of genes based on the linkage disequilibria generated in natural populations.

968B

Evolution of gene expression in Diptera. Distinct features of patterning gene expression in *Anopheles gambiae* early embryo. Y. Goltsev¹, B. Peterson¹, W. Hsiong², G. Lanzarro², M. Levine¹. 1) MCB, UC Berkeley; 2) Entomology department, UC Davis.

There is a general belief that evolution of cis-regulatory DNAs drives changes in animal morphology. Yet this has not been tested rigorously. We have compared the expression profiles of critical embryonic determinants between the mosquito *Anopheles gambiae* and the fruitfly *Drosophila melanogaster*, two relatively close species which belong to the large order Diptera namely to its sister subgroups: sub-order Nematocera and sub-order Brachycera.

The majority of orthologue gene pairs that were in the scope of our analysis manifest similar expression patterns, which correlates with evolutionary proximity of these two animals. Yet we found several significant changes in the profiles of 'core' patterning genes including altered patterns of A.g.Hox-3 (zen), A.g.Hunchback, and A.g.Sog.

Our further analysis of cis-regulatory regions for A.gambiae genes in transgenic D.melanogaster showed that changes in gene expression patterns result from changes in these non-coding DNAs. Moreover in the case of A.g.sog a distinct structure of the cis-regulatory region seems to direct the establishment of the particular morphology of extraembryonic ectoderm which differs in many aspects from amnioserosa of the fruitfly.

Finally in the course of our studies we developed experimental procedures which make it possible to analyze gene expression in *Anopheles gambiae* embryos by whole mount RNA in situ hybridization. This procedure was largely inapplicable before due to distinct features of Nematoceran egg structure namely non-transparent and impermeable endochorions.

969C

An Evo-Devo approach leading to a structural-functional model of Troponin genes in insects. R. Herranz, J. Mateos, J.A. Mas, E. Garcia-Zaragoza, M. Cervera, R. Marco. Bioquímica, Fac. Medicina (UAM), Madrid, Madrid, SPAIN.

The Troponin complex in *D. melanogaster* is built by three subunits, Troponin C, the calcium sensor component, and Troponin T and I, more structural proteins. While TpnC is encoded by multiple genes in insects (see accompanying poster), the TpnT and TpnI proteins are encoded by single genes. Developmental and muscle type variability is obtained in them by alternative splicing. Their isoform expression pattern is essentially conserved in two additional Drosophila species, *D. subobscura* and *D. virilis*. Single orthologous genes have been identified in *Drosophila pseudoobscura*, *Anopheles gambiae* and *Apis mellifera* sequenced genomes. This evolutionary approach has allowed the location of a newly described exon in the 3-half of the Troponin T coding region, differentially expressed in *Drosophila* indirect flight muscle. Equivalent differential isoform variable regions are found in the Troponin I gene sequences. All Tpn isoforms (T, I and C) have co-evolved, building a species and muscle specific Troponin complex. The most intriguing variations among these insects correspond to the IFM specific isoforms including the presence of Proline/Alanine rich sequences. In Drosophilidae TpnI isoform, PAANGKA repetitions appear in exon 3. Drosophilidae TmII gene produces two TmH isoforms with APPAEGA repetitions in exons 14 and 15, while *Anopheles* only contains one isoform with such an exon. *Apis* TpnI lacks exon 3 and the APPAEGA sequence is found at the TpnI carboxyterminus. We are currently completing the identification of the different isoform sequences and their expression patterns in *Apis* musculature. It is quite likely that all these sequences play a role in the thin/thick filament interaction involved in the stretch activation phenomenon leading to the IFM asynchronous contraction. All these findings in combination with the Troponin C gene data in insects allows us to propose a general scheme for the Troponin complex evolution and its subfunctionalisation in insects.

970A

Diversification and independent evolution of TpnC genes in insects. R. Herranz¹, J. Mateos¹, C. Diaz¹, T. P. Nguyen², T. L. Lovato², R. M. Cripps², R. Marco¹. 1) Bioquímica, Fac. Medicina (UAM), Madrid, Madrid, SPAIN; 2) Dep Biology, U. of New Mexico, Albuquerque, NM 87131-1091, USA.

The success of the genomic sequencing programs in insects allows the discovery of additional family members of genes encoding known functions, i.e. the Troponin C (TpnC). We have found two new TpnC genes in *Drosophila melanogaster*, DmTpnCIIIb (41F) and DmTpnCII (25D), increasing to five the number of Troponin C genes identified in this species. DmTpnCIIIb has a more restricted tissue specificity than the rest of the TpnC genes and, with the chromosomally linked DmTpnCIIIa (41C), is expressed specifically in the adult thorax. DmTpnCII is expressed during development more broadly than the rest. Finally, DmTpnCIa (73F) and DmTpnCIb (47D) show a high embryonic/larval expression and in adults are expressed almost exclusively in the abdomens. These characteristics are conserved in other drosophilids including their developmental pattern of expression. Insect TpnCs can be divided in three groups based on primary sequence properties, allowing a systematic classification of newly identified genes. Similar sets can be identified in *Anopheles gambiae* (one Type I gene, one Type II gene and four Type III genes clustered in a 32 kb chromosome region) and *Apis mellifera* (one Type I gene, two Type II related genes and two Type III genes tandemly organized in 5 kb). The pattern of expression of the *A. mellifera* genes essentially agree with the pattern in Drosophilidae, providing further functional support to the classification. A model for the evolution of the TpnC genes is proposed, suggesting that the rapid increase in number and sequence specialization of the adult Type III isoforms could play an important role in the acquisition of asynchronous indirect flight function in insects. The hymenoptera TpnCs data indicates that this specialization was evolutionarily achieved independently in different insect orders by both convergent and divergent processes.

971B

Reproductive fitness related variations among Indian Drosophila species. S. Rajpurohit, P. Tyagi, A. Bhardwaj, R. Parkash. Department of Biosciences, MD Universty, Rohtak, Haryana, India.

Mating propensity and fertility were studied in four *Drosophila* species populations collected from different geographical sites of Indian subcontinent. The result of mating propensity and fertility revealed statistically significant variations among different populations with respect to latitude, body weight, fertility and mating speed. Northern populations showed greater mating activity and fertility as compared to that of southern tropical populations. Thus, there is a positive correlation between mating activity and fertility and also with latitude in all the four *Drosophila* species. The data suggest that the males are more subjected to intra sexual selection. Several populations of the all four species at the usual temperature at 25 C. However an analyses was extended six populations of *Drosophila melanogaster* across full thermal range for mating propensity as well as mating speed. A negative

correlation was observed mating propensity and mating speed in the *Drosophila melanogaster*. Body size variation are correlated with mating propensity in all the species. The latitudinal slope values for mating propensity do not differ significantly for different species while offspring production (fertility) has evidenced significant divergence between species.

972C

Naturally segregating QTL affecting wing shape of *Drosophila melanogaster*. J. G. Mezey. Center for Population Biology, University of California, Davis, Davis, CA, 95616.

Variation in vein placement and wing shape of *Drosophila melanogaster* depends on many genes dispersed throughout the genome. To identify promising regions where these genes may be located, I perform a QTL analysis of wing shape, surveying natural variation in *D. melanogaster*. The analysis confirms the picture that many loci have the potential to affect vein placement and also demonstrates that the number of QTL segregating for these phenotypes in natural populations is likely to be very large. This implies that: (1) the mutation target for wing shape variation that does not affect functionality of the wing is large and (2) responses to directional selection on vein placement are likely to involve a large number of QTL. The analysis suggests a number of candidate loci responsible for natural variation in vein placement. Comparison among the locations of putative QTL identified to those of previous studies of wing shape indicates that each study has surveyed different QTL. A test of correspondence indicates that, despite this, there is more genomic clustering of QTL than expected at random on the third chromosome.

973A

Mitochondrial genotype interacts with insulin signaling and dietary restriction to determine *Drosophila* longevity. R. Wagaman¹, E. Goldstein², D. Rand^{1,2}. 1) Molecular and Cellular Biology, Brown University, Providence, RI; 2) Ecology and Evolutionary Biology, Brown University, Providence, RI.

There is a growing literature suggesting that mitochondrial decline is a major cause of aging in diverse organisms. Few studies have used direct manipulations of mitochondrial genotype to examine the genetic basis of mitochondrial aging, or other fitness traits. We have used introgression crosses to bring the mtDNA from *D. simulans* into the nuclear genetic background of *D. melanogaster*. These sim-mel flies should exhibit a disruption of coadapted nuclear-mitochondrial interactions and a variety of biochemical and fitness traits. Here we show that the mtDNA genotype interacts with different wild type backgrounds, with the dosage of mutant chico alleles, and with dietary restriction to determine the longevity of flies. *D. melanogaster* flies carrying *D. simulans* mtDNA were crossed to different wild strains, and to the chico1 mutant. Different nuclear x mtDNA combinations show either longevity extension or reduction, depending on the combination of nuclear and mitochondrial genotypes. The *D. simulans* mtDNA haplotype interacts with the chico1 mutation in a dose-dependent manner. The longevity extension effects of chico1 are significantly reduced by the *D. simulans* mtDNA, and this effect is about half as strong in chico1/+ heterozygotes as in chico1/chico1 homozygotes. MtDNA genotype also interacts with diet to modulate longevity. Flies cultured on a 50% diet showed extended longevity, but flies carrying mtDNA from *D. simulans* displayed significantly smaller longevity extension than those carrying native *D. melanogaster* mtDNA. In a related set of experiments, flies carrying *D. simulans* mtDNA showed reduced survivorship under desiccation and starvation conditions. The results suggest that mtDNA genes interact with insulin and dietary signaling pathways to modulate aging and survival.

974B

Misregulation of the transcriptome in *Drosophila* species hybrids is mediated through the nucleolar protein Hmr. T. W. Herreman, K. P. White. Genetics, Yale University, New Haven, CT.

The mechanisms underlying genetic isolation between related species are not well understood. In hybrid crosses between *D. melanogaster* females and *D. simulans* males, the male offspring survive until the third larval instar but are under-developed and fail to pupariate or undergo metamorphosis. We analyzed the transcriptional activity of hybrid genomes in early larval males who carried one chromosomal complement from each parent species. We found that, when compared to sib-mated intraspecific controls, only a small fraction of the genome is misregulated in hybrids. We then used a mutation in *D. melanogaster* that rescues hybrid male lethality (*Hmr*). Surprisingly, although *Hmr* flies appear morphologically normal, they differ considerably in their genomic transcription profile from wild type. However, in hybrid males that carry a mutant *Hmr* allele, 60% of the small set of transcripts misregulated in non-*Hmr* hybrids are rescued to near wild type levels. Furthermore, we find that the Hmr protein localizes to the nucleolus, and this localization is disrupted in the rescued hybrid animals. This raises the possibility that Hmr acts to mediate chromosome-nucleolar interactions and that the primary basis for hybrid male lethality in these species involves nucleolar defects that are reflected on the level of transcript abundance.

IMMUNE SYSTEM AND CELL DEATH**975C**

***Enterococcus faecalis* gastrointestinal tract colonization of *Drosophila melanogaster* by commensal strains and strains of known virulence.** C.R. Cox¹, M.S. Gilmore². 1) Oklahoma Center for Neuroscience, University of Oklahoma Health Sciences Center, Stanton L. Young Biomedical Research Center, Oklahoma City, Oklahoma; 2) Department of Ophthalmology, University of Oklahoma Health Sciences Center, Dean A. McGee Eye Institute, Oklahoma City, Oklahoma.

Enterococci exist as commensals in the GI tract of a broad range of mammalian and insect hosts and are leading causes of nosocomial infection. Enterococci are the leading etiological agents of surgical site infections, the second leading cause of blood stream infections and the third leading cause of nosocomial urinary tract infections. With a view toward identifying host and bacterial factors that govern enterococcal GI colonization in a tractable system, we examined the natural colonization pattern of *Drosophila*. In a survey of laboratory stocks and wild-captured isolates we found *Drosophila* to be naturally colonized with several species of enterococci including *E. faecalis*, *E. faecium* and *E. durans*. All fly stocks were found to be colonized with 30 to 150 colony forming units per fly. *Drosophila* were cured of natural enterococcal colonization by growth on erythromycin-containing media, and subsequently colonized with isogenic strains of *E. faecalis* varying in expression of a cytolytic toxin. *Drosophila* were also colonized with a clinical isolate of *E. faecalis* associated with a hospital outbreak. Colonization patterns were characterized by fluorescent immunostaining of *Drosophila* thin sections, and enterococci were found to occur primarily in the crop, ventriculus, and posterior intestine. The effects of colonization on physiological parameters, including time to reproduction, brood size and body mass were also measured, although no statistical difference was noted for flies colonized with varying strains of *E. faecalis*. Natural carriage, together with a wide availability of *Drosophila* mutants, make the fly an ideal host for such studies.

TECHNIQUES AND GENOMICS**976A**

Microarray analysis of dTOR - Target of rapamycin in *Drosophila melanogaster*. S. Buch, J.D. Katzenberger, M. Bauer, I. Zinke, M. Bonaus, M.J. Pankratz. Forschungszentrum Karlsruhe, Institut fuer Genetik, Karlsruhe, Germany.

Studies in yeast revealed a mutation in two genes, TOR1 and TOR2, which conferred resistance to rapamycin, an antiproliferative drug. It has been shown that TOR is a growth regulator in dividing cells; it positively controls protein synthesis by modulating the activities of translational components like 4EBP and pS6K. Homologues of TOR exist in several organisms, among them mammals and *Drosophila*. Studies in yeast showed that TOR signalling pathway responds to nutrients and dTor mutant flies resemble flies that suffer amino acid deprivation. Another nutrient dependent pathway is insulin signalling pathway, which controls cell size and cell number. In contrast to this, TOR signalling pathway affects cell size, but not cell number. This implies that these two pathways together coordinate cell growth in response to the availability of nutrients and the presence of growth factors. We are especially interested in the analysis of these two pathways.

Drosophila gives us a great model organism to further investigate nutrient dependent gene regulation. Our focus is the investigation of dTOR mutants and the overexpression of dTOR by microarray analysis. This revealed that dTOR is involved in protein degradation, amino acid metabolism and tRNA synthesis. We found changes in glycine cleavage system, SAM Cycle, glutathione metabolism and in case of the overexpression induction of several heat shock proteins. Additionally, genes involved in sugar and fat metabolism were found to be differentially expressed. With this data we try to clarify the role of TOR signalling in *Drosophila*.

977B

Expression profile of *Anopheles gambiae* in response to infection by *Plasmodium falciparum*. J.R. Perusse¹, S.M. Kanzok², L. Zheng², K.P. White¹. 1) Department of Genetics, Yale University, New Haven, CT; 2) School of Epidemiology and Public Health, Yale University, New Haven, CT.

The recent sequencing of the *Anopheles gambiae* genome allows the development of a new set of tools to apply toward malaria research. Gene expression of mosquitoes challenged with the parasite has begun to be explored, but mostly using experimental systems that do not occur in nature. For example, expression profiling has been performed to explore the *Anopheles gambiae* response to rodent malaria (*Plasmodium berghei*). However, it has been shown that certain well-characterized genes involved in the immune response are expressed differently in *Anopheles gambiae* infected with *Plasmodium falciparum* or *P. berghei*. To determine the complete genomic response to the human malarial pathogen *P. falciparum*, we are using high density oligonucleotide microarrays to

identify genes expressed in *Anopheles gambiae* after initial establishment of infection. Future experiments will seek to understand the specificity of the immune response of the mosquito to its coevolved parasite by comparing its transcriptional response to that elicited by the mouse malarial parasite.

978C

Genome-wide screening at the Drosophila RNAi Screening Center. S.L. Armknecht, I.T. Flockhart, M. Booker, J. Murphy, S. Talala, N. Ramadan. DRSC, Dept. of Genetics, Harvard Medical School, Boston, MA.

The completion of the genome sequences of a number of organisms, including yeast, *C. elegans*, *Drosophila*, and humans has now given us the opportunity to look comprehensively at gene functions not only in specific organisms but also across species. Further, the evolutionary conservation between species generates important insights into the functional organization of cellular pathways. Thus, the most critical step following the completion of full genome sequences is to develop methodologies that allow the systematic and rapid analysis of gene functions generated by these large-scale projects.

To characterize gene functions on a genome-wide scale in *Drosophila*, we have generated, in collaboration with Dr. Renato Paro's group (Heidelberg, Germany), a library of double-stranded RNAs (dsRNAs) directed against all predicted open reading frames. This resource can now be used to conduct high-throughput cell-based RNAi screens to identify genes involved in various assays. Treatment of *Drosophila* cells in culture with dsRNAs readily leads to partial or complete elimination of the corresponding cellular protein. Using robotic technologies, this library of dsRNAs can be screened rapidly in specific cell-based assays. These screens provide a powerful methodology to identify all the proteins encoded by the genome that interfere with a specific assay.

In May 2003, the NIH granted funding (NIGMS RO1-GM067761, Norbert Perrimon) to make this technology available to the community. At this time, a centralized RNAi screening center, the Drosophila RNAi Screening Center (DRSC), was established at Harvard Medical School. Information regarding screening at the DRSC and the available resources will be presented.

979A

Generation of large site-directed terminal deficiencies on the fourth chromosome. R.S. Sousa-Neves, T. Lukacsovich, J.L. Marsh. Dev and Cell, UCI, Irvine, CA.92697.

The fourth chromosome has been one of the most inaccessible regions of the fly genome. However, it harbors a number of conserved genes whose function remain largely unknown. In order to improve the mapping capabilities of genes on the fourth chromosome and provide a way of correlating existent mutants to known transcripts, we generated a series of P element induced terminal deficiencies. The genetic and molecular characterization of these deficiencies show that they remove as much as 900kb of the euchromatic right arm of the fourth chromosome. Together they allow the subdivision of the region 102B2-102D6 in five discrete regions of known genetic composition.

980B

Compositional features of transcriptional code and discovery of cis-regulatory modules (CRMs) in the genome of Drosophila. D. Papatsenko, S. Small. Dept Biol, New York Univ, New York, NY.

Transcription regulatory signals in genomes of higher eukaryotes contain information responsible for correct spatio-temporal gene expression. The regulatory signals are found in discrete segments of the genomic DNA: proximal promoters and cis-regulatory modules (CRMs), located far from transcription start sites. Detection of transcriptional signals (CRMs) and interpretation of their function are among most important though challenging tasks of modern biology. Many of current computational recognition techniques available for the detection of *Drosophila* CRMs rely on presence of dense groups (regulatory clusters) of binding sites in the genome of *Drosophila*. However, the redundant nature of the genome and presence of large quantity of non-functional matches (and non-functional groups) for the binding motifs reduce performance of this popular technique. In order to improve CRM detection methods we incorporated new biological features into the clustering recognition models. We have shown that spacing and orientation of binding sites in the most known *Drosophila* CRMs follows specific rules, defining site composition in the functional CRMs. Based on the compositional information extracted from known enhancers (<http://homepages.nyu.edu/~dap5/PCL/appendix2.htm#crms>) we have generated a number of highly selective recognition models for developmental CRMs containing clusters of sites for Bicoid, Krppel and some other most robust developmental regulators. Genome-wide search using these models with consequent annotation of the obtained results has revealed novel developmental CRMs in a number of loci of known developmental genes (odd-skipped, sloppy-paired, drumstick, nubbin) as well as in many loci of computed genes. The annotated list of putative CRMs, obtained in these computational searches is available from our web resource: <http://homepages.nyu.edu/~dap5> (Direct targets of maternal and gap genes).

981C

Transposon-based innovative method for sequencing highly repetitive heterochromatic DNA in BAC/oriV clones. M. Mendez Lago^{1,2}, J. Wild¹, J.P. Abad², A. Martin-Gallardo³, A. Villasante², W. Szybalski¹. 1) McArdle Lab., University of Wisconsin, Madison, WI; 2) Centro Biología Molecular (CBMSO, CSIC-UAM), Madrid, Spain; 3) Servicio Interdepartamental de Investigación (SIIdI) UAM, Madrid, Spain.

The contiguous finished sequence from rigorously assembled contigs is needed to understand the role of heterochromatin regions in chromosome behavior. However, when using the current aligning approaches, based on sequence overlaps, it has been hardly possible to align the newly acquired repetitive heterochromatic sequences, because they are often nearly identical. Moreover, some present methods are laborious and not open to the extensive mapping technologies currently employed by sequencing centers. Here we present a novel transposon (Tn)-based strategy for sequencing heterochromatic BACs that contain *oriV*, which permits DNA amplification in *trfA* hosts. First, we modified Tn5/*oriV* by adding rare restriction sites, I-SceI and PI-SceI. Using this Tn, we constructed a library of BAC/*oriV* clones with two PI-SceI sites. One site was at a fixed position in the backbone of the BAC plasmid, while the other was in Tn, randomly inserted into the cloned heterochromatic DNA. In the next step, sequences (500-1000 nt) are to be collected, from the outward priming sites in Tn, from several hundreds of such clones. Finally, all Tns were precisely mapped using PFGE and appropriate hybridization probes from the BAC vector and transposon. Combined results of sequencing and determination of Tns precise positions and orientation will permit us to assemble the entire sequence of the heterochromatic clone, since the principle of our approach is the assembly of all the newly acquired sequences according to their physical positions instead of aligning by the conventional search for overlaps. This strategy, we hope, especially when automated by optical mapping with labeled indexers, will become crucial for heterochromatin sequencing.

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Nutrinomics: A combined genomic and proteomic approach to identify nutrition controlled components of metabolic pathways in Drosophila. S. Walther, I. Zinke, M.J. Pankratz. Forschungszentrum Karlsruhe, Inst Genetics, Karlsruhe, Germany.

In view of the rising obesity and nutrition dependent diseases in first world countries, the control of food and caloric intake becomes more and more an important issue of research. Single components are necessary for the survival of the whole organism and interact with several surrounding teammates in order to sustain homeostasis. Many proteins involved in these pathways are known for a long time now, but their regulation is still not well investigated. In microarray experiments with sugar fed larvae of *Drosophila* several genes were regulated. One of these genes showed an extreme upregulation upon sugar in comparison to normal fed larvae and was called sugarbabe (*sug*). *sug* is a transcription factor expressed mainly in the gut, the fat body and the malpighian tubules, suggesting that it may be involved in metabolic regulation. Putative target genes were identified using a heat shock GAL4-UAS system to overexpress *sug* in wild types and subsequent microarray analysis. Three out of the top five downregulated genes were lipases (CG8093, CG6271, CG6277), leading to the conclusion that *sug* represses lipases and shuts down fat breakdown. The binding abilities of *sug* to its target genes were determined by bandshifts with specific promoter regions of three lipases. Starting with overlapping 300 bp constructs and scaling them down to 30 bp for two of the three lipases (CG8093 and CG6277) a preliminary core consensus sequence (CAN/CTTTTAANG) that binds to *sug* in vitro could be predicted.

A proteomic approach uses the power of 2D Gels to investigate the protein-pool of *Drosophila* larvae under different feeding conditions. According to the microarray experiments we will first investigate the proteom response to sugar and starvation conditions. Proteins that show a high regulation or a posttranslational modification will be picked and analysed via MALDI-TOF.

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