

46th Annual Drosophila Research Conference

PROGRAM ADDENDUM

Thursday, March 31

- **Correction to the Program**

Plenary Session I – Grand Ballroom

10:30 AM George W. Beadle Award Presentation
Presenter: Thomas W. Cline, University of California-Berkeley
Recipient: Thomas C. Kaufman, Indiana University, Bloomington

- **Platform Presentation Cancellation and Replacement**

Neural Physiology and Biology – Golden West Room

4:15 PM **Cancelled:** Functional analyses of *fru^M*-expressing neurons for their role in regulating courtship activation and male arousal. **David H. Tran.**

4:15 PM **Replaced by:** Visual cues mediate the quality of *Drosophila melanogaster* courtship behavior. **Joy E. C. Hatzidakis, Devanand S. Manoli, Bruce S. Baker.** (Formerly poster presentation/board 742A)

Friday, April 1

- **Platform Presentation Author Addition**

Pattern Formation I – Town and Country Room

11:00 AM Regulation of wing proliferation by the Dpp morphogen gradient. **Dragana Rogulja,**
Additional author: Kenneth D. Irvine, Waksman Institute, Piscataway, NJ.

Saturday, April 2

- **Platform Presenter Substitution**

Drosophila Models of Human Diseases – California Room

4:15 PM A living reporter for APP Gamma-secretase activity identifies novel genes. **To be presented by Ming Guo** instead of Garrett Gross.

- **Workshop Addition: Golden West Room**

9:30 PM–11:30 PM **Endocrine Regulation of Growth and Metabolism**

Organizers: **Seung K. Kim**, Stanford University, Stanford, California; **Eric Rulifson**, University of Pennsylvania, Philadelphia.

In this workshop, guest speakers from several fields including developmental biology, signal transduction and behavioral research, will highlight recent advances in our understanding of endocrine regulation of growth and metabolism. Discussions will emphasize emerging parallels between *Drosophila* endocrine models and their potential significance to human endocrine biology and related diseases. One goal of this session is to show how areas of endocrine research dominated by studies of mammalian models are increasingly available for study in *Drosophila*. Areas of research to be covered include development of cells producing insulin, differentiation of cells that sense and regulate glucose and other metabolites, neuropeptide signals that regulate response to environmental stress, and discovery of signaling pathways that mediate responses to these endocrine signals. Speakers include Seung Kim (Stanford University), Ping Shen (Univ. of Georgia, Athens), Volker Hartenstein (UCLA), Eric Rulifson (Univ. of Pennsylvania), R.J. Wessels (Burnham Institute, La Jolla, California), Pierre Léopold (CNRS, Nice), and Sean Oldham (Burnham Institute).

• **Poster Abstract Correction:**

1 st Author	Poster #	Additional Author
Allison Bardin	685A	François Schweisguth, Ecole Normale Supérieure, Paris, France

• **Poster Presenter Changes:**

1 st Author	Poster #	Substitute Presenter
Manika Pal-Bhadra	271A	S.N.C.V.L. Pushpavalli
Veronique Monnier	916A	Christelle Lasbleiz

• **Poster Presentations Cancelled:**

Poster Author	Poster #
Jesus Mateos	209B
Pedro Fernandez-Funez	513C
Thomas Klein	516C
Andreas Bergmann	526A
Horacio M. Frydman	589A
Pinky Kain	688A
Subhabrata Pal	930C
Meng Wang	943A

• **Additional Exhibitors:**

VIEWPOINT LIFE SCIENCES, INC. Booth 206
 410 Elm Avenue
 Otterburn Park, QC, J3H 4B5, Canada
 Telephone: (450) 464-9632 Fax: (450) 464-0837
 E-mail: info@viewpointlifesciences.com
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• Late Poster Abstracts (see complete text of abstracts following these listings):

First Author/ Presenter	Poster #	Abstract Title and Co-Authors
MEIOSIS, MITOSIS, AND CELL DIVISION		
Catherine C. Baker	960C	Characterization of the novel meiotic arrest mutant <i>ozzie</i> . Catherine C. Baker, Margaret T. Fuller. Developmental Biology, Stanford University School of Medicine, Stanford, CA.
Byung S. Gim	961A	Regulation of Cyclin B and meiotic cell cycle progression during spermatogenesis. Byung S. Gim, Margaret T. Fuller. Dev Bio, Beckman Ctr. B300, School of Medicine, Stanford U, Stanford, CA.
Yasushi Izumi	962B	Regulation of mitotic spindle orientation in <i>Drosophila</i> neuroblasts. Yasushi Izumi¹, Thomas Raabe², Fumio Matsuzaki¹. 1) Laboratory for Cell Asymmetry, RIKEN, Kobe, Japan; 2) Institute for Medical Radiation and Cell Research, University of Wuerzburg, Wuerzburg, Germany.
Masa-Toshi Yamamoto	963C	Preliminary analysis of mitochondrial segregation at meiotic divisions in male sterile mutants of <i>Drosophila melanogaster</i> . Masa-Toshi Yamamoto¹, Takashi Ohsako¹, Jun-ichi Kawano². 1) <i>Drosophila</i> Genetic Resource Ce, Kyoto Inst Technology, Kyoto, Japan; 2) School of Health Sciences, Kyushu Univ. of Health and Welfare, Miyazaki Japan.
CYTOSKELETON AND CELLULAR BIOLOGY		
Nichole D. Bond	964A	The role of β <i>FTZ-F1</i> in fat-body dissociation during metamorphosis. Nichole D. Bond, Archana Nelliot, Deborah K. Hoshizaki. Biological Sciences, University of Nevada Las Vegas, Las Vegas, NV.
Masayasu Hirano	965B	Cell shape determines epithelial patterning in the embryonic epidermis. Masayasu Hirano, Obianuju Dike, Christy Tanner, Erica Smith, Simon Collier. Biological Sciences, Marshall University, Huntington, WV.
Archana Nelliot	966C	Control of Programmed Tissue Dissociation in <i>Drosophila melanogaster</i> . Archana Nelliot, Nichole Bond, Deborah Hoshizaki. Department of Biological Sciences, University of Nevada Las Vegas, Las Vegas, NV.
Tatsuhiko Noguchi	967A	Myosin VI accelerates Arp2/3 complex based actin network formation during individualization of <i>Drosophila</i> spermatogenesis. Tatsuhiko Noguchi¹, Marta Lenartowska^{1,2}, Aaron Rogat¹, Kathryn Miller¹. 1) Department of Biology, Washington University in St. Louis, St. Louis, MO; 2) Laboratory of Developmental Biology, Institute of General and Molecular Biology, Nicolaus Copernicus University, Torun, Poland.
GENOME AND CHROMOSOME STRUCTURE		
Nathalie Dostatni	968B	Exploring chromatin state inheritance through cell division: role of the chromatin assembly factor 1 (CAF-1). Nathalie Dostatni, Benjamin Klapholz, Sophie Hamel, Genevieve Almouzni, Bruce Dietrich. UMR 218 Nuclear Dynamics and Genome Plasticity, Curie Institute, Paris, France.
Nina S Dudnik	969C	Histone H2A/H2B ` immers rapidly exchange at many sites in the <i>Drosophila</i> genome. Nina S Dudnik, Brian E Schwartz, Kami Ahmad. BCMP, Harvard Medical School, Boston, MA.
REGULATION OF GENE EXPRESSION		
Timothy A. Blauwkamp	970A	Direct Transcriptional Repression by Wnt Signaling. Timothy A. Blauwkamp, Ming Fang, Kenneth M. Cadigan. MCDB Dept., University of Michigan, Ann Arbor, MI.
Lodovica Borghese	971B	Transcriptional profiling of migratory cells. Lodovica Borghese¹, William C. Eades², Ann Atzberger¹, Ross L. Cagan², Pernille Rørth¹. 1) European Molecular Biology Laboratory, Heidelberg, Germany; 2) Washington University School of Medicine, Saint Louis, MO.

Olivier Crauk	972C	Understanding the transcriptional synergy between the two morphogens Bicoid and Hunchback. Olivier Crauk, Nathalie Dostatni . UMR 218, Inst Curie & CNRS, Paris, France.
Chantal Dauphin-Villemant	973A	Transcriptional regulation of Halloween genes plays an important role in the control of <i>Drosophila</i> ecdysteroid biosynthesis. Chantal Dauphin-Villemant, Jean-Philippe Parvy . FRE2852 CNRS – Groupe Biogenese des Steroides, Université P. et M. Curie, Bat A, 5eme et., case 29, 7 quai Saint-Bernard, 75005 Paris, France.
Torsten Fauth	974B	Characterization of <i>Drosophila</i> EcR and USP in a mammalian cell culture system. Torsten Fauth^{1,2}, Josh Beatty¹, Margarethe Spindler-Barth², Vincent C. Henrich¹ . 1) Institute for Health, Science, and Society, University of North Carolina-Greensboro, Greensboro, NC 27402-6170; 2) General Zoology and Endocrinology, University Ulm, 89081 Ulm, Germany.
	975C	Withdrawn.
Zhe Han	976A	A myocardin-related transcription factor regulates activity of serum response factor in <i>Drosophila</i> . Zhe Han, Xiumin Li, Jiang Wu, Eric Olson . Dept Molecular Biol, Univ Texas SW Medical Ctr, Dallas, TX.
Aleksandar S. Necakov	977B	In vivo screening for nuclear receptor agonists and antagonists in flies. Aleksandar S. Necakov, Heidi M. Sampson, Henry Krause . Banting and Best Department of Medical Research, University of Toronto, 112 College Street, Toronto, Ontario, Canada M5G 1L6.
Yutaka Nibu	978C	dDrap1 mediates short-range repression. Yutaka Nibu . Dept Cell & Developmental Biol, Cornell Univ/Weill Medical Col, New York, NY.
Vanya Rasheva	979A	Mutations of the insulator protein dCTCF cause homeotic transformations. Vanya Rasheva, Ying Kong, Victoria Meller . Dept. of Biological Sci., Wayne State University, Detroit, MI 48202, av6197@wayne.edu.
Ye Tao	980B	Expression, Regulation and Function of Toll Gene in <i>Drosophila</i> Heart Development. Ye Tao¹, Jianbo Wang¹, Ingolf Reim², Kathleen Gajewski¹, Manfred Frasch², Robert A. Schulz¹ . 1) Department of Biochemistry & Molecular Biology, University of Texas M.D. Anderson Cancer Center, Houston, TX; 2) Brookdale Center for Molecular, Cell and Developmental Biology, Mount Sinai School of Medicine, New York, NY.
SIGNAL TRANSDUCTION		
Takashi Adachi-Yamada	981C	Cell-cell communication mediated by LRR protein Fish-lips suppresses removal of ectopic ventral appendage cells. Takashi Adachi-Yamada^{1,2}, Toshiyuki Harumoto¹, Kayoko Sakurai³, Ryu Ueda⁴, Kaoru Saigo⁵, Michael B. O'Connor^{6,7}, Hiroshi Nakato^{3,6} . 1) Grad. School of Sci. Technol., Kobe University, Kobe, Hyogo, JAPAN; 2) SORST, Japan Science and Technology Agency; 3) Dept. of Biol., Faculty of Sci., Tokyo Metropolitan University, Hachioji, Tokyo, JAPAN; 4) Genetic Strains Research Center, National Institute of Genetics, Mishima, Shizuoka, JAPAN; 5) Dept. of Biophys. Biochem., Grad. School of Sci., University of Tokyo, Tokyo, JAPAN; 6) Dept. of Genet., Cell Biol. Develop., University of Minnesota, Minneapolis, USA; 7) Howard Hughes Medical Institute.
Shannon L. Ballard	982A	Examination of BMP Signaling in the Regulation of Larval Growth. Shannon L. Ballard, Kristi A. Wharton . Dept MCB, Brown Univ, Providence, RI.
Claire R. Davies	983B	The Role of TOR signaling in the regulation of cardiac aging in <i>Drosophila</i> . Claire R. Davies, Robert J. Wessells, Erin Fitzgerald, Sean Oldham, Rolf Bodmer . Cancer Center, The Burnham Institute, La Jolla, CA.

Chun Han	984C	<i>Drosophila</i> Glypicans Dally and Dally-like shape the extracellular Wingless morphogen gradient in the wing disc. Chun Han ^{1,2} , Dong Yan ^{1,2} , Tatyana Belenkaya ¹ , Xinhua Lin ^{1,2} . 1) Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA; 2) The Graduate Program in Molecular and Developmental Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45229, USA.
Tetsuya Okajima	985A	Chaperone Activity of Protein O-fucosyltransferase 1 promotes Notch Receptor Folding. Tetsuya Okajima , Aiguo Xu , Liang Lei , Kenneth D. Irvine . HHMI, Waksman Inst, Rutgers Univ, Piscataway, NJ.
Parthive H. Patel	986B	Phenotypic analysis of cells with reduced <i>Tsc2</i> activity. Parthive H. Patel , Fuyuhiko Tamanoi . Molecular Biology Institute, UCLA, Los Angeles, CA. 90095.
Elayne Provost	987C	Mutations in a GST-containing zinc-finger protein suppress the <i>prune-Killer of prune</i> lethal interaction. Elayne Provost ¹ , Grafton Hersperger ¹ , Lisa Timmons ² , Wen Qi Ho ¹ , Evelyn Hersperger ¹ , Rosa Alcazar ¹ , Allen Shearn ¹ . 1) Biology Department, Johns Hopkins University, Baltimore, MD; 2) Department of Molecular Biosciences, University of Kansas, Lawrence, KN.
Christy Reedy	988A	Activation and modification of <i>slipper</i> , a <i>Drosophila</i> MLK in the JNK pathway, during dorsal closure. Christy Reedy , Beth Stronach . Biological Sciences, University of Pittsburgh, Pittsburgh, PA.
Chao Tong	989B	Hedgehog signaling activity of Smoothed requires phosphorylation by protein kinase A and casein kinase I. Chao Tong , Jianhang Jia , Bing Wang , Liping Luo , Jin Jiang . Center for Developmental Biolo, UT Southwestern Medical Center, Dallas, TX.
Alexander N. R. Weber	990C	Activation of the Toll Ligand Spätzle. Alexander N.R. Weber ¹ , Martin C. Moncrieffe ¹ , Jean-Luc Imler ² , Nicholas J. Gay ¹ . 1) Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge CB2 1GA, United Kingdom; 2) UPR 9022 – CNRS, Institut de Biologie Moléculaire et Cellulaire, 15 rue René Descartes, 67084 Strasbourg cedex, France.
Idella Christine Wilson	991A	Screen for modifiers of a dominant gain of function phenotype. Idella Christine Wilson , Tonia Von Ohlen . Biology, Kansas State University, Manhattan, KS.
Dong Yan	992B	Role of proteoglycan in FGF signaling during tracheal branching and mesoderm migration in <i>Drosophila</i> . Dong Yan ^{1,2} , Xinhua Lin ^{1,2} . 1) Division Developmental Biology, Cincinnati Children's Hospital; 2) Molecular and Developmental Biology Program, University of Cincinnati, Cincinnati, OH.
Hideki Yoshida	993C	Functional analysis of <i>Drosophila</i> alpha 1-3 fucosyltransferase during development. Hideki Yoshida ^{1,2} , Tomomi Ichimiya ^{1,2} , Ryu Ueda ^{2,3} , Satoshi Goto ^{2,4} , Shoko Nishihara ^{1,2} . 1) Bioinformatics, Soka University, Hachioji, Japan; 2) CREST, JST; 3) Genet. Strains, Natl. Inst. Fac. Pham., Misawa, Japan; 4) MITILS., Machida, Tokyo, Japan.
PATTERN FORMATION		
Miranda J. Butler	994A	<i>Tarsel-less</i> , a gene involved in a new cell signaling event in <i>Drosophila</i> appendages. Miranda J. Butler , Jose I. Pueyo , Maximo I. Galindo , Sarah A. Bishop , Juan Pablo Couso . School Life Sciences, University of Sussex, Brighton, United Kingdom.
Claudia M. Mizutani	995B	<i>ind</i> and <i>msh</i> are differentially sensitive to graded BMP signaling in the neuroectoderm. Claudia M. Mizutani , Francisco F. Esteves , Evyia Vitola , Ethan Bier . Dept Biol, Univ California, San Diego, San Diego, CA.
Charles Sackerson	996C	The dependence of <i>even-skipped</i> early stripe 1 on Eve reveals a functional phase of <i>eve</i> expression which precedes early stripe expression. Charles Sackerson . Dept Biol, Iona Col, New Rochelle, NY.
Elizabeth A. Silva	997A	The role of the tumour suppressor Fat in early differentiation and patterning of the <i>Drosophila</i> eye. Elizabeth A. Silva , Helen McNeill . Developmental Patterning, LRI, Cancer Research UK, London, UNITED KINGDOM.

Meng-Ping Tu	998B	Compartmental cross-talk during wing disc size regulation. Meng-Ping Tu, Laura A. Johnston. Dept Genetics & Development, Columbia Univ, Col P&S, New York, NY.
John H. Yoder	999C	The recent origin of two Abdominal-B proteins and their relationship to reduction of the number of abdominal segments in Diptera. John H. Yoder, Sean B. Carroll. University of Wisconsin and HHMI, Madison, WI.
GAMETOGENESIS AND SEX DETERMINATION		
Thomas Fellner	1000A	Phenotypic and molecular characterization of lucky luke (luke) and rantanplan (rpn), two novel genes required for stem cell division or stem cell maintenance. Thomas Fellner¹, Cordula Schulz², Margaret T. Fuller¹. 1) Stanford University School of Medicine, Department of Developmental Biology, Stanford, CA; 2) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Harriet Lorena Harris	1001B	Life and Death in Drosophila: <i>Wolbachia</i> throws the switch. Harriet Lorena Harris¹, Zoe L. Veneti², Gregory D. D. Hurst², Henk R. Braig³. 1) Biology, Concordia University College, Edmonton, Alberta, CANADA; 2) Biology, University College London, London, UK; 3) Biological Sciences, University of Wales Bangor, Bangor, Gwynedd, UK.
Daniel Kirilly	1002C	BMP Signaling is Required for Controlling Somatic Stem Cell Self-Renewal in the <i>Drosophila</i> Ovary. Daniel Kirilly^{1,2}, Eric Spana³, Norbert Perrimon⁴, Richard Padgett⁵, Ting Xie^{1,2}. 1) Stowers Institute for Medical Research, Kansas City, MO; 2) Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS; 3) Department of Biology, Duke University, Durham, NC; 4) Howard Hughes Medical Institute, Department of Genetics, Harvard Medical School, Boston, MA; 5) Waksman Institute, Rutgers University, Piscataway, NJ.
Jerome Quintero	1003A	Altered cellularization times, rather than the X:A ratio, appear to determine the sex of haploid and triploid embryos. Jerome Quintero, Hong Lu, James W. Erickson. Dept. of Biology, Texas A&M University, College Station, TX.
Jessica Rivera	1004B	The role of <i>α-endosulfine</i> in the proliferative response to nutrition of the <i>Drosophila</i> ovary. Jessica Rivera, Daniela Drummond-Barbosa. Cell & Developmental Biology, Vanderbilt University, Nashville, TN.
Rongwen Xi	1005C	Stem Cell Self-Renewal Controlled by Chromatin Remodeling Factors. Rongwen Xi¹, Ting Xie^{1,2}. 1) Stowers Institute for Medical Research, Kansas City, MO 64110, USA; 2) Department of Anatomy and Cell Biology, University of Kansas School of Medicine, 3901 Rainbow Boulevard, Kansas City, KS 66160, USA.
ORGANOGENESIS		
Raquel Marco-Ferreres	1006A	Overexpression of troponin T in Drosophila muscles causes a decrease in the levels of thin filament proteins. Raquel Marco-Ferreres, Juan J. Arredondo, Margarita Cervera. Departamento de Bioquímica and Instituto de Investigaciones Biomédicas, UAM-CSIC, Madrid, Spain.
I. Reim	1007B	The <i>Tbx20</i> -related genes <i>midline</i> and <i>H15</i> are required for the proper patterning and differentiation of the dorsal vessel by activating cardioblast-specific <i>tinman</i> expression. I. Reim¹, P. C. H. Lo¹, J. Mohler², M. Frasch¹. 1) Mol., Cell & Dev. Biology, Mt. Sinai School of Medicine, New York, NY; 2) Dept. of Biol. Sci., Barnard College, New York, NY.
NEUROGENETICS AND NEURAL DEVELOPMENT		
Angela Becker	1008C	Gene expression profiling during gliogenesis in Drosophila. Angela Becker¹, Benjamin Altenhein¹, Boris Beckmann², Christian Busold², Jörg Hoheisel², Gerd Technau¹. 1) Institute of Genetics, Mainz, Germany; 2) DKFZ Heidelberg, Germany, Member of the Heidelberg FlyArray Consortium.
Takako Isshiki	1009A	Aging of neuroblast-during the late stages of embryogenesis and larval period. Takako Isshiki, Ayumi Kusano. Research Strains Center, National Institute of Genetics, Mishima, Shizuoka, Japan.

Marta Morey	1010B	New regulators of R7 targeting specificity on the X chromosome. Marta Morey, Aljoscha Nern, Tory Herman, Larry Zipursky. HHMI, Dpt Biological Chemistry, D.Geffen Sch. Medicine, UCLA, Los Angeles, CA.
Luyuan Pan	1011C	Drosophila Model of Fragile X Syndrome. Luyuan Pan, Yong Q. Zhang, Heinrich Matthies, Elvin Woodruff III, Kendal Broadie. Dept. of Biological Science, Kennedy Center for Research on Human Development, Vanderbilt University, Nashville, TN. 37215.
Susana Peralta	1012A	Clathrin-dependent endocytosis role in pupal eye development. Susana Peralta¹, Javier Vinós^{1,2}. 1) Dpto Bioquímica, Universidad Autónoma de Madrid, Spain; 2) Instituto de Investigaciones Biomédicas, CSIC, Madrid, Spain.
Anuradha Ratnaparkhi	1013B	A novel role for <i>fog</i> as a factor involved in axo-glial interactions in Drosophila. Anuradha Ratnaparkhi, Kai Zinn. Biology, Caltech, Pasadena, CA.
Hongyan Wang	1014C	DmRic8 mediates asymmetric cell divisions through regulating heterotrimeric G proteins in neuroblasts and sensory organ precursors. Hongyan Wang, Kian Hong Ng, Hongliang Qian, William Chia, Fengwei Yu. Temasek Life Sciences Lab, Singapore, Singapore, Singapore.
Yoshihiro Yuasa	1015A	Developmental context for Notch-dependent PROS expression in longitudinal glial cells. Yoshihiro Yuasa¹, Yasushi Hiromi^{1,2,3}. 1) Dept Developmental Genetics, National Inst Genetics, Mishima, Japan; 2) SOKENDAI; 3) CREST, JST, Japan.
Fengwei Yu	1016B	Locomotion defects regulate heterotrimeric G protein signaling via two distinct modes of action during Drosophila neuroblast asymmetric divisions. Fengwei Yu¹, Hongyan Wang¹, Hongliang Qian¹, Rachna Kaushik², Mary Bownes³, Xiaohang Yang², William Chia¹. 1) Temasek Life Sciences Laboratory, National University of Singapore, Singapore117604; 2) Institute of Molecular and Cell Biology, 61 Biopolis Drive, Proteos, Singapore 138673; 3) Institute of Cell and Molecular Biology, University of Edinburgh, King's Buildings, Edinburgh, EH9 3JR UK.

NEURAL PHYSIOLOGY AND BEHAVIOR

Nigel Atkinson	1017C	slowpoke induction underlies ethanol tolerance in Drosophila. Nigel Atkinson, Roshani Cowmeadow, Alfredo Ghezzi, Yazan Al-Hasan, Harish Krishnan. Section Neurobiology, Univ Texas, Austin, Austin, TX.
Ginger E. Carney	1018A	The p24 intracellular trafficking proteins and oviposition behavior. Ginger E. Carney. Dept Biol, Texas A&M Univ, College Station, TX.
L.S. Corley	1019B	Candidate Acps in the stalk eyed fly <i>Cyrtodiposis dalmanni</i> . L. S. Corley, E. McConnell, K. Kraaijeveld, P. Hadrill, K. Fowler, A. Pomiankowski, T. Chapman. Biology, University College London, United Kingdom.
Jesús Romero-Pozuelo	1020C	Frequenin finds her sister and both modulate synaptic form and function. Jesús Romero-Pozuelo¹, Jeffrey Dason², Harold L Atwood², Alberto Ferrús¹. 1) Instituto Cajal, CSIC, Madrid, Spain; 2) Dept. of Physiology, U. of Toronto, Toronto (Ontario), Canada.
Anne F. Simon	1021A	Dissociation between extension of longevity and resistance to stress in alleles of the steroid biosynthesis gene <i>dare</i> . Anne F. Simon, David E. Krantz. UCLA, Neuropsychiatric Institute, Los Angeles, CA.
Scott Waddell	1022B	Dorsal Paired Medial neurons are transiently required for Drosophila Olfactory Memory. Scott Waddell, Alex C. Keene. Department of Neurobiology, UMass Medical School, Worcester, MA 01605.

EVOLUTION AND QUANTITATIVE GENETICS

Esther Betran	1023C	Fast protein evolution in a parental gene and its young retroposed derived gene of germline expression in Drosophila. Esther Betran, Mansi Motiwale. Dept Biol, Univ Texas, Arlington, Arlington, TX.
Deb Hamilton	1024A	The effects of Auto Fluorescent Intensity on Mate Choice Among Populations of Drosophila from Evolution Canyon. Deb Hamilton, Dr. Tom Wolf. Dept Biol, Washburn Univ, Topeka, KS.

Mary A. Knox	1025B	Evolutionary analysis of the <i>swallow</i> gene. Mary A. Knox, Belinda Awuor, Edwin C. Stephenson. Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL.
Nykolaus P. Reed	1026C	<i>Osiris 16</i> is an Essential Gene in <i>Drosophila melanogaster</i> . Nykolaus P. Reed¹, Alan C. Christensen², Douglas R. Dorer¹. 1) Dept Microbiology, Meharry Medical Col, Nashville, TN; 2) School of Biological Sciences, University of Nebraska, Lincoln, NE.
Claudia Rohde	1027A	Chromosomal evolution of sibling species of the <i>Drosophila willistoni</i> group. Chromosomal arm IIR. Claudia Rohde¹, Ana C. L. Garcia¹, Victor H. Valiati², Vera L. S. Valente¹. 1) Departamento de Genética, Universidade Federal do Rio Grande do Sul - UFRGS, Porto Alegre, RS, Brazil, claudiarohde@yahoo.com; 2) Ciências da Saúde, Universidade do Vale do Rio dos Sinos - UNISINOS, São Leopoldo, RS, Brazil.
IMMUNE SYSTEM AND CELL DEATH		
Jerell R. Aguila	1028B	Resistance to Starvation: The Role of Larval-Derived Fat Cells in the Adult. Jerell R. Aguila, Justin W. Suszko, Deborah K. Hoshizaki. Biological Sciences, University of Nevada Las Vegas, Las Vegas, NV.
Sylvain Brun	1029C	The MAPKKK D-Mekk1 regulates the expression of turandot stress genes in response to injury in <i>Drosophila</i> . Sylvain Brun¹, Sheila Vidal¹, Paul Spellmann², Kuniaki Takahashi³, Herve Tricoire⁴, Bruno Lemaitre¹. 1) Centre de Genetique Moleculaire, CNRS, 91198 Gif-sur-Yvette, France; 2) Department of Molecular and Cell Biology and Howard Hughes Medical Institute, University of California at Berkeley, Berkeley, CA 94720-3200, USA; 3) National Institute of Genetics, Mishima, Shizuoka 411-8540, Japan; 4) Institut Jacques Monod, 2 place Jussieu, 75251 Paris, France.
Kathleen A. Galindo	1030A	Generation and Characterization of Debcl null animals, a <i>Drosophila</i> Pro-apoptotic Bcl-2 Family Member. Kathleen A. Galindo, Arisha Patel, John M. Abrams. Cell Biology, UT Southwestern Medical Center, Dallas, TX.
Shu Kondo	1031B	Dual role of <i>Drosophila</i> caspases in execution of apoptosis and resumption of mitosis after DNA damage-induced cell cycle arrest. Shu Kondo^{1,2,3}, Yasushi Hiromi^{2,3}, Masayuki Miura^{1,3}. 1) Department of Genetics, Grad. Sch. Pharm. Sci., University of Tokyo, Japan; 2) Division of Developmental Genetics, NIG, Mishima, Japan; 3) CREST, JST, Japan.
TECHNIQUES AND GENOMICS		
Venky N. Iyer	1032C	Annotating the newly sequenced <i>Drosophila</i> genomes using <i>Drosophila melanogaster</i> . Venky N. Iyer¹, Daniel A. Pollard², Michael B. Eisen^{1,2,3}. 1) Dept. of Molecular and Cell Biology, UC Berkeley, Berkeley, CA; 2) Biophysics Graduate Group, UC Berkeley, Berkeley, CA; 3) Genome Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA.
Oswaldo Marinotti	1033A	Genome-wide analysis of gene expression in adult <i>Anopheles gambiae</i> . Oswaldo Marinotti¹, Eric Calvo², Nguyen Quang K.¹, Dissanayake Sumudu¹, Nirmala Xavier¹, Ribeiro Jose M. C.², James Anthony A.¹. 1) Molecular Biology and Biochemistry, Univ. California Irvine, Irvine, CA; 2) Laboratory of Malaria and Vector Research, National Institutes of Health, Rockville, MD.
James P. Mohler	1034B	Development of Insect Retroviral Vectors for Somatic and Field Transformation. James P. Mohler, Mansi Mehta, Rosio Ramos, Sana Ali. Biological Sciences, Barnard College, New York, NY.
Heeren Patel	1035C	A new family of direct-drive <i>dfd::YFP</i> balancers. Heeren Patel, Tien Le, ZhiGuo Liang, Stephanie Ray, Matthew Slovitt, Gita Sivasubramaniam, Marcus Yu, Greg Beitel. BMBCB, Northwestern University, Evanston, IL.

Maria G. Samsonova	1036A	A prototype of Laboratory Information Management System for processing and analysis of confocal images of gene expression patterns. Maria G. Samsonova¹, Andrei S. Pisarev¹, Ekaterina G. Poustelnikova¹, Konstantin N. Kozlov¹, Ekaterina M. Myasnikova¹, John Reinitz² . 1) Dept. of Computational Biology, State Polytechnical University, St. Petersburg, Russia; 2) Stony Brook University, NY.
K. S. Siebert	1037B	Insertional mutagenesis systems in the red flour beetle, <i>Tribolium castaneum</i> . K. S. Siebert¹, M. D. Lorenzen², Y. Park¹, S. J. Brown³, R. W. Beeman² . 1) Dept. of Entomology, Kansas State University, Manhattan, KS; 2) USDA-ARS-GMPRC, Manhattan, KS; 3) Div. of Biology, Kansas State University, Manhattan, KS.
Bo Wang	1038C	High throughput collection of <i>Drosophila</i> embryos for homozygous lethal mutants based on <i>deformed</i> driven YFP expression. Bo Wang¹, Julia Thompson¹, Greg Beitel², Rock Pulak¹ . 1) Union Biometrica, 35 Medford Street, Suite 101, Somerville, MA 02143; 2) Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208.
DROSOPHILA MODELS OF HUMAN DISEASES		
Michelle L. Beaucher	1039A	MMPs alter the invasive ability of <i>lethal giant larvae</i> mutant tumors. Michelle L. Beaucher¹, Evelyn Hersperger¹, Andrea Page-McCaw², Allen Shearn¹ . 1) Dept Biol, Johns Hopkins Univ, Baltimore, MD; 2) Dept Biol, Rensselaer Polytechnic Inst, Troy, NY.
Laura Ciapponi	1040B	The role of the <i>Drosophila</i> Nbs protein in telomere protection. Laura Ciapponi, Giovanni Cenci, Claudia Berdini, Maurizio Gatti . University of Rome "La Sapienza", Department of Genetics and Molecular Biology, Rome, Italy.
Afifa Khan	1041C	An enhancer/suppressor screen for genes that function in the Lkb1 pathway, and characterisation of the Lkb1 phenotype in <i>Drosophila melanogaster</i> . Afifa Khan, Ruth Wheeler, Helen McNeill . Developmental Patterning, Cancer Research UK, London, United Kingdom.
Young-II Kim	1042A	<i>kal-1</i> , the <i>Drosophila</i> orthologue of human Kallmann syndrome gene KAL1, is required for the neurite branching of embryonic motorneurons. Young-II Kim, Wonseok Son, Sun-Mi Woo, Ook Joon Yoo . Dept Life Sci, KAIST, DeaJeon, South Korea.
Udai Bhan Pandey	1043B	A <i>Drosophila</i> model recapitulating key clinical features of spinal and bulbar muscular atrophy. Udai Bhan Pandey, Zhiping Nie, Stephanie Ciosek, J. Paul Taylor . Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.
Rui Sousa-Neves	1044C	<i>datilógrafo (dati)</i> encodes a zinc finger transcription factor required for proper locomotor activity in <i>Drosophila</i> . Rui Sousa-Neves¹, Claudia Mieko Mizutani², Tamas Lukacsovich¹, Judith Purcell¹, J. L. Marsh¹ . 1) Dept Dev & Cell Biol, Univ California, Irvine, Irvine, CA; 2) Section of Cell and Developmental Biology, University of California, San Diego, La Jolla, CA.

MEIOSIS, MITOSIS, AND CELL DIVISION

960C

Characterization of the novel meiotic arrest mutant *ozzie*. Catherine C. Baker, Margaret T. Fuller. Developmental Biology, Stanford University School of Medicine, Stanford, CA.

In multicellular organisms, cell division must be under higher-order regulation, so that specific cell types can undergo specialized cell cycles or stop cell division altogether as part of their differentiation program. These specialized cell cycles incorporate cell-type specific inputs, such as transcriptional states and cell size, to modify the activity of the kinases and phosphatases which make up the core cell cycle machinery.

In *Drosophila*, the meiotic cell cycle in males is regulated so that the G2/M transition of meiosis I is delayed until the transcription program responsible for expression of many terminal differentiation genes in primary spermatocytes has been turned on. This critical cross-regulatory mechanism acts in part through regulation of the *Twine/Cdc25* phosphatase, an essential positive regulator of the G2/M transition. Although *twine* mRNA is expressed, translation of *twine* depends on activity of the RNA-binding protein Boule. In turn, accumulation of Boule protein depends on wild-type function of 5 genes required for the transcription program for spermatid differentiation. The *can*, *mia*, *rye*, *nht*, and *sa* genes encode testis-specific TBP-associated factors (tTAFs) expressed only in primary spermatocytes and are required for the normal transcription of spermatid differentiation genes during meiotic prophase.

The novel meiotic arrest mutant *ozzie* (*ozz*) may represent a pathway parallel to that of Boule. *ozz* mutant spermatocytes fail to enter meiotic division, and undergo negligible differentiation. In *ozz* testes, Boule protein accumulates to wild-type levels, but *twine-lacZ* is not translated. We will present additional data on mRNA and protein expression in *ozz* mutants, including results of immunostaining with anti-Cyclin B and anti-Fzo antibodies. Preliminary deficiency mapping data will also be presented.

961A

Regulation of Cyclin B and meiotic cell cycle progression during spermatogenesis. Byung S. Gim, Margaret T. Fuller. Dev Bio, Beckman Ctr. B300, School of Medicine, Stanford U, Stanford, CA.

In *Drosophila* male primary spermatocytes, Cyclin B and *cdc2* form a complex, the essential cell cycle kinase, to control the G2/M transition to drive cells into meiotic division. Expression of Cyclin B protein during male meiotic prophase is regulated post-transcriptionally: although *Cyclin B* mRNA is transcribed in early primary spermatocytes, accumulation of the protein is delayed until just before the spermatocytes enter meiotic division⁽¹⁾. This delay is a key feature of the meiotic cell cycle, leading to the extended G2 period of meiotic prophase. To identify the mechanism(s) responsible for the delay in Cyclin B protein accumulation, we generated several Cyclin B-GFP reporter constructs, with a spermatocyte specific promoter and combinations of 5' and 3' untranslated region (UTR) and genomic DNA of *Cyclin B*. The results indicated that the 3' UTR of *Cyclin B* has a function for delayed protein expression in spermatocytes, suggesting regulation by translational control. To screen for candidate *trans*-acting regulators of Cyclin B protein levels, we used antibodies against Cyclin B protein to stain testes from males mutant for genes encoding predicted RNA-binding proteins (RBPs) expressed in the testis. We identified mutants in two different genes where Cyclin B protein was expressed prematurely in spermatocytes, suggesting the genes might act directly or indirectly to repress translation of Cyclin B in young spermatocytes. (1) White-Cooper *et al.*, 1998 *Development* 125, 125-134.

962B

Regulation of mitotic spindle orientation in *Drosophila* neuroblasts. Yasushi Izumi¹, Thomas Raabe², Fumio Matsuzaki¹. 1) Laboratory for Cell Asymmetry, RIKEN, Kobe, JAPAN; 2) Institute for Medical Radiation and Cell Research, University of Wuerzburg, Wuerzburg, Germany.

In *Drosophila* embryos, neuroblasts delaminate from the epithelial cell layer and repeatedly undergo asymmetric divisions. Mitotic neuroblasts reorientate the spindle along the apical-basal axis to ensure unequal segregation of the basal determinants whereas epithelial cells symmetrically divide parallel to the embryo's surface. Recently, we suggested that spindle orientation in both neuroblasts and epithelial cells is regulated by a common mechanism involving the receptor-independent Pins-Gai signaling. To investigate the mechanism by which the Pins-Gai complex orients the spindle, we conducted the co-immunoprecipitation experiments and identified Mushroom body defect (Mud) as a binding protein for Pins. Mud is a large coiled-coil protein, whose mutations have been reported to affect the mushroom body morphology. We found that Mud and Pins colocalizes at the apical cortex in neuroblasts and at the lateral cortex in epithelial cells, whereas Mud (but not Pins) also distributes to the centrosomal region in both cell types. Cortical Mud localization depends on *pins* and *Gai*. In *mud* mutants, spindle orientation and the centrosomal structure are impaired in both neuroblasts and epithelial cells. These results suggest that Mud has dual functions in spindle regulation, (1) orienting the spindle through the interactions with both Pins and astral microtubules and (2) proper organization of the centrosome.

963C

Preliminary analysis of mitochondrial segregation at meiotic divisions in male sterile mutants of *Drosophila melanogaster*. Masa-Toshi Yamamoto¹, Takashi Ohsako¹, Jun-ichi Kawano². 1) *Drosophila* Genetic Resource Ce, Kyoto Inst Technology, Kyoto, JAPAN; 2) School of Health Sciences, Kyushu Univ. of Health and welfare, Miyazaki JAPAN.

The mechanisms and regulation of mitochondrial segregation at cell division may vary among mitosis, oogenesis and spermatogenesis. In spermatogenesis, unlikely in other cell divisions, daughter cells must receive equal amount of mitochondria to make all sperm mobile and functional. In order to investigate if genetic regulation is involved in the mitochondrial segregation in spermatogenesis of *Drosophila melanogaster*, we first tried to collect strains of which fertility is reduced and varied among individuals, assuming a certain proportion of the daughter cells at the first meiotic divisions receive mitochondria just enough to produce functional sperm. We isolated 17 strains of which males are partially fertile (3 to 30% fertility compared to that of the heterozygotes). Three strains show nebenkerns abnormal in sizes and numbers in the spermatocytes. We here present preliminary cytological observations of two strains, *ms(2)kn66* and *ms(3)o33*. The *ms(2)kn66* mutant shows different sized mitochondria derivatives but regular numbers of nuclei in each spermatocyte when examined by phase contrast microscope. The *ms(3)o33* mutant shows irregular combinations of nuclei and mitochondria derivatives resulting in multiple association among them revealed by electronmicroscopy of the elongating spermatids.

CYTOSKELETON AND CELLULAR BIOLOGY

964A

The role of β FTZ-F1 in fat-body dissociation during metamorphosis. Nichole D. Bond, Archana Nelliott, Deborah K. Hoshizaki. Biological Sciences, University of Nevada Las Vegas, Las Vegas, NV.

During metamorphosis ecdysone signaling mediates several distinct biological responses; larval tissues such as the salivary glands and midgut undergo autophagy, while imaginal discs proliferate and differentiate to form the adult structures. Based on recent data, we have determined that fat-body dissociation and survival of the adult animal is dependent upon tissue-specific signaling of ecdysone in the fat body. The genetic cascade triggered by ecdysone in the fat body is likely to involve a unique set of known ecdysone downstream target genes.

We present data that identifies a known primary target of ecdysone signaling as a key component in fat-body dissociation. We find that the transcription factor gene β ftz-F1 is sufficient to initiate the dissociation process both *in vivo* and *in vitro* but is not sufficient for the final steps involving cell detachment. Completion of dissociation requires ecdysone.

965B

Cell shape determines epithelial patterning in the embryonic epidermis. Masayasu Hirano, Obianuju Dike, Christy Tanner, Erica Smith, Simon Collier. Biological Sciences, Marshall University, Huntington, WV.

Epithelial cells of similar affinity normally pack as regular hexagons. However, a simple array of hexagonal cells limits the possibilities for cell-based epithelial patterning. Epithelial cell shape and packing can be changed, even without remodeling cell junctions, by forces that distort the epithelium. The denticle cells of the ventral embryonic epidermis appear to be subjected to a ventrolateral force that transforms them into elongated rectangles that pack like bricks in a wall. The anterior-posterior boundaries of adjacent denticle cells form a continuous straight edge along which the rows of F-actin rich 'predentacles' emerge. We show that driving expression of a dominant negative form of the actin cytoskeletal regulator Rac1 in a single row of denticle cells eliminates denticles within that row but also affects denticle patterning in adjacent denticle rows. This implicates the actin cytoskeleton not just in denticle formation but also in denticle cell organization perhaps through propagating the force that causes denticle cell shape. We also show that mutations in the Planar Cell Polarity (PCP) Effector genes alter denticle cell shape and packing resulting in disorganized denticle patterning. We hypothesize that the PCP Effector proteins act to stabilize cell interactions during the application of a ventrolateral force to allow normal denticle cell shape and packing. The role of non-muscle myosin and components of the NDR kinase signaling pathway in denticle cell organization will also be discussed.

966C

Control of Programmed Tissue Dissociation in *Drosophila melanogaster*. Archana Nelliott, Nichole Bond, Deborah Hoshizaki. Department of Biological Sciences, University of Nevada Las Vegas, Las Vegas, NV.

In Dipterans, metamorphosis is characterized by the loss and transformation of larval tissues as the animal prepares for adult life. In response to elevated levels of the steroid molting hormone 20-hydroxy ecdysone (referred to hereafter as ecdysone) most larval tissues undergo autophagic cell death. The larval fat body,

however, appears refractive to cell death and instead dissociates into individual cells. These cells are metabolically active and are believed to serve as a fuel source for the metamorphosing animal.

Little is known about the control of programmed tissue dissociation and thus, fat-body dissociation provides a novel system to examine the regulation and mechanism of tissue dissociation. Using fluorescent cell markers, we have established the first detailed study of this process in live animals. The developmental profile of dissociation reveals that the gross, as well as cellular, morphological changes associated with it are divisible into three stages that are concurrent with changes in the ecdysone titer. Hence, we have begun characterizing animals in which ecdysone signaling has been specifically disrupted in the fat body. Results of these studies will be reported.

967A

Myosin VI accelerates Arp2/3 complex based actin network formation during individualization of *Drosophila* spermatogenesis. Tatsuhiko Noguchi¹, Marta Lenartowska^{1,2}, Aaron Rogat¹, Kathryn Miller¹.

1) Department of Biology, Washington University in St. Louis, St. Louis, MO; 2) Laboratory of Developmental Biology, Institute of General and Molecular Biology, Nicolaus Copernicus University, Torun, Poland.

In order to understand the *in vivo* function of myosin VI, we extensively analyzed the *Drosophila* myosin VI mutant during the final stage of spermatogenesis. During individualization, 64 actin cones divides 64 synthical spermatids into individual sperms. It has been reported that myosin VI localizes at the front of each actin cone and without myosin VI individualization process fails. Previously we reported that, *in vitro* culture of myosin VI mutant spermatogenic cysts, individualization stops in the middle of the cyst and the actin cones cannot maintain the proper accumulation of actin during individualization. In this report, we analyzed the actin cone structure more in detail. S1 labeling demonstrates that the wild type actin cone has two distinct structural domains. The front half has densely packed meshwork of actin filaments. In contrast, the rear half of actin cone has long actin bundles parallel to longitudinal axis of the cone. Arp2/3 complex and cortactin localize to the front of the actin cone. In addition, vast majority of actin filaments are oriented with its pointed end facing towards the front of the cone. In myosin VI mutant, significant filament loss occurred predominantly in the front half of the cone. ATP extraction of GFP-myosin VI from permeabilized cysts demonstrated that myosin VI motor domain is important for localization. FRAP of GFP-myosin VI demonstrate that myosin VI can stay bound on actin cone over a few minutes. This suggests that myosin VI is localizing to front edge of the actin cone protecting pointed ends of filaments, and structurally stabilizes actin structure by holding filaments by its motor domain, rather than involved in membrane trafficking event as is usually suggested.

GENOME AND CHROMOSOME STRUCTURE

968B

Exploring chromatin state inheritance through cell division: role of the chromatin assembly factor 1 (CAF-1). Nathalie Dostatni, Benjamin Klapholz, Sophie Hamel, Genevieve Almouzni, Bruce Dietrich. UMR 218 Nuclear Dynamics and Genome Plasticity, Curie Institute, Paris, France.

To better understand the maintenance of epigenetic marks through cell division, we are studying the Chromatin Assembly Factor-1 (CAF-1), unique for strictly assembling nucleosomes onto newly synthesised DNA. It comprises three subunits and is largely conserved through evolution, from yeast to human. We have generated a null allele for p180 (p1801), the gene coding for the *Drosophila* CAF-1 large subunit. The phenotype of this loss-of-function is lethal at 48 hours of larval development and is fully rescued by transgenic ubiquitous expression of p180. Interestingly, full rescue of the *Drosophila* mutant is also obtained with the mouse and human homologs, indicating that despite a weak conservation among CAF-1 large subunits (26% of primary sequence identity), the essential function of the *Drosophila* CAF-1 p180 is common to its mouse and human homologs. A sensitized rescue system of p1801-induced lethality allowed us to show that p180 genetically interacts with an allele of the histone-donor Asf1 encoding a protein unable to bind H3/H4 histones. This provides evidence that the essential function of CAF-1 *in vivo* requires histones. The p1801-induced lethality is first associated with a reduction in the number of larval neuroblasts undergoing mitosis. Interestingly, this lethality can be prevented by driving most dividing cells into G0 by food starvation, indicating that it is linked to cell cycle defects. To understand these defects at the cellular level, we are currently analyzing germline and somatic mutant clones. At last, an interaction between the murine CAF-1 large subunit and the Heterochromatin Protein 1 (HP1) has been characterized biochemically (Murzina et al, Mol. Cell, 1999), revealing a potential link between nucleosome assembly and a higher order of chromatin organisation. We have shown that this interaction also occurs in *Drosophila* and we are currently testing its involvement in CAF-1 function *in vivo*.

969C

Histone H2A/H2B dimers rapidly exchange at many sites in the *Drosophila* genome. Nina S Dudnik, Brian E Schwartz, Kami Ahmad. BCMP, Harvard Medical School, Boston, MA.

Models for transcription from a chromatin template predict that nucleosomes are either remodeled or disassembled to clear a path for polymerases. At least some disassembly must occur during transcription, because we observe that histone H3 is replaced by variant H3.3 at transcribed loci. The structure of the nucleosome would require that H2A/H2B dimers be removed to replace H3. We tested this model by examining if dimers undergo transcription-coupled replacement. A heat shock-induced pulse of H3.3 accumulates at the heat shock loci, which were active at the time of the pulse. We produced a pulse of GFP-tagged H2A and H2B, and examined polytene chromosomes for patterns of histone incorporation at timepoints following recovery. In contrast to H3.3, tagged H2A and H2B accumulate rapidly in the nucleolus and then redistribute to many euchromatic sites. At early timepoints, approximately 7 sites are strongly labeled and many bands show weaker staining. Overall, bands get brighter and by one hour, several hundred sites contain tagged histones with comparable intensities. We conclude that the exchange of histone dimers is much faster than replacement of H3.3. Furthermore, none of the target sites for dimer incorporation correspond to the heat shock loci. In fact, staining for RNA polymerase II shows that most sites of transcription are not sites of rapid exchange of dimers, and conversely, most sites of rapid exchange are not transcribed loci. Nucleosome disassembly at transcribed regions might occur without dimer exchange if old dimers are reused. However, this does not explain why non-transcribed sites rapidly acquire tagged histones. We are examining whether DNA replication or transcription by RNA pol III can account for the bulk of dimer exchange.

REGULATION OF GENE EXPRESSION

970A

Direct Transcriptional Repression by Wnt Signaling. Timothy A. Blauwkamp, Ming Fang, Kenneth M. Cadigan. MCDB Dept., University of Michigan, Ann Arbor, MI.

The canonical Wnt signaling pathway is a highly conserved signal transduction cascade that controls the transcription of genes involved in cell growth, differentiation, apoptosis and transformation. While several directly activated genes have been identified in a variety of organisms, very few directly repressed genes have been characterized. The goal of my research is to determine if, and how, individual genes in the same cell respond differently Wnt signaling, some being activated while others are repressed at the same time.

Using microarray analysis of a *Drosophila* embryonic cell line, I have identified several genes that are transcriptionally repressed by Wnt signaling, as well as several other genes that are activated by Wnt signaling at the same time. At least two of the activated genes and one of the repressed genes are directly regulated by Wnt signaling, as shown by TCF binding to the Wnt-responsive enhancers and by the ability to regulate transcription in the absence of protein synthesis. Reporter constructs for the three directly regulated genes identified thus far have been generated and the minimal DNA sequences conferring Wnt-mediated activation or repression are being identified. By comparing the minimal enhancers from activated genes to minimal enhancers from repressed genes I hope to determine how individual genes are specified for activation or repression by Wnt signaling.

971B

Transcriptional profiling of migratory cells. Lodovica Borghese¹, William C. Eades², Ann Atzberger¹, Ross L. Cagan², Pernille Rørth¹. 1) European Molecular Biology Laboratory, Heidelberg, Germany; 2) Washington University School of Medicine, Saint Louis, MO.

Border cells perform an invasive migration during normal ovary development in *Drosophila*. The migratory process is under tight transcriptional control. In order to characterize transcriptional changes associated with border cell migration, we first developed a protocol to specifically purify migratory border cells from ovaries. We then compared gene expression profiles of border cells and follicle cells, i.e. migratory and non-migratory cells from the same epithelium. We identified about 300 genes whose expression was significantly increased in border cells relative to follicle cells (at least by a two fold difference). The *Drosophila* C/EBP transcription factor, *Slbo*, is required for border cell migration to occur. We further compared gene expression profiles of wild type and *slbo* mutant border cells. We identified about 200 genes whose expression was significantly increased in wild type migratory border cells. The expression of 100 genes was increased in both comparisons. These results have been validated by quantitative real time PCR, in situ expression and antibody staining. Among the transcripts enriched in border cells we found several previously known to be expressed and/or have function in border cell migration. We also found genes not previously associated with border cell migration; for example a group of 10 genes related to muscle function and the *six4* transcription factor. We have performed functional analyses in border cells for many of these genes and we are currently analyzing further genes for which mutant alleles are available.

972C

Understanding the transcriptional synergy between the two morphogens Bicoid and Hunchback.

Olivier Crauk, Nathalie Dostatni. UMR 218, Inst Curie & CNRS, Paris, France.

The Bicoid (Bcd) and Hunchback (Hb) morphogens are transcription factors acting in synergy in the establishment of the anterior part of the *Drosophila* embryo. Although Bcd is clearly a genuine transcription factor able to directly activate its target genes, this property has never been clearly established for Hb, which rather acts during development as a direct transcriptional repressor. Our aims are to understand the molecular mechanism leading to the synergy observed between Bcd and Hb. We have shown that this process can be separated into two components: i) at the beginning of cellularisation the expression domain of a target gene of both Bcd and Hb (Hb3Bcd3-lacZ) is clearly expanded towards the posterior when compared to a target gene of Bcd only (Bcd3-lacZ); ii) at the end of cellularisation the level of expression of Hb3Bcd3-lacZ is clearly increased when compared to the one of Bcd3-lacZ. In order to study the correlation between these two components of the synergy, we are currently developing a quantitative RT-PCR approach on small numbers of staged embryos. To identify the functional domains of Bcd involved in the synergy, we have analysed deleted forms of Bcd: we have shown that the alanine-rich repression domain (A) and the N-terminal region of the protein, including the serine-threonine-rich activation domain (ST), are involved in this process. Moreover, experiments using Gal4-derived transcription factors composed of the Gal4 DNA binding domain fused to different domains of Bcd, and reporter genes either for Gal4 alone or for Gal4 and Hb, allowed us to identify the Bcd domains sufficient for the synergy. We found that both the ST activation domain and the A repression domain are involved and we are currently analysing which component of the synergy is affected by each of these two domains. We propose that the synergy results from two distinct and redundant mechanisms: i) the first one is a direct positive effect of Hb on Bcd via its ST domain; ii) the second one involves the A repression domain of Bcd and likely a negative control by Hb.

973A

Transcriptional regulation of Halloween genes plays an important role in the control of *Drosophila* ecdysteroid biosynthesis.

Chantal Dauphin-Villemant, Jean-Philippe Parvy. FRE2852 CNRS - Groupe Biogenese des Steroides, Université P. et M. Curie, Bat A, 5eme et., case 29, 7 quai Saint-Bernard, 75005 Paris, France.

20-Hydroxyecdysone is the primary endocrine signal that mediates developmental transitions in insects. Using an enzyme immunoassay, we showed that the molting hormone titer is strictly correlated with the steroidogenic capacity of L3 ring glands.

The biosynthetic pathway of ecdysteroids is still incompletely understood but involves a cascade of hydroxylations catalyzed by cytochrome P450 enzymes (CYPs). Phantom (phm), disembodied (dib) and shadow (sad) code respectively for CYP306A1, CYP302A1 and CYP315A1, catalyzing the last steps in ecdysteroid biosynthesis. A temporal correlation was observed between the expression of these enzymes in *Drosophila* ring gland and the dynamics of ecdysone production during the third instar, suggesting that hormone pulses depend on transcriptional regulation of steroidogenic enzymes.

The identity of transcription factors putatively involved in such regulation has been investigated. Using clonal analysis, levels of two steroidogenic enzymes, Phantom (PHM) and Disembodied (DIB), were shown to be very reduced in ftz transcription factor 1 (ftz-f1) mutant ring gland cells whereas there was no effect of the without children (woc) mutation, suggesting that FTZ-F1 regulates phm and dib expression. Since β FTZ-F1 is the homolog of the vertebrate steroidogenic factor 1 (SF1), which plays a key role in the differentiation of vertebrate steroidogenic organs through transcriptional regulation of steroidogenic enzymes, this study emphasizes the strong parallels between insects and vertebrates with respect to the regulatory mechanisms of steroidogenesis.

This work was carried out in association with: Catherine Blais, Frédéric Bernard, Annick Maria, James T. Warren, Anna Petryk, Lawrence I. Gilbert and Michael B. O'Connor.

974B

Characterization of *Drosophila* EcR and USP in a mammalian cell culture system. Torsten Fauth^{1,2}, Josh Beatty¹, Margarethe Spindler-Barth², Vincent C. Henrich¹. 1) Institute for Health, Science, and Society, University of North Carolina-Greensboro, Greensboro, NC 27402-6170; 2) General Zoology and Endocrinology, University Ulm, 89081 Ulm, Germany.

The functional ecdysteroid receptor in *Drosophila melanogaster* is a heterodimer composed of the ecdysone receptor (EcR) and Ultraspiracle (USP). Combinations of three natural EcR isoforms and different VP16-USP fusion proteins have been tested in a mammalian cell culture system (Chinese hamster ovary) for transcriptional activity, ligand-binding, and binding to the *hsp27* EcRE. The three different EcR isoforms showed different ligand-binding affinities, differences in trans-activation capabilities, and differences in DNA-binding affinity. In all cases, induction by muristerone A also increased DNA-binding affinity in cellular extracts. Several EcR and USP mutations involving amino acid residues possibly important for specific receptor subfunctions were also tested in

the cell culture system to examine their effects on the transcriptional activity induced by ecdysteroids (murA and 20-hydroxyecdysone), in order to develop specific hypotheses for subsequent in vivo testing. One mutation in EcR, K497E, affects an amino acid shared by all three isoforms and causes a constitutive activation in only the EcR-B2 isoform. The elevated transcriptional activity is accompanied by elevated affinity for an *hsp27* EcRE in gel shift assays, suggesting that the amino acid residue involves a repressor interaction that is B2 specific.

975C

Withdrawn.

976A

A myocardin-related transcription factor regulates activity of serum response factor in Drosophila.

Zhe Han, Xiumin Li, Jiang Wu, Eric Olson. Dept Molecular Biol, Univ Texas SW Medical Ctr, Dallas, TX.

Serum response factor (SRF) regulates genes involved in cell proliferation, migration, cytoskeletal organization and myogenesis. Myocardin and myocardin-related transcription factors (MRTFs) act as powerful transcriptional coactivators of SRF in mammalian cells. We describe an MRTF from *Drosophila*, called DMRTF, which shares high homology with the functional domains of mammalian myocardin and MRTFs. DMRTF forms a ternary complex with and stimulates the activity of *Drosophila* SRF, which has been implicated in branching of the tracheal (respiratory) system and formation of wing interveins. A loss-of-function mutation introduced into the DMRTF locus by homologous recombination results in abnormalities in tracheal branching similar to those in embryos lacking SRF. Mis-expression in wing imaginal discs of a dominant negative DMRTF mutant also causes a diminution of wing interveins, whereas over-expression of DMRTF results in excess intervein tissue, abnormalities reminiscent of SRF loss- and gain-of-function phenotypes, respectively. Over-expression of these DMRTF mutants in mesoderm and in the tracheal system also perturbs mesoderm cell migration and tracheal branching, respectively. We conclude that the interaction of MRTFs with SRF represents an ancient protein partnership involved in cytoplasmic outgrowth and cell migration during development.

977B

In Vivo Screening for Nuclear Receptor Agonists and Antagonists in Flies. Aleksandar S. Necakov, Heidi M. Sampson, Henry Krause. Banting and Best Department of Medical Research, University of Toronto, 112 College Street, Toronto, Ontario, Canada M5G 1L6.

Nuclear receptors comprise a family of transcription factors whose activation/repression state can be modulated by the binding of small molecules. The identification of ligands capable of binding to particular nuclear receptors is central to understanding the mechanisms through which these receptors elicit their function. To date, 18 nuclear receptor genes have been identified in the *Drosophila melanogaster* genome. However, cognate ligands for 17 of the 18 receptors have not yet been identified. As part of a project to identify the remaining ligands, we have developed an in vivo screen that allows for the dynamic visualization of ligand mediated nuclear receptor activation in live *Drosophila* tissues. Each of the 18 fly nuclear receptor ligand binding domains has been fused to the DNA binding domain of Gal4 and introduced into flies under control of a heat-shock inducible promoter. Activity of the chimeric transcription factors requires the presence of ligand, and is visualized with the use of a GAL4-dependent GFP reporter. Nine of the 18 chimeric proteins were found to exhibit unique spatio-temporal activity patterns during embryonic and larval development. Using the Ecdysone receptor (EcR) fusion protein, we show that these limited activity patterns are ligand dependent and can be expanded both temporally and spatially by exogenously provided ligand. We are currently carrying out high throughput chemical screens to identify potential nuclear receptor agonists and antagonists. These lead compounds may lead to the development of new species-specific pesticides.

978C

dDrap1 mediates short-range repression. Yutaka Nibu. Dept Cell & Developmental Biol, Cornell Univ/Weill Medical Col, New York, NY.

Transcriptional repression is essential for patterning the gene expression in the early *Drosophila* embryo. We have isolated and characterized the *Drosophila* CtBP (dCtBP) protein, a corepressor of several short-range transcriptional repressors that inhibits adjacent activators within 100 bp. Our preliminary data suggest that dCtBP interacts with the *Drosophila* homologue of Drap1 (dDrap1) in vitro and in yeast. It is known that mammalian Drap1 forms a heterodimer with Dr1 via histone-fold motifs and increases the repression activity of Dr1. The Dr1/Drap1 complex interacts with TBP through Dr1 and blocks the subsequent recruitment of TFIIA and TFIIIB on the promoter. It is unknown, however, whether Drap1/Dr1 can mediate short-range repression. We have found that dDrap1 fused to the Gal4 DNA-binding domain represses the reporter gene expression in transgenic embryos in a manner consistent with short-range repression. Importantly, our data show that *Drosophila* Dr1 (dDr1) cannot repress in a similar condition. Further, we expressed dCtBP or dDrap1 in wing discs. We will discuss the role of the interaction between dCtBP and dDrap1.

979A

Mutations of the insulator protein dCTCF cause homeotic transformations. Vanya Rasheva, Ying Kong, Victoria Meller. Dept. of Biological Sci., Wayne State University, Detroit, MI 48202, av6197@wayne.edu.

The CTCF (CCCTC binding factor) protein is known to possess enhancer blocking function at chromatin insulator in vertebrates. The structure of CTCF, with 11 zinc fingers, is highly conserved. A *Drosophila* ortholog of CTCF, dCTCF, has demonstrated similar binding site specificity and transcription repression (Moon et al., *EMBO Rep.*, 2005; 6: 165 - 170) It was also shown that dCTCF maintains the enhancer blocking function of the insulator *Fab-8*, a cis-acting regulatory element of the *Abd-B* (*Abdominal-B*) locus. We identified a P-element insertion mutant of the *CTCF* gene which is viable and fertile. P-element excision was used to generate mutations, 10 of which are lethal in the late pupal stage. Homozygous mutants display homeotic transformation of posterior abdominal segments to a more anterior fate, a phenotype similar to that displayed by *Fab-8* mutants. This suggests that dCTCF, like its mammalian orthologue, acts to establish or maintain chromatin insulators.

980B

Expression, Regulation and Function of Toll Gene in Drosophila Heart Development. Ye Tao¹, Jianbo Wang¹, Ingolf Reim², Kathleen Gajewski¹, Manfred Frasch², Robert A. Schulz¹. 1) Department of Biochemistry & Molecular Biology, University of Texas M.D. Anderson Cancer Center, Houston, TX; 2) Brookdale Center for Molecular, Cell and Developmental Biology, Mount Sinai School of Medicine, New York, NY.

The dorsal vessel of *Drosophila* is a contractile linear tube that resembles the vertebrate heart at early developmental stages. During *Drosophila* heart development, precursor cells from the mesoderm undergo specification, migration, patterning and differentiation, and finally form the mature organ. This process is precisely regulated and a series of genes have been found to be involved in it. Here we show the expression, regulation and function of one such gene-Toll. Toll encodes a transmembrane protein expressed in the developing *Drosophila* heart, from the stage that bilateral cardioblasts emerge till the stage of heart formation. By analyzing the upstream regulatory sequence of Toll gene, we found a 305-bp minimal heart enhancer that resembles the specific heart expression pattern of the Toll gene when it drives LacZ or GFP expression. Within the DNA sequence of this minimal heart enhancer, we found evolutionarily conserved T-box protein consensus binding sites and Tinman binding site essential for Toll heart enhancer activity. We further proved that the activity of this 305-bp heart enhancer is controlled by the key transcriptional factors of *Drosophila* heart development, the T-box member Dorsocross1 and the homeodomain protein Tinman. The phenotypes of Toll mutant embryos show distinct abnormalities in heart morphology, which suggests Toll has an important role in the alignment and migration of the cardioblasts during heart formation.

SIGNAL TRANSDUCTION

981C

Cell-cell communication mediated by LRR protein Fish-lips suppresses removal of ectopic ventral appendage cells. Takashi Adachi-Yamada^{1,2}, Toshiyuki Harumoto¹, Kayoko Sakurai³, Ryu Ueda⁴, Kaoru Saigo⁵, Michael B. O'Connor^{6,7}, Hiroshi Nakato^{3,6}. 1) Grad. School of Sci. Technol., Kobe University, Kobe, Hyogo, JAPAN; 2) SORST, Japan Science and Technology Agency; 3) Dept. of Biol., Faculty of Sci., Tokyo Metropolitan University, Hachioji, Tokyo, JAPAN; 4) Genetic Strains Research Center, National Institute of Genetics, Mishima, Shizuoka, JAPAN; 5) Dept. of Biophys. Biochem., Grad. School of Sci., University of Tokyo, Tokyo, JAPAN; 6) Dept. of Genet., Cell Biol. Develop., University of Minnesota, Minneapolis, USA; 7) Howard Hughes Medical Institute.

Growth, patterning and apoptosis are interdependent during development. For example, cells that choose an abnormal fate in a developing field are frequently removed. An interesting issue in this process that needs to be resolved is the identification of protein(s) by which cells discern their correct fate from an abnormal fate. In order to examine this issue, we have carried out a forced expression of the dioxin receptor homolog Spineless (Ss) ectopically in the wing imaginal disc. The presence of mosaic clones ectopically expressing Ss results in a local transformation of organ identity from wing into a segment of the ventral appendages such as leg and antenna. The transformed cells subsequently activate c-Jun N-terminal Kinase to undergo cell death in an autonomous or nonautonomous manner depending on their position in the wing disc, suggesting that a cell-cell interaction is, at least in some cases, involved in the detection of transformed cells. However, both the autonomous cell death and nonautonomous cell death caused by Ss are similarly suppressed by a novel Leucine-Rich Repeat (LRR) family transmembrane protein, Fish-lips (Fili), that may interact with surrounding normal cells. These data support a mechanism in which lacking some membrane proteins helps to recognize the presence of transformed cells and directs these cells to an apoptotic fate in order to remove them from the normal developing field.

982A

Examination of BMP Signaling in the Regulation of Larval Growth. Shannon L. Ballard, Kristi A. Wharton. Dept MCB, Brown Univ, Providence, RI.

Bone Morphogenetic Proteins (BMPs) are a family of extracellular signaling molecules belonging to the Transforming Growth Factor β (TGF- β) superfamily. In vertebrates and invertebrates, BMPs regulate developmental processes such as cell proliferation, cell fate specification, and apoptosis. Mutations in the BMP 2, 4 ortholog, *decapentaplegic* (*dpp*) as well as in the BMP 5,6,7,8 ortholog, *glass bottom boat* (*gbb*), produce several defects in growth and development of larvae. *gbb* was named for the clarity of the mutant larvae, a phenotype similar to that exhibited by starved larvae. In addition, *gbb* mutant larvae exhibit defects in the size of larval and imaginal tissues as well as exhibit a developmental delay. Mutations in downstream components of the BMP signaling cascade, including the receptors and intracellular signal transducers, exhibit similar growth phenotypes. We have begun to investigate the relationship between these mutant BMP growth phenotypes and that of starved larvae as well as larvae mutant for pathways known to affect nutritional uptake.

983B

The Role of TOR signaling in the regulation of cardiac aging in *Drosophila*. Claire R Davies, Robert J Wessells, Erin Fitzgerald, Sean Oldham, Rolf Bodmer. Cancer Center, The Burnham Institute, La Jolla, CA.

Cardiac dysfunction is the most common cause of death in the elderly and is a problem of increasing clinical relevance. Recent heart performance studies in *Drosophila* have shown that mutations in the insulin signaling pathway minimized the decline of cardiac performance with age. Downstream of insulin-IGF signaling the pathway branches into the nutrient-sensitive target-of-rapamycin (TOR) pathway. Preliminary heart performance assays with mutants of the TOR signaling pathway (*S6K* null mutants and two heteroallelic combinations of *dTOR* mutants) have shown that altering the TOR pathway can also provide cardiac protection during aging. Investigations have focused on determining the mechanisms by which the TOR signaling pathway influences cardiac function during aging. Specifically, growth, metabolism and autophagy are being analyzed, as these are processes that are known to be regulated by the TOR pathway. Differences were found in the growth and metabolism in the whole fly of these mutants and therefore studies of these processes have been extended to analyzing specific effects in the heart. Firstly, to determine whether TOR mediated cardiac protection is due to altered growth, the size and morphology of cells in the heart are being analyzed. Secondly, to investigate the metabolic state within the heart, glycogen and lipid levels are being assessed. Thirdly, the levels of autophagy are being assessed using an autophagy marker.

984C

***Drosophila* Glypicans Dally and Dally-like shape the extracellular Wingless morphogen gradient in the wing disc.** Chun Han^{1,2}, Dong Yan^{1,2}, Tatyana Belenkaya¹, Xinhua Lin^{1,2}. 1) Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA; 2) The Graduate Program in Molecular and Developmental Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45229, USA.

Drosophila Wingless (Wg) is the founding member of the Wnt family of secreted proteins. During the wing development, Wg acts as a morphogen whose concentration gradient provides positional cues for wing patterning. The molecular mechanism(s) of Wg gradient formation is not fully understood. Here, we systematically analyzed the roles of glypicans Dally and Dally-like protein (Dlp), the Wg receptors Frizzled (Fz) and Fz2, and the Wg co-receptor Arrow (Arr) in Wg gradient formation in the wing disc. We demonstrate that both Dally and Dlp are essential and have different roles in Wg gradient formation. The specificities of Dally and Dlp in Wg gradient formation are at least partially achieved by their distinct expression patterns. To our surprise, although Fz2 was suggested to play an essential role in Wg gradient formation by ectopic expression studies, removal of Fz2 activity does not alter the extracellular Wg gradient. Interestingly, removal of both Fz and Fz2, or Arr causes enhanced extracellular Wg levels, which is mainly resulted from up-regulated Dlp levels. We further show that Notum, a negative regulator of Wg signaling, down-regulates Wg signaling mainly by modifying Dally. Lastly, we demonstrate that Wg movement is impeded by cells mutant for both *dally* and *dlp*. Together, these new findings suggest that the Wg morphogen gradient in the wing disc is mainly controlled by combined actions of Dally and Dlp. We propose that Wg establishes its concentration gradient by a restricted diffusion mechanism involving Dally and Dlp in the wing disc.

985A

Chaperone Activity of Protein O-fucosyltransferase 1 promotes Notch Receptor Folding. Tetsuya Okajima, Aiguo Xu, Liang Lei, Kenneth D. Irvine. HHMI, Waksman Inst, Rutgers Univ, Piscataway, NJ.

Notch proteins act as receptors for a conserved signaling pathway that effects numerous cell fate decisions. The extracellular domains of Notch receptors consist largely of tandemly repeated EGF domains. Disruption of a single EGF domain can dominantly perturb Notch function or trafficking, as in the human congenital syndrome CADASIL, indicating that the folding of Notch must be tightly controlled. Here, we show that in *Drosophila*, protein O-fucosyltransferase 1 (OFUT1), previously identified as an enzyme that catalyzes a specific

glycosylation of EGF domains, also has a distinct, chaperone activity. Although fucosylation has been thought to occur in the Golgi, OFUT1 is an ER protein, and its ER localization is essential for function in vivo. OFUT1 is required for the trafficking of wild-type Notch out of the ER, and can partially rescue defects associated with Notch point mutations. This apparent ability of OFUT1 to facilitate folding of Notch does not require its fucosyltransferase activity. These observations suggest a novel mechanism for quality control, in which a glycosyltransferase binds to its substrate in the ER to facilitate normal folding, and raise the possibility that OFUT1 gene therapy might be used to treat CADASIL. Reference: Okajima T et al. Science 2005.

986B

Phenotypic analysis of cells with reduced *Tsc2* activity. Parthive H. Patel, Fuyuhiko Tamanoi. Molecular Biology Institute, UCLA, Los Angeles, CA. 90095.

The TSC tumor suppressor complex consists of a heterodimer of two proteins, Tsc1 (tuberin) and Tsc2 (hamartin). In humans, loss of either *Tsc1* or *Tsc2* results in the condition, tuberous sclerosis, characterized by the formation of benign hamartomatous lesions which can develop into malignant tumors. The Tsc complex has been described to be regulated negatively by insulin signaling (via PKB/Akt) and positively regulated by the cellular energy pathway (LKB, AMPK). RNA interference experiments in both *Drosophila* and mammalian cultured cells have demonstrated Tsc2 as a negative regulator of cell growth mediated by Rheb/TOR signaling. S2 cells treated with *Tsc2* dsRNA display an increase in Rheb/TOR signaling (as determined by increased S6K phosphorylation). Due to the ease of using dsRNA interference with *Drosophila* cultured cells, we are characterizing additional phenotypes of cells with decreased TSC activity and testing putative candidate genes that may regulate Rheb/TOR signaling. Understanding the Tsc/Rheb/TOR signaling module in *Drosophila* will elucidate mechanisms of human disease such as cancer and diabetes.

987C

Mutations in a GST-containing zinc-finger protein suppress the *prune-Killer of prune* lethal interaction.

Elayne Provost¹, Grafton Hersperger¹, Lisa Timmons², Wen Qi Ho¹, Evelyn Hersperger¹, Rosa Alcazar¹, Allen Shearn¹. 1) Biology Department, Johns Hopkins University, Baltimore, MD; 2) Department of Molecular Biosciences, University of Kansas, Lawrence, KN.

The *prune* (*pn*) gene is predicted to encode a phosphodiesterase. Null mutants in the *pn* gene are viable; the only detectable phenotype is a brownish-purple eye color. The *abnormal wing disc* (*awd*) gene encodes a nucleoside diphosphate kinase. *Killer of prune* (*Kpn*) is a missense mutation in the *awd* gene resulting in the substitution Pro97Ser in the protein. While *Kpn* has no phenotype by itself, a single copy is lethal when present in *pn* mutants. The mechanism of the *pn-Kpn* lethal interaction is unknown. To better understand the interaction between *pn* and *Kpn*, we undertook a suppressor screen. Transgenic *Kpn* flies were mutagenized with EMS and several dominant suppressor mutations were recovered. These mutations are recessive lethal and allelic. The *Su(Kpn)* gene is located within 84C6-8. The gene was identified by sequencing of mutant alleles as CG10065 (dGFZF) a GST-containing zinc finger protein. Current studies are aimed at understanding the molecular mechanism of action of *Su(Kpn)* in the *pn-Kpn* interaction.

988A

Activation and modification of *slipper*, a *Drosophila* MLK in the JNK pathway, during dorsal closure.

Christy Reedy, Beth Stronach. Biological Sciences, University of Pittsburgh, Pittsburgh, PA.

The c-Jun N-terminal kinase (JNK) pathway, a type of mitogen-activated protein kinase (MAPK) pathway, has been characterized for its role in many cellular morphogenetic processes, as well as for apoptosis and cell differentiation. One specific process, dorsal closure, occurs in the *Drosophila* embryo. During this phase of development, JNK signaling is observed at the leading edge cells of the epithelial layer involved in closure. JNK activity is required in these cells to modulate the cytoskeleton and cell shape, allowing the epidermis to close on the dorsal side of the embryo. The mixed lineage kinase (MLK), *slipper*, is the JNKKK which is responsible for activation of the pathway during dorsal closure. Currently, it is not known what the upstream signals are that begin this process. We are interested in examining how *slpr* becomes activated and have taken two different approaches to answer this question. The first involves testing known candidates which have been proposed to interact with *slpr*. *Misshapen*, a JNKKKK, is a gene indicated by experimental evidence to play a role in dorsal closure. We hypothesize that *msn* acts directly upstream of *slpr* in the pathway. Other unknown candidates can be identified through pull-down assays and silver staining. The second approach we are using is an attempt to pinpoint genetic interactions in a non-biased manner. A genetic screen was conducted using genomic deletions in the second chromosome which enable scanning for regions which cause a specific phenotype. We have found eight regions which either enhance or suppress a *slpr* mutant phenotype. We believe that one or more of these areas could be partly responsible for the wide variety of phenotypes that we see and may also be indicative of proteins involved in parallel pathways. Smaller deleted regions of the chromosome will be used to narrow down these modifying regions. These experiments allow us to test both genetically and biochemically for the role of *slpr* in JNK signal transduction.

989B

Hedgehog signaling activity of Smoothed requires phosphorylation by protein kinase A and casein kinase I. Chao Tong, Jianhang Jia, Bing Wang, Liping Luo, Jin Jiang. Center for Developmental Biology, UT Southwestern Medical Center, Dallas, TX.

The Hedgehog (Hh) family of secreted proteins governs cell growth and patterning in animal development. The Hh signal is transduced by the seven-transmembrane protein Smoothed (Smo); however, the mechanism by which Smo is regulated remains largely unknown. Here we show that protein kinase A (PKA) and casein kinase I (CKI) regulate Smo cell-surface accumulation and activity in response to Hh. Blocking PKA or CKI activity in the *Drosophila* wing disc prevents Hh-induced Smo accumulation and attenuates pathway activity, whereas increasing PKA activity promotes Smo accumulation and pathway activation. We show that PKA and CKI phosphorylate Smo at several sites, and that phosphorylation-deficient forms of Smo fail to accumulate on the cell surface and are unable to transduce the Hh signal. Conversely, phosphorylation-mimicking Smo variants show constitutive cell-surface expression and signalling activity. Furthermore, we find that the levels of Smo cell-surface expression and activity correlate with its levels of phosphorylation. Our data indicate that Hh induces progressive Smo phosphorylation by PKA and CKI, leading to elevation of Smo cell-surface levels and signalling activity.

990C

Activation of the Toll Ligand Spätzle. Alexander N.R. Weber¹, Martin C. Moncrieffe¹, Jean-Luc Imler², Nicholas J. Gay¹. 1) Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge CB2 1GA, United Kingdom; 2) UPR 9022 - CNRS, Institut de Biologie Moléculaire et Cellulaire, 15 rue René Descartes, 67084 Strasbourg cedex, France.

The Toll receptor is a signalling mediator in *Drosophila* dorsoventral patterning during embryogenesis and in innate immune defences to Gram-positive bacteria and fungi. Egg deposition or pathogen recognition, respectively, lead to activation of the Toll ligand Spätzle via a proteolytic cascade. Spätzle consists of an N-terminal pro-domain that lacks secondary structure, and a C-terminal 106 amino acid domain (C106) which adopts a cystine knot fold. In dorsoventral patterning the protease Easter is responsible for Spätzle activation and it has been postulated that Easter processing releases an active fragment that corresponds to the Spätzle C-terminal domain. In fact, recombinant Spätzle whose pro-domain has been removed by tryptic proteolysis binds to and activates the Toll receptor whereas the untreated pro-protein is inactive. However, it remains unclear whether complete removal of the Spätzle pro-part is required for activation or whether specific endoproteolytic backbone cleavage by Easter is sufficient to activate Spätzle.

To study Spätzle activation we generated a recombinant protein with a TEV protease cleavage site inserted at the proposed site of Easter cleavage. Our data show that treatment with TEV severs the Spätzle N- and C-terminal domains but does not lead to dissociation of pro-part and C106. In fact both domains remain tightly but non-covalently associated after backbone cleavage. Nonetheless, the cleaved product binds to the Toll ectodomain and is capable of activating the Toll pathway in *Drosophila* Schneider cells. The ED₅₀ was similar to that of isolated C106 suggesting that backbone cleavage but not dissociation of the Spätzle pro-part is sufficient to render C106 active. The implications of these findings are discussed.

991A

Screen for modifiers of a dominant gain of function phenotype. Idella Christine Wilson, Tonia Von Ohlen. Biology, Kansas State University, Manhattan, KS.

Three pathways that work together to form the Central Nervous System (CNS) of the developing *Drosophila* embryo are Dorsal, Decapentaplegic (Dpp) and Epidermal Growth Factor Receptor (EGFR). These signaling pathways guide essential genes to necessary expression. A computational approach used to search the genome for clusters of transcription factor binding sites aided us in identifying new genes potentially regulated by these pathways. Using *in situ* hybridization, the gene CG10479 was found to show expression in the lateral column of the CNS as well as in larval imaginal tissues of the eye and wing. Further *in situ* hybridizations and genetic analysis showed CG10479 to be positively regulated by Dorsal and negatively regulated by EGFR. CG10479 encodes a Src Homology 2 (SH2) domain which suggests binding to phosphotyrosines. This action often plays a key role in phosphotyrosine signaling cascades, including Jun-Kinase (JNK) and Map-Kinase (MAPK). Using the UAS/Gal4 misexpression system, we have ectopically expressed CG10479 in the thorax, producing abnormal dorsal closure of the notum (using a pannier driver) and a strongly reduced scutellum (using an apterous driver). A suppressor/enhancer screen will provide us with an answer to the question of whether this gene is involved in the JNK and/or MAPK pathways as well as identify any potential interacting partners. Future projects include a deficiency screen to determine a loss of function phenotype and a loopless RNAi hairpin transgene as a mutagenesis technique.

992B

Role of proteoglycan in FGF signaling during tracheal branching and mesoderm migration in *Drosophila*. Dong Yan^{1,2}, Xinhua Lin^{1,2}. 1) Division Developmental Biology, Cincinnati Children's Hospital; 2) Molecular and Developmental Biology Program, 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 essential for the signaling activities of two *Drosophila* FGF receptors Heartless (Htl) and Breathless (Btl), which are required for mesoderm migration and tracheal branching, respectively. Embryos mutant for sugarless and sulfateless, two genes encoding the homologs of UDP-D-glucose dehydrogenase and heprin/heparan sulfate N-deacetylase/N-sulfotransferase, are defective in both Htl and Btl signaling. However, currently it is unclear which HSPG core proteins 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 (Dlp), 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, as well as another core protein syndecan, in FGF signaling in mesoderm migration and tracheal branching. Our data indicate that both HSPG core proteins and HS GAG biosynthesis enzymes can contribute to the specificity of FGF signaling.

993C

Functional analysis of *Drosophila* alpha 1-3 fucosyltransferase during development. Hideki Yoshida^{1,2}, Tomomi Ichimiya^{1,2}, Ryu Ueda^{2,3}, Satoshi Goto^{2,4}, Shoko Nishihara^{1,2}. 1) Bioinformatics, Soka University, Hachioji, Japan; 2) CREST, JST; 3) Genet. Strains, Natl. Inst. Fac. Pharm., Misawa, Japan; 4) MITILS. Machida, Tokyo, Japan.

Antibodies raised against horseradish peroxidase (HRP) recognize neural specific carbohydrate antigens (HRP epitopes) in *Drosophila* and other insects. Although the antibodies have been used as specific neural markers in studies on neural development, the function of the HRP epitopes is still unknown. In order to elucidate the function of the HRP epitopes during neural development in *Drosophila*, we reduced the level of alpha 1-3 fucosyltransferase (FucTA), which synthesizes an essential part of the HRP epitopes, by transgenic RNA interference during development. Reduction of FucTA in the wing imaginal discs resulted in increase of chemosensory bristles, thickening of wing veins and notching at the wing margin. The increase of chemosensory bristles phenotype was enhanced by reduction of the *Notch* or *Su(H)* gene. Furthermore, immunohistochemical analyses using antibodies revealed that knocking down of FucTA induced ectopic proneural gene expression and decreased Notch protein and signaling. These data suggest that FucTA has an important role on the Notch signaling during neural development in *Drosophila*. A detailed characterization of FucTA will be presented.

PATTERN FORMATION

994A

***Tarsel-less*, a gene involved in a new cell signalling event in *Drosophila* appendages.** Miranda J. Butler, Jose I. Pueyo, Maximo I. Galindo, Sarah A. Bishop, Juan Pablo Couso. School Life Sciences, University of Sussex, Brighton, United Kingdom.

We have identified a new gene with a crucial role in appendage development in *Drosophila*. In the leg, this gene, which we call *tarsel-less* (*tal*), is required for the development of the whole tarsal region from tarsus one to five. In a mutant for *tal* these regions do not develop. The phenotype is not due to cell death, but involves an alteration of the morphogenesis of the disc. The gene is not only expressed and required in the presumptive tarsal region, but also in antennae and wings, and in embryonic tissues of ectodermal origin which, undergo an invagination without losing their epithelial integrity. In the tarsus, *tal* controls the expression of *rotund* and *spineless*, and its expression is intercalated between those of *Bar* and *dacshund*. Interestingly, *tal* acts in a non-autonomous manner. We propose that *tal* identifies a gene product involved in a cell signalling process which a) activates *rotund* and *spineless* expression and b) represses *Bar* and *dacshund* expression.

995B

***ind* and *msh* are differentially sensitive to graded BMP signaling in the neuroectoderm.** Claudia M. Mizutani, Francisco F. Esteves, Evyia Vitola, Ethan Bier. Dept Biol, Univ California, San Diego, San Diego, CA.

The extracellular protein Short-gastrulation (Sog) blocks the BMP morphogen Decapentaplegic (Dpp) from repressing neural gene expression in the neuroectoderm. *sog* is initially expressed throughout the neuroectoderm and then fades in a dorsal-to-ventral fashion. As Sog levels decrease, the neuroectoderm is sequentially subdivided into three D/V domains defined by the expression of the homeobox genes *vnd*, *ind* and *msh*. The tight temporal correlation between these events suggests that a Dpp gradient may form within the

neuroectoderm by diffusion from the adjacent ectoderm, which provides cues for positioning the domains of neuroectodermal gene expression. Since BMP signaling represses neural gene activity, this BMP gradient might function by repressing expression of neural genes in a dosage sensitive fashion. To test this hypothesis, we created lateralized embryos that lack normal D/V polarity but express uniform levels of nuclear Dorsal, and asked whether the neural patterning could be restored along the A/P axis in response to a stripe of *dpp* expression. In embryos that express mid-neuroectodermal levels of Dorsal, *ind* is ubiquitously expressed, while *msh* is repressed by *ind*. When Dpp signaling is activated in a stripe in these embryos, *ind* and *msh* form two distinct domains with *msh* expression nearest to the Dpp source. These results indicate that *ind* is more sensitive to BMP repression than *msh*. *msh* expression is confined to cells with low levels of *ind*, presumably due to relief of *ind* mediated repression of *msh*. We also provide evidence that *ind* is more sensitive than *msh* to Dpp repression in embryos with a normal Dorsal gradient, but varying levels of Sog and Dpp. We propose that graded BMP signaling may have been the primary ancestral mechanism for subdividing the neuroectoderm in both vertebrates and invertebrates and that D/V patterning mediated by Dorsal was added subsequently during the evolution of *Drosophila*.

996C

The dependence of *even-skipped* early stripe 1 on *Eve* reveals a functional phase of *eve* expression which precedes early stripe expression. Charles Sackerson. Dept Biol, Iona Col, New Rochelle, NY.

Expression of the pair-rule gene *even-skipped* (*eve*) is traditionally considered to be composed of early stripes, late stripes, and secondary expression in neural, muscle, and proctodeal primordia. However, *eve* expression is first detected before cellularization as a broad gap-like band extending from about 50 - 75% egg length. We have investigated whether this pre-early expression could be responsible for the *Eve*-dependence of *eve* early stripe 1. Elimination of the early stripe 1 enhancer from an *eve* locus transgene allows the expression of pre-early *Eve* in the absence of forming early stripe 1. In this background, an early stripe 1 reporter is expressed. Early stripe 1 expression is also rescued by *Eve* expressed from the *hunchback* promoter. We propose that pre-early *eve* is a functional phase of *eve* expression, required for early stripe 1, and hence for downstream events such as cephalic furrow formation and *Deformed* expression.

997A

The role of the tumour suppressor *Fat* in early differentiation and patterning of the *Drosophila* eye. Elizabeth A. Silva, Helen McNeill. Developmental Patterning, LRI, Cancer Research UK, London, UNITED KINGDOM.

Fat is an atypical member of the cadherin superfamily that has a well-documented role in planar cell polarity, including specification of dorsal versus ventral polarity of the ommatidia. Widely recognized but less understood is the function of *Fat* as a tumor suppressor. Hypomorphic allele combinations result in larvae with exceedingly overgrown imaginal discs, usually resulting in death by pupal stages. We are investigating this role for *Fat* and its consequences on patterning of the fly eye using cell biological and genetic approaches.

998B

Compartmental cross-talk during wing disc size regulation. Meng-Ping Tu, Laura A. Johnston. Dept Genetics & Development, Columbia Univ, Col P&S, New York, NY.

Developing *Drosophila* organs, such as wing imaginal discs, possess intrinsic information that allows them to reach an appropriate and reproducible size. The mechanism by which organ size is sensed and monitored during development is unknown, but it may require the subdivision of cells into compartments. The earliest compartmental subdivision in the fly separates anterior (A) from posterior (P) cells, permanently partitioning them and their progeny. Mosaic wings containing A and P compartments that grow at significantly different rates are normal size at the end of development. This and other observations have led to the idea that each compartment contributes to overall size by growing independently of the other. We are testing this hypothesis by analyzing specific growth parameters when the A and P compartments are induced to grow at different rates. We are using the *rp14* mutant, Minute (3)66D, to create mosaic discs in which cells in one compartment are rescued by the expression of an *Rpl14* transgene. Although M(3)66D wing discs are smaller than controls at early stages of development, the larvae develop with normal timing until the third instar, when they require an additional day prior to wandering stage. By wandering stage, wing disc size is the same in wildtype, RPL14-rescued, and non-rescued M(3)66D mutants. We find that expression of the rescuing *Rpl14* transgene in posterior M(3)66D cells shortens the extended larval period by half, and increases the size of the posterior compartment throughout the third instar. Remarkably, RPL14-rescue of the M(3)66D P compartment also affects the rate of development of the A compartment. We will present this and other data suggesting that cross-talk occurring between the A and P compartments controls their rate of growth.

999C

The recent origin of two Abdominal-B proteins and their relationship to reduction of the number of abdominal segments in Diptera. John H. Yoder, Sean B. Carroll. University of Wisconsin and HHMI, Madison, WI.

The posterior Hox gene Abdominal-B (AbdB) represents a unique example of generating functional complexity among the Hox genes of *Drosophila*. Dual promoters drive two transcript classes generating distinct protein isoforms. These proteins are expressed in non-overlapping patterns in posterior abdominal segments (A5-A10) and have discrete genetic functions. The two transcript classes (m and r) share four coding exons, while the m-transcript has a unique 5' exon encoding a Q-rich N-terminus. The r protein is expressed only in pA8-A10 where it represses m transcription.

Previous investigations of AbdB expression in other insects and arthropods have not distinguished possible m and r specific transcripts, however these studies consistently observed patterns reflecting *Drosophila* r-specific expression (pA8-A10). These observations suggest primitive insects either lack an m-equivalent protein or that m and r functions reside either in proteins whose expression overlap or a single protein with composite functions. It has been suggested that the anterior expansion of AbdB expression in *Drosophila* contributed to the reduced size of its abdomen (7 adult segments compared to 10 in primitive insects).

The abdomen of basal diptera is reduced to 8 adult segments, exhibiting an intermediate morphology between primitive insects and *Drosophila*. We have investigated the evolution of the AbdB gene in the Dipteran lineage and have found that the two transcript classes and expression patterns have recent origins. Basal diptera have a single AbdB transcript expressed in A8-A10 but possess a 5' exon with significant similarity to that of the *Drosophila* m transcript. The r-transcript and protein therefore represent a derived character of Brachycerous diptera. Furthermore, it appears a single AbdB protein in basal diptera is capable of promoting posterior segment reduction.

GAMETOGENESIS AND SEX DETERMINATION

1000A

Phenotypic and molecular characterization of lucky luke (luke) and rantanplan (rpn), two novel genes required for stem cell division or stem cell maintenance. Thomas FELLNER¹, Cordula SCHULZ², Margaret T. FULLER¹. 1) Stanford University School of Medicine, Department of Developmental Biology, Stanford, CA; 2) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

The continuous supply of highly differentiated but short-lived cell types, such as blood, skin, intestinal epithelium, as well as male germ cells, throughout the life of an organism depends on the regulated behavior of adult stem cells. Such cells are relatively undifferentiated and possess a long-term potential to divide and produce two types of daughter cells - those that retain stem cell identity and those that initiate differentiation along a defined lineage. Due to these characteristics, adult stem cells harbor promising potential for regenerative medicine. The *Drosophila* male germ line is emerging as a powerful model system for the study of the mechanisms that regulate stem cell behavior in situ, in the context of their normal microenvironment. In a forward genetic screen for viable but male-sterile mutants we identified several new mutants that affect male germ line stem cell behavior. In two intriguing mutants, luke and rpn, we observed that testes lack earlier stages of spermatogenesis while later stages are present, indicating that spermatogenesis is initiated but that stem cell function is not maintained. Further analysis using specific markers revealed that the hub, a cluster of somatic cells at the testis apical tip that functions as a component of the germ line stem cell niche, is retained in the mutants and surrounded by stem cells. This indicates that male germ line stem cells in these two mutants are initially functional but lose their capability to produce differentiating daughters over time. Thus wild-type function of luke and rpn could be required for stem cells to continue dividing or for stem cell daughters to differentiate along their defined lineage or to escape cell death. The phenotypic and molecular characterization of these new genes will be the topic of my presentation.

1001B

Life and Death in *Drosophila*: *Wolbachia* throws the switch. Harriet Lorena Harris¹, Zoe L. Veneti², Gregory D. D. Hurst², Henk R. Braig³. 1) Biology, Concordia University College, Edmonton, Alberta, CANADA; 2) Biology, University College London, London, UK; 3) Biological Sciences, University of Wales Bangor, Bangor, Gwynedd, UK.

Drosophila species, like many other insects, are hosts to the maternally inherited endosymbiont *Wolbachia pipientis*. *Wolbachia* is the causative agent of such diverse reproductive anomalies as thelytokous parthenogenesis in parasitoid wasps (all embryos develop as females), cytoplasmic incompatibility (CI) in dipterans (embryos resulting from matings between uninfected females and infected males die) and male-killing in *D. bifasciata* (male embryos are selectively killed). Both parthenogenesis and CI appear to be brought about by a modification in the behaviour of zygotic chromosomes prior to the first mitotic division. In *D. simulans*,

Wolbachia (WRi strain) somehow modifies the sperm during maturation and, following fertilization, the male pronucleus forms fragmented chromosomes which do not enter mitosis in synchrony with the female chromosomes. The result is aneuploidy and embryonic death. In crosses between males and females which are both infected with the same *Wolbachia* strain, the modified sperm is rescued and development of the embryos proceeds normally. In contrast, the mechanism of *Wolbachia*-induced male killing has not previously been determined. Our recent results show that male-killing in *D. bifasciata* involves pathological changes in XY embryos beginning at stage 12 and leading to apoptosis. Unlike the infection of *D. melanogaster* by Wmel, which causes premature death of the flies, the male-killing infection does not involve excess proliferation of *Wolbachia*, nor does it involve the inappropriate expression of the Sex-lethal gene as seen in *D. melanogaster* infected with the male-killing bacterium *Spiroplasma*. Thus, phylogenetically related *Wolbachia* utilize very different strategies to manipulate their hosts, which suggests that this bacterium has evolved diverse cellular mechanisms for both propagation and survival. These mechanisms will be the subject of this talk.

1002C

BMP Signaling is Required for Controlling Somatic Stem Cell Self-Renewal in the *Drosophila* Ovary.

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Stem cells have the capacity to self-renew and produce differentiated progeny that replenish lost cells in the adult tissues. They have been shown to be regulated by microenvironments or niches. However, extrinsic signals from niches still remain poorly defined in many systems. Somatic stem cells (SSCs) in the *Drosophila* ovary have emerged as a powerful system for identifying extrinsic signals that control stem cell self-renewal. In this study, we show that *Gbb*, a *Drosophila* homologue of BMP5-8, is required for controlling SSC self-renewal and proliferation. SSCs express the BMP responsive gene, *Dad*, indicating that they are capable of responding to the BMP signal. SSC/follicle cell progenitor proliferation is retarded in *gbb* mutants. The marked SSCs mutant for BMP downstream signal transducers, such as *punt*, *tkv* and *mad*, are lost much faster and divide slower than the marked wild-type ones, indicating that the BMP signaling is essential for controlling SSC maintenance and division. Moreover, we show that the SSCs defective in BMP signaling lose the capacity to self-renew but not viability. Overexpression of a constitutively active type I receptor *tkv* prolongs SSC lifespan. Therefore, our study clearly demonstrates that BMP signaling controls SSC self-renewal and proliferation in the *Drosophila* ovary. Since BMPs are expressed in various stem cell compartments of the adult mammalian tissues and the stem cell self-renewal property has been conserved from *Drosophila* to mammals, we would expect that BMP signaling controls self-renewal and proliferation of adult stem cells in other systems.

1003A

Altered cellularization times, rather than the X:A ratio, appear to determine the sex of haploid and triploid embryos.

Jerome Quintero, Hong Lu, James W. Erickson. Dept. of Biology, Texas A&M University, College Station, TX.

Sex determination is a textbook example of how two-fold differences in protein concentrations signal cell fates. Conventionally, *Sex-lethal* (*Sxl*) is activated by an X chromosome to autosome ratio (X:A) of 1.0, but left inactive if the X:A is the male value 0.5.

We and others have posited that sex determination is better explained as an X-counting process rather than as a X:A ratio-determining one. Accordingly, X dose is defined by the male or female concentrations of four X-linked signal element proteins (XSEs); with the female XSE concentrations activating the *Sxl*/*Pe* promoter. Three facts, however, appear to better support the classic X:A model. First, an autosomal element, *dpn*, exists. Second, haploid 1X/1A cells are female. Third, triploid 2X/3A flies (X:A = .67) are sexual mosaics.

We examined the action of the autosomal repressor *Dpn* and the effects of haploidy and triploidy on *Sxl*/*Pe*. Elimination of *Dpn* binding sites caused ectopic *Pe* activity in males and an earlier onset in females. However, expression was always higher in females, implying that the sexes are molecularly distinct in the absence of autosomal and maternal inhibitors. To determine how ploidy influences sex, we examined *Pe* in haploid and triploid embryos. The female character of haploids, and the intersexuality of 2X triploids, appear primarily to be consequences of altered cellularization. Haploid embryos express *Sxl* because they undergo an extra cell-cycle that increases the number of X chromosomes, rather than because their X:A = 1.0. Conversely, premature cellularization in triploids prevents 2X/3A embryos from fully activating *Pe* and, thus, from establishing stable uniform *Sxl* expression. Our findings imply that *Sxl*/*Pe* responds primarily to the differential dose of the XSE genes, rather than to the value of the X:A ratio, with autosomal and maternal repressors fine-tuning the X-counting process.

1004B

The role of α -endosulfine in the proliferative response to nutrition of the *Drosophila* ovary. Jessica Rivera, Daniela Drummond-Barbosa. Cell & Developmental Biology, Vanderbilt University, Nashville, TN.

It is poorly understood how adult tissues adjust proliferation rates in response to external stimuli such as nutrition, and our laboratory studies this question in the *Drosophila* ovary. We have previously shown that germline and follicle cells adjust their growth and division rates in response to nutrition, and that this response requires the insulin pathway and the α -endosulfine homologue, a potential regulator of insulin secretion. Our goal is to study the *in vivo* molecular mechanism of action and the regulation of *Drosophila* α -endosulfine (*dendos*).

α -endosulfine has been proposed to induce insulin secretion by binding to the regulatory subunit, SUR1, of a K_{ATP} channel, based on studies in cultured mammalian pancreatic β cells. Other studies suggest that α -endosulfine binds to Ca^{2+} channels and inhibits insulin release. These data are therefore inconclusive and did not result from *in vivo* analysis. We have shown that *Dendos* is expressed in the ovary and brain, which is the source of insulin-like peptides. *dendos* is also required non-cell autonomously for the proliferative response to nutrition, suggesting that it acts via a secreted factor. We hypothesize that *Dendos* binds and regulates the *Drosophila* SUR1 homolog (*Sur*) *in vivo* to promote insulin-like peptide secretion and regulate cell proliferation in the ovary. We have detected *sur* expression by RT-PCR in the adult ovary and head, two sites of *Dendos* expression. Our laboratory has also generated antisera against the *Drosophila* *Sur* protein and we are currently characterizing their specificity. To determine if *Dendos* acts via *Sur*, we are currently studying the loss of function phenotype by analyzing a *piggyBac* insertion in *sur* and by performing RNAi with inverted repeat transgenes. An *in vitro* expression cloning (IVEC) screen is also in progress to identify *Dendos* interacting proteins, which may include regulators of its activity.

1005C

Stem Cell Self-Renewal Controlled by Chromatin Remodeling Factors. Rongwen Xi¹, Ting Xie^{1,2}.

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One of the most important issues in stem cell biology is to define niche signals and intrinsic factors that control a stem cell's self-renewal. Even though quite a few niche signals have been identified in different systems, it remains unclear how stem cells respond to niche signals at the chromatin level. Germline stem cells (GSCs) and somatic stem cells (SSCs) in the *Drosophila* ovary represent attractive systems to study relationships between stem cells and extrinsic signals. Here, we report that ATP-dependent chromatin remodeling factors, ISWI and DOM, control GSC and SSC self-renewal in the *Drosophila* ovary, respectively. The GSCs mutant for *iswi* are defective in responding to extrinsic BMP signals that are essential for controlling GSC self-renewal and are thus lost rapidly due to differentiation. Furthermore, *iswi* is also required for promoting GSC division. Finally, *dom* is required for maintaining SSCs. Therefore, our study, for the first time, shows that the ATP-dependent chromatin remodeling factors control stem cell self-renewal and further suggests that different stem cell types could utilize different classes of ATP-dependent chromatin remodeling factors to control their self-renewal.

ORGANOGENESIS

1006A

Overexpression of troponin T in *Drosophila* muscles causes a decrease in the levels of thin filament proteins. Raquel Marco-Ferreres, Juan J. Arredondo, Margarita Cervera. Departamento de Bioquímica and Instituto de Investigaciones Biomédicas, UAM-CSIC, Madrid, Spain.

Formation of the contractile apparatus in muscle cells requires co-ordinated activation of several genes and the proper assembly of their products. To investigate the role of TnT (troponin T) in the mechanisms that control and co-ordinate thin-filament formation, we generated transgenic *Drosophila* lines that overexpress TnT in their indirect flight muscles. All flies that overexpress TnT were unable to fly, and the loss of thin filaments themselves was coupled with ultrastructural perturbations of the sarcomere. In contrast, thick filaments remained largely unaffected. Biochemical analysis of these lines revealed that the increase in TnT levels could be detected only during the early stages of adult muscle formation and was followed by a profound decrease in the amount of this protein as well as that of other thin-filament proteins such as tropomyosin, troponin I and actin. The decrease in thin-filament proteins is not only due to degradation but also due to a decrease in their synthesis, since accumulation of their mRNA transcripts was also severely diminished. This decrease in expression levels of the distinct thin-filament components led us to postulate that any change in the amount of TnT transcripts might trigger the down-regulation of other co-regulated thin-filament components. Taken together, these results suggest the existence of a mechanism that tightly co-ordinates the expression of thin-filament genes and controls the correct stoichiometry of these proteins. We propose that the high levels of unassembled protein might act as a sensor in this process.

1007B

The *Tbx20*-related genes *midline* and *H15* are required for the proper patterning and differentiation of the dorsal vessel by activating cardioblast-specific *tinman* expression. I. Reim¹, P.C.H. Lo¹, J. Mohler², M. Frasch¹. 1) Mol., Cell & Dev. Biology, Mt. Sinai School of Medicine, New York, NY; 2) Dept. of Biol. Sci., Barnard College, New York, NY.

Two classes of T-box genes, the *Tbx6*-related *Dorsocross* genes (*Doc1-3*) and the *Tbx20* orthologs, *midline* (*mid* a.k.a. *H15r/nmr2*) and *H15*, are expressed in the *Drosophila* equivalent of the heart, i.e., the dorsal vessel. While *Doc* genes are expressed more broadly in the early cardiogenic mesoderm and later in a subset of mature cardioblasts, *mid* and *H15* are specifically expressed in all cardioblasts during embryogenesis. We demonstrate that *mid* and *H15* have important roles in establishing the metameric patterning of cardioblast identities, but not in specifying cardioblasts as such. Mutant embryos lacking the activity of *mid*, or both *mid* and *H15*, form dorsal vessels with largely normal numbers of cardioblasts and pericardial cells. Furthermore, the mutant cardioblasts express general cardioblast markers such as Mef2 at normal levels. However, the expression of *tinman* (*tin*), which normally occurs in four out of six cardioblasts in each hemisegment, is almost completely abolished. Conversely, overexpression of *mid* throughout the mesoderm can ectopically activate *tin*, with and without extra cardioblast formation. These genetic data suggest that *tin* is a downstream target of *mid*. DNase I footprinting has revealed several binding sites for the T-box domain of *mid* within the tinC cardiac enhancer of *tin*, implying that *mid* may directly regulate the late cardiac expression of *tin*. In contrast to *tin*, expression of the *Doc* genes, which is normally restricted to two Tin-negative cardioblasts per hemisegment, is strongly expanded into the majority of cardioblasts in *mid* mutant and *mid* + *H15*-deficient embryos. Altogether our data demonstrate that *mid*, and to a lesser degree *H15*, are required for the normal functional diversification of cardioblasts, including re-activation of *tin* and the expression of *tin*-dependent terminal differentiation genes in a segmental subpopulation of cardioblasts.

NEUROGENETICS AND NEURAL DEVELOPMENT

1008C

Gene expression profiling during gliogenesis in *Drosophila*. Angela Becker¹, Benjamin Altenhein¹, Boris Beckmann², Christian Busold², Jörg Hoheisel², Gerd Technau¹. 1) Institute of Genetics, Mainz, Germany; 2) DKFZ Heidelberg, Germany, Member of the Heidelberg FlyArray Consortium.

The expression of the master regulator gene glial cells missing (*gcm*) induces gliogenesis in the embryonic central nervous system. Whereas the function of *gcm* as a binary switch between neuronal and glial fate is well described, little is known about further genes that promote glial fate and regulate glial cell differentiation. Towards identifying genes acting downstream of *gcm*, we performed a genome-wide microarray screen. In order to achieve as high an accuracy as possible, we designed two antagonistic screens: ectopic expression of *gcm* in the CNS to transform all presumptive neurons into glial cells, and the *gcm* loss-of-function, which shows a lack of all lateral glial cells. Both gain- and loss-of-function mutants were compared to wildtype on a whole-genome microarray („Flyarray“, Heidelberg) comprising about 21.400 predicted genes. For both screens we accomplished a time course experiment throughout embryogenesis. These methods enabled us to identify differentially regulated genes from different developmental stages and from different mutant conditions. Here we present the outcome of our screen and show that carefully selected combinations of filters regarding expression intensity, fold regulation and reproducibility are useful tools to achieve a reliable detection of target genes in microarray experiments. By examining the gain- of-function screen and the loss-of-function screen separately as well as in combination we could select genes showing different temporal expression profiles throughout gliogenesis. Temporal expression of these genes in all or subsets of glial cells correlates to our microarray data as revealed by in situ-hybridization.

1009A

Aging of neuroblast-during the late stages of embryogenesis and larval period. Takako Isshiki, Ayumi Kusano. Research Strains Center, National Institute of Genetics, Mishima, Shizuoka, Japan.

During development, neuroblasts generate diverse cell types in an invariant order, changing their property over time. Although substantial progresses have been made in understanding the molecular mechanisms of how neuroblasts generate diverse cell types over time, they still remain largely unknown. We previously showed that *Drosophila* embryonic neuroblasts sequentially express the transcription factors Hunchback, Krüppel, Pdm and Castor over time. However, most neuroblasts divide 10 times on average after the onset of Castor expression during embryogenesis. Thus, there must be subsequent molecular mechanisms to regulate temporal specification in a neuroblast lineage.

For the purpose of identifying yet unknown factors involved in temporal specification within neuroblast lineage, we searched for the genes whose transcripts are expressed in the embryonic CNS in a temporally regulated

manner. As a result, we found a few transcription factors expressed later than Castor in most neuroblasts. In *castor* mutant embryos, expression of the newly found transcription factors disappears, though neuroblasts continue asymmetric divisions and generation of their progeny. These indicate that Castor plays crucial roles for proceeding developmental program controlling temporal specification at the late stages. To enable further investigation of temporal specification at the late stages, we have elucidated the precise order of expression of the late stage specific transcription factors within a lineage. In addition, we have revealed that larval neuroblasts go through part of the exact sequence of the expressions observed in embryonic neuroblasts at the late stages. Hence, both neuroblasts appear to utilize the same molecular mechanisms despite their differences in the period and number of division. This fact strongly suggests that intrinsic mechanisms within a neuroblast lineage have significant roles in temporal specification throughout development.

1010B

New regulators of R7 targeting specificity on the X chromosome. Marta Morey, Aljoscha Nern, Tory Herman, Larry Zipursky. HHMI, Dpt Biological Chemistry, D.Geffen Sch. Medicine, UCLA, Los Angeles, CA.

We are studying the molecular mechanisms by which neurons select the appropriate targets using the R7 photoreceptor neurons in *Drosophila* as a model system. R7 neurons are sensitive to UV light and project to a specific layer in the medulla, a part of the optic lobe. Proteins involved in the R7 targeting process include three cell surface receptors: the homophilic cell adhesion molecule N-cadherin (N-cad) and two receptor tyrosine phosphatases Lar and PTP69D. In the absence of N-cad or Lar, R7 growth cones initially target to the correct layer in the medulla, but retract to the R8 layer at later stages. To identify additional genes required for R7 targeting, we have screened mosaic animals in which only the R7 neurons are mutant. This screen used an R7-dependent behavioral selection (i.e. the choice between UV and green light), as a primary assay. Subsequent single cell histological analysis of the isolated behavioral mutants identified three main classes of targeting phenotypes: layer selection defects, abnormal projections within the correct layer and ectopic localization of a synaptic marker. On the X chromosome mutants of all three classes were identified. Among these, three have a layer selection phenotype similar to *N-cad*. One of them, which we named *curta*, has been characterized molecularly. It encodes a conserved protein of unknown function. *curta* appears to genetically interact with *N-cad* and has a similar developmental phenotype. We are taking both genetic and biochemical approaches to understand the role of *curta* in R7 targeting and to uncover its relationship with other genes required in this process.

1011C

***Drosophila* Model of Fragile X Syndrome.** Luyuan Pan, Yong Q Zhang, Heinrich Matthies, Elvin Woodruff III, Kendal Broadie. Dept. of Biological Science, Kennedy Center for Research on Human Development, Vanderbilt University, Nashville, TN. 37215.

Fragile X syndrome (FraX) is the most common form of inherited mental retardation. The disease is caused by the silencing of *fragile X mental retardation 1 (fmr1)* gene, which encodes the RNA binding translational regulator FMRP. Recent work has focused on putative roles of FMRP in regulating the development and plasticity of neuronal synaptic connections. *Drosophila* has sole homolog of human *fmr1*, *dfmr1*. Our lab firstly reported the *Drosophila* model of Fragile X syndrome. We found dFMRP is a negative regulator of neuronal architecture and synaptic differentiation in both peripheral and central nervous systems. Loss of dFMRP causes the over extension of both axon and dendrites, and abnormal pre-synaptic terminal structure. We also found a potent direct downstream target of dFMRP, Futsch, which is the fly homolog of mammalian MAP1B. We found the evidence that dFMRP regulates the Cytoskeleton dynamics. Loss of dFMRP causes the miss of central pair microtubules in the *Drosophila* sperm tail axoneme, which is another major dFMRP expression tissue except CNS. The proteomic analysis we did identified several potential functional targets of dFMRP. The finding of two important factors in monoamine synthesis pathways regulated by dFMRP, indicated the regulation function of dFMRP on dopamine level in CNS. In the next work, we will continuously make efforts on finding novel downstream targets and functional modifier of dFMRP, and go deep into the molecule mechanism of dFMRP in CNS.

1012A

Clathrin-dependent endocytosis role in pupal eye development. Susana Peralta¹, Javier Vinós^{1,2}. 1) Dpto Bioquímica, Universidad Autónoma de Madrid, Spain; 2) Instituto de Investigaciones Biomédicas, CSIC, Madrid, Spain.

We are studying the role of Clathrin-dependent endocytosis in the development of *Drosophila* retina during pupal stages. Analysis of retinal development of a mutant allele in the Clathrin Heavy Chain, *Chc4*, indicates a direct role of Clathrin-dependent endocytosis in secondary and tertiary pigment cells maturation and long-term survival, but not in their specification.

The *Chc4* pupal retina shows a correct number of ommatidial cells, yet its development is delayed and mechanosensory organs are misplaced with a higher frequency than in control retinas. More strikingly, all

secondary and tertiary pigment cells in the *Chc4* retina die during retinal maturation. This cell death phenotype is due to apoptosis, since it is suppressed by expression of the baculovirus apoptotic inhibitor p35 in retinal cells. The apoptotic cell death of retinal pigment cells requires Notch function. This result suggests that Clathrin-dependent endocytosis participates in Notch control of excess retinal cells removal by apoptosis.

We have sequenced the *Chc4* allele and have found one change in a conserved alanine position 1076 to a threonine. We have cloned the *Chc* wild type sequence with this change in a P-UAS vector and generated different transgenic lines. Using the UAS- GAL 4 system expression we have shown that this aminoacidic change is responsible for the *Chc4* phenotypes.

1013B

A novel role for *fog* as a factor involved in axo-glia interactions in *Drosophila*. Anuradha Ratnaparkhi, Kai Zinn. Biology, Caltech, Pasadena, CA.

The gene *folded gastrulation (fog)* encodes a secreted glycoprotein whose role in epithelial morphogenesis during gastrulation in *Drosophila* embryos has been well characterized. Absence of Fog results in an irregular ventral furrow. We have now examined Fog's roles during nervous system development. We find that *fog* is expressed in a subset of longitudinal glia in the embryonic CNS. We studied the consequences of loss of Fog for CNS development by examining *fog* mutant embryos in which *fog*'s early phenotype was rescued by *fog* expression from the *huckebein* promoter. We also eliminated Fog expression in the glia using transgenic *fog* RNAi (UAS*dsfog*). Both perturbations affect the formation of the lateral longitudinal fascicles in the CNS.

We also examined potential relationships between Fog and the receptor tyrosine phosphatase DPTP52F. We had observed that a *fog*-like ventral furrow phenotype is present in *Ptp52F* mutants (Alice Schmid and K.S., unpublished), suggesting that Fog might signal through DPTP52F expressed on the ventral furrow cells. We find that overexpression of Fog in neurons results in ectopic midline crossing in the CNS. This phenotype is suppressed in *Ptp52F* mutant backgrounds, suggesting that DPTP52F is required for transduction of the Fog signal that leads to midline crossing. In summary, our data suggest that Fog is a glial signal that affects axon guidance, and that some of Fog's effects on the neurons may be mediated through DPTP52F.

1014C

DmRic8 mediates asymmetric cell divisions through regulating heterotrimeric G proteins in neuroblasts and sensory organ precursors. Hongyan Wang, Kian Hong Ng, Hongliang Qian, William Chia, Fengwei Yu. Temasek Life Sciences Lab, Singapore, Singapore, Singapore.

Asymmetric cell division is a universal mechanism utilised to generate cellular diversity during development. The *Drosophila* embryonic central nervous system (CNS) derives largely from neural progenitors called neuroblasts (NBs), which undergo asymmetric cell divisions along the apical-basal axis to give rise to two daughters of distinct fate and size. Heterotrimeric G proteins G α i and G β play important but distinct roles in asymmetric division of *Drosophila* neuroblasts. Here we describe that a novel player Ric8 is a putative GEF for G α i and is involved in asymmetric cell division. Ric8 is required for the proper localization of G α i, Pins and Insc in dividing NBs. Ric8 is also involved in spindle orientation of NBs and mitotic domain 9 cells. Ric8 complexes with G α i and Pins and interacts preferentially with GDP-bound form of G α i. All our data suggest that Ric8 completes a receptor-independent activation of G protein cycle in asymmetric cell division of *Drosophila* neuroblasts.

1015A

Developmental context for Notch-dependent PROS expression in longitudinal glial cells. Yoshihiro Yuasa¹, Yasushi Hiromi^{1,2,3}. 1) Dept Developmental Genetics, National Inst Genetics, Mishima, Japan; 2) SOKENDAI; 3) CREST, JST, Japan.

Notch signaling is utilized in choosing binary cell fates in many aspects of organogenesis. The outcome of Notch activation is context-dependent, resulting in the activation of different target genes in each developmental process. The molecular nature of the developmental context that provides target specificity is poorly understood. One process that Notch activity determines a particular cell type in the nervous system is the subtype specification of longitudinal glial cells in the embryonic central nervous system. In each hemisegment, ten longitudinal glial cells are generated from a single precursor, longitudinal glioblast, that divides while migrating towards the midline. These glial cells comprise two subtypes, because six of them activate Notch signaling and express a homeodomain transcription factor Prospero (PROS), whereas the other four are PROS-negative. PROS expression is dependent on Notch activation, and conversely, when Notch is artificially activated all ten longitudinal glial cells become PROS-positive. To identify the developmental context for Notch-dependent glial subtype specification we are analyzing the regulation of PROS expression in the longitudinal glia. We found that PROS expression in the longitudinal glia requires three transcriptional factors: homeodomain protein REPO, ets transcription factor Pointed and AT-rich binding protein Dead Ringer/Retain

(DRI). An enhancer element of the *pros* gene that recapitulates the expression pattern of PROS in the longitudinal glial cells includes the consensus binding sites for all three transcriptional factors. These results suggest that these three transcriptional factors provide a context for Notch-dependent PROS expression in the longitudinal glia.

1016B

Locomotion defects regulate heterotrimeric G protein signalling via two distinct modes of action during *Drosophila* neuroblast asymmetric divisions. Fengwei Yu¹, Hongyan Wang¹, Hongliang Qian¹, Rachna Kaushik², Mary Bownes³, Xiaohang Yang², William Chia¹. 1) Temasek Life Sciences Laboratory, National University of Singapore, Singapore 117604; 2) Institute of Molecular and Cell Biology, 61 Biopolis Drive, Proteos, Singapore 138673; 3) Institute of Cell and Molecular Biology, University of Edinburgh, King's Buildings, Edinburgh, EH9 3JR UK.

Heterotrimeric G proteins mediate asymmetric division of *Drosophila* neuroblasts. Free G $\beta\gamma$ appears to be crucial for the generation of an asymmetric mitotic spindle and consequently daughter cells of distinct size. However, how G $\beta\gamma$ is released from the inactive heterotrimer remains unclear. Here we show that Locomotion defects (Loco) interacts and colocalises with Gai and, through its GoLoco motif, acts as a guanine nucleotide dissociation inhibitor (GDI) for Gai. Simultaneous removal of the two GoLoco motif proteins, Loco and Pins, results in defects which are essentially indistinguishable from those observed in *G β 13F* or *Gy1* mutants, suggesting that Loco and Pins act synergistically to release free G $\beta\gamma$ in neuroblasts. Furthermore, the RGS domain of Loco can also accelerate the GTPase activity of Gai to regulate the equilibrium between the GDP and the GTP bound forms of Gai. Thus, Loco regulates heterotrimeric G protein signalling via two distinct modes of action during *Drosophila* neuroblast asymmetric divisions.

NEURAL PHYSIOLOGY AND BEHAVIOR

1017C

slowpoke induction underlies ethanol tolerance in *Drosophila*. Nigel Atkinson, Roshani Cowmeadow, Alfredo Ghezzi, Yazan Al-Hasan, Harish Krishnan. Section Neurobiology, Univ Texas, Austin, TX.

Ethanol is one of the most commonly used drugs in the world. Research in the last decade has suggested that the rapid effects of ethanol are caused by direct interaction with ligand-gated ion channels, thereby altering their function and leading to an overall depression of the nervous system. We are interested in the compensatory mechanisms used by the nervous system to counter the effects of ethanol intoxication. We have found that the ion channel Slowpoke plays a very important role in the response to these drugs. Slowpoke is a calcium-activated potassium channel that is expressed widely throughout the musculature and nervous system of the fly. We have shown that transcription of the slowpoke gene is increased after ethanol sedation and that up-regulation is coincident with the acquisition of rapid alcohol tolerance. Flies that carry slowpoke null mutations are unable to acquire tolerance, and flies that over-express slowpoke display an inherent alcohol resistance. While some other channel genes also increase in expression after sedation, mutations in these channel genes did not prevent the acquisition of tolerance. Therefore slowpoke appears to play an unusual and critical role in the production of alcohol tolerance.

1018A

The p24 intracellular trafficking proteins and oviposition behavior. Ginger E. Carney. Dept Biol, Texas A&M Univ, College Station, TX.

Drosophila reproductive behaviors are regulated by the expression of many genes and fall under the control of transcription factors in the sex-determination hierarchy-- *Sex-lethal*, *doublesex*, *fruitless*, and *dissatisfaction*. The mating behavior of *Drosophila* females changes during the course of their adult life and is dependent upon age and mating status. Young females reject male mating advances, but once they are approximately 1 day old they become receptive to a male's overtures. In mated females, components found in the male's ejaculate stimulate the processes of ovulation and egg-laying behavior and also render the females unreceptive to mating for several days. We have identified mutations in several genes that result in loss of egg-laying behavior. One of these genes, *logjam*, has amino acid sequence homology to intracellular vesicle trafficking proteins called p24 (EMP24/GP25) proteins (Carney and Taylor, 2003) whose functions within cells are controversial. Loss-of-function mutations in two other p24 genes, *baiser* and *eclair*, cause impaired egg-laying ability as well as a maternal effect on embryonic patterning (Bartoszewski et al. 2004).

p24 proteins have been postulated to serve as cargo receptors for recognition of proteins that must be loaded into cytoplasmic vesicles, to function in vesicle biogenesis, to function as quality control agents in movement of proteins through the secretory system, and to aid in correct protein folding and posttranslational modification. We have characterized the developmental expression patterns for all of the p24 genes and find that they are

widely expressed. Our particular interest is the *logjam* mRNA, which is expressed in the central nervous system (CNS) as well as eggs that are midway through the oogenic process. We are currently characterizing *logjam* mutants at the molecular level to understand the function of this gene product in an essential reproductive behavior.

1019B

Candidate Acps in the stalk eyed fly *Cyrtodipsis dalmanni*. L.S. Corley, E. McConnell, K. Kraaijeveld, P. Hadrill, K. Fowler, A. Pomiankowski, T. Chapman. Biology, University College London, London, United Kingdom.

The stalk eyed fly *Cyrtodipsis dalmanni* is a lekking species subject to strong sexual selection due to female mate preference for larger male eyespan, with extremely high rates of multiple mating. Under natural conditions, males form nocturnal leks that attract up to 20 females which then mate several times with the resident male. The leks are labile, reforming each evening. Females also mate opportunistically when they encounter males during the day. This mating system promotes severe sperm competition between ejaculates of different males. Similar behaviour is seen under laboratory conditions; both lekking and opportunistic mating occurs at high rates, with males and females mating between 10-30 times per day. Males clearly gain from multiple mating as this enables them to transfer more sperm. Females also gain direct benefits from multiple mating as single or few matings do not provide enough sperm to ensure high fertility. However, the degree of multiple mating far exceeds what is necessary to ensure full fertility, and we now have evidence that females continuously exposed to males suffer longevity costs compared to female-only controls. *C. dalmanni* allows us to investigate the mechanisms underlying sexual conflict in a species with strong female mate choice, lekking behaviour and very high rates of multiple mating. We conducted a PCR Suppressive Subtractive Hybridization (SSH) screen to identify genes expressed specifically in the male accessory glands. We have isolated several candidate Acps and they include 7 trypsin/serine protease genes, a hydrogen ATPase gene, a putative fibroin gene, an alkaline phosphatase gene, and an L21 ribosomal protein.

1020C

Frequenin finds her sister and both modulate synaptic form and function. Jesús Romero-Pozuelo¹, Jeffrey Dason², Harold L Atwood², Alberto Ferrús¹. 1) Instituto Cajal, CSIC, Madrid, Spain; 2) Dept. of Physiology, U. of Toronto, Toronto (Ontario), Canada.

The *Drosophila* frequenin gene (*frq*) encodes a Ca²⁺-binding protein specifically expressed in the nervous system. Homologous genes, also called Neuronal Calcium Sensor 1 (NCS1), are conserved from yeast to human. They form part of the Neuronal Calcium Sensor (NCS) family of proteins, characterized by 4 putative EF-hand Ca²⁺-binding motifs and one N-terminal myristoylation domain. It has been shown that *frq* modulates synaptic efficacy at the *Drosophila* and *Xenopus* neuromuscular junctions (NMJ) (Pongs et al., 1993; Olafsson et al., 1995). How *frq*/NCS1 is producing this modulatory effect, is not completely clear. Some studies suggest that *frq* regulates the cellular phospholipase C mediated exocytosis pathway by its interaction with the Pi4KB (PiK1 in yeast), a key enzyme of the phosphoinositides metabolism (Hendricks et al., 1999; Koizumi et al., 2002; Zhao et al., 2001). Other studies, however, point to *frq* as a Ca²⁺-dependent modulator of ion channels at the presynaptic cell, possibly A type K⁺ channels (Poulain et al., 1993; Nakamura et al., 2001) or voltage-gated Ca²⁺ channels (Tsujiyama et al., 2002.; Wang et al., 2001). We have found a new gene in *Drosophila*, named *frq2*, that encodes a protein with a 94.7% of amino acid identity with the previously cloned frequenin (now named *frq1*). Both genes are expressed specifically in the nervous system throughout development; however, *frq1* is more abundant than *frq2*. Both reach their maximal expression levels in the late embryo, 1st instar larvae and adult stages. Aiming to understand the functional differences between the two, and their respective mechanism of action, we produced genetically modified flies in which the expression of these genes is altered. We have used the UAS/GAL4 system to over- or under-express *frq1*, *frq2* or both, and to analyze the functional consequences of these manipulations. We show here the corresponding effects on the structure, number of synapses and synaptic responses of the larval NMJ.

1021A

Dissociation between extension of longevity and resistance to stress in alleles of the steroid biosynthesis gene *dare*. Anne F. Simon, David E. Krantz. UCLA, Neuropsychiatric Institute, Los Angeles, CA.

We have previously demonstrated a steroid control of longevity in *Drosophila melanogaster* (Simon et al., Science, 2003, 299: 1407-1410). In this study, we report the effects of mutants of another gene involved in the biosynthesis of steroids (*Drosophila* *adrenoxin reductase: dare*¹ and *dare*³). Our experiments show that these mutants tested as homozygotes or heterozygotes display an increase in resistance to dry starvation. In contradiction, only the heterozygotes mutants present an increased longevity compared to their controls. These results show dissociation between stress-resistance and longevity depending on the strength of the allele studied, and suggest that variations in steroid levels may have different effects on longevity and stress-resistance.

1022B

Dorsal Paired Medial neurons are transiently required for *Drosophila* Olfactory Memory. Scott Waddell, Alex C. Keene. Department of Neurobiology, UMass Medical School, Worcester, MA 01605.

We previously determined that the *Drosophila amnesiac (amn)* gene is highly expressed in Dorsal Paired Medial (DPM) neurons. DPM neurons ramify throughout the mushroom body lobes which suggests that their memory function may be the modulation of information coding in the mushroom body. Perturbation of DPM neuron function with a *shibire*^{ts1} transgene leads to memory loss that mimics that seen in *amn* mutants. Here we present a detailed analysis of DPM neuron function in olfactory conditioning using tight temporal control of DPM neuron inactivation by expression of a *shibire*^{ts1} transgene. DPM neuron output is only required during the consolidation phase for middle term odor memory and is dispensable during acquisition and recall. These data indicate that DPM neurons are transiently required for memory stabilization.

EVOLUTION AND QUANTITATIVE GENETICS

1023C

Fast protein evolution in a parental gene and its young retroposed derived gene of germline expression in *Drosophila*. Esther Betran, Mansi Motiwale. Dept Biol, Univ Texas, Arlington, TX.

We study in detail the evolution of one young retroposed gene, CG13732, and its parental gene, CG15645. These two genes were predicted genes with unknown function. We inferred parental and derived looking at the fingerprints of the retroposition process and revealed that CG13732 is present only in 4 species of *Drosophila*: *D. melanogaster*, *D. simulans*, *D. sechellia* and *D. mauritiana*. Analysis of polymorphism for the derived gene and divergence data for both parental and derived genes reveals that both genes produce functional proteins and they are changing at a very fast rate (KA/KS~0.53). Interestingly, the significantly negative value of Fay and Wu's H in the Non-african sample reveals an excess of variation at high frequency. We discuss if the excess of variation at high frequency could be explained by positive selection in the region or demography. In addition, the putative proteins for these genes show high level of length variation (fixed differences of codon deletions and insertions). The comparative expression pattern shows that both genes express in the same adult tissues (male and female germline) in *Drosophila melanogaster*. Expression of these genes in other species is being studied. We argue that the fast rate of evolution of these genes could be related to their putative germline function.

1024A

The Effects of Auto Fluorescent Intensity on Mate Choice Among Populations of *Drosophila* from Evolution Canyon. Deb Hamilton, Dr. Tom Wolf. Dept Biol, Washburn Univ, Topeka, KS.

Many factors have been identified which play a role in the mate choice of species which make up this diverse genus. These varied factors include behavior, visual stimuli, acoustic signals, and circadian rhythms. Focusing of the role of visual stimuli alone one may identify many cues, which are important in courtship. With important visual cues ranging from wing displays to female locomotor activity it is easy to appreciate, the essential role visual information has in mate choice and ultimately speciation. Our group has shown that *Drosophila* exhibit fluorescence upon exposure to UV light and that species express different patterns of facial fluorescence. Building on this work, we attempt to discern and quantify differences in facial fluorescent intensity between two populations of *Drosophila melanogaster* collected from opposite slopes of Evolution Canyon, which show evidence of population specific mate preference. Early findings suggest that populations from different sides of the canyon exhibit different fluorescent intensities and continuing work investigates the hypothesis that auto fluorescence is among the visual stimuli that contribute to mate choice.

1025B

Evolutionary analysis of the *swallow* gene. Mary A. Knox, Belinda Awuor, Edwin C. Stephenson. Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL.

In higher Diptera, establishment of the anterior-posterior embryonic axis relies on maternally-supplied molecules that are localized at both the anterior and the posterior tips of the egg. While the posterior organizing center is evolutionarily ancient, the anterior morphogen, the *bicoid* mRNA/protein, is an evolutionary novelty that is apparently limited to higher Diptera. We are trying to understand the origin and evolution of the anterior localization mechanism, by performing an evolutionary comparison of the genes that play known roles in this process. All of the known components in anterior localization have homologs in *Anopheles gambiae* or more-distantly related animals, and characterized roles in other known processes in *D. melanogaster*. These genes may thus have been co-opted from other cellular processes. The exception is *swallow*, which appears to act only in anterior mRNA localization in *melanogaster*, and which has no identifiable homologs in *Anopheles*. The *swallow* gene shows several unusual features: (1) it has evolved rapidly within *Drosophila*, (2) it has generated extant pseudogenes in (probably) two different lineages within the *melanogaster* group, (3) it has moved from one genomic position to another, also within the *melanogaster* group. We will present an analysis of the

evolution of *swallow* and its pseudogenes, and of its movement within the genome. Our goal is to identify the origins of *swallow*, an apparently novel gene that acts in a developmentally-important process, by tracing its history in *Drosophila* and other higher Diptera.

1026C

Osiris 16 is an Essential Gene in *Drosophila melanogaster*. Nikolaus P. Reed¹, Alan C. Christensen², Douglas R. Dorer¹. 1) Dept Microbiology, Meharry Medical Col, Nashville, TN; 2) School of Biological Sciences, University of Nebraska, Lincoln, NE.

Drosophila melanogaster has a unique locus that is both haplo-lethal and triplo-lethal. This locus is known as the *Triplo-lethal locus* and has been mapped to a 340 kb interval at 83DE on chromosome 3. A novel gene family has been identified within the *Triplo-lethal locus* called *Osiris*. The *Osiris* gene family consists of twenty homologous genes with unknown function. *Osiris* genes share paired cysteines near the amino terminus, four hydrophobic blocks, a block rich in tyrosine and histidine, and the conserved sequence AQXLAY. The four hydrophobic blocks suggest that these are type 1 transmembrane proteins. Previous research has suggested that members of the *Osiris* family are essential genes in *Drosophila*. It is being reported here that *Osiris 16* is an essential gene in *Drosophila melanogaster*. RNA interference has shown that ubiquitous knockdown of *Osiris 16* confers lethality.

1027A

Chromosomal evolution of sibling species of the *Drosophila willistoni* group. Chromosomal arm IIR.

Claudia Rohde¹, Ana C. L. Garcia¹, Victor H. Valiati², Vera L. S. Valente¹. 1) Departamento de Genética, Universidade Federal do Rio Grande do Sul - UFRGS, Porto Alegre, RS, Brazil, claudiarohde@yahoo.com; 2) Ciências da Saúde, Universidade do Vale do Rio dos Sinos - UNISINOS, São Leopoldo, RS, Brazil.

The phylogenetic relationships among nine entities of *Drosophila* belonging to the *willistoni* subgroup were investigated by establishing the homologous chromosomal segments of IIR chromosome, Muller's element B (equivalent to chromosome 2L of *D. melanogaster*). The sibling species of the *Drosophila willistoni* group investigated include *D. willistoni*, *D. tropicalis tropicalis*, *D. tropicalis cubana*, *D. equinoxialis*, *D. insularis* and four semispecies of the *D. paulistorum* complex. The phylogenetic relationships were based on the existence of segments in different triads of species, which could only be produced by overlapping inversions. Polytene banding similarity maps and break points of inversions between species are presented. The fact disclosed by the cytological studies on the four principal species (*D. willistoni*, *D. t. tropicalis/D. t. cubana*, *D. equinoxialis* and *D. paulistorum* superspecies) is that each one of the segregating inversions found in them is completely restricted to a single species. Closely related entities as *D. paulistorum* semispecies accumulate fewer rearrangements and share longer conserved segments in IIR than distantly related species, as *D. willistoni* and *D. insularis*. Therefore, the estimated number of inversions fixed in the IIR chromosome during the divergence of *D. willistoni* subgroup studied was of at least 7 inversions. The implications of the chromosomal data for the phylogeny of the species and comparisons with molecular data are discussed.

IMMUNE SYSTEM AND CELL DEATH

1028B

Resistance to Starvation: The Role of Larval-Derived Fat Cells in the Adult. Jerell R Aguila, Justin W Suszko, Deborah K Hoshizaki. Biological Sciences, University of Nevada Las Vegas, Las Vegas, NV.

Metamorphosis is characterized by an extraordinary transformation from the larval to the adult state. The imaginal cells proliferate to give rise to the adult tissues while the larval tissues are degraded through the process of programmed cell death. A striking exception to this loss of larval tissues is the fat body, which undergoes tissue dissociation into individual cells. These larval-derived fat cells survive as independent cells through metamorphosis and are believed to function as a nutritional reservoir to fuel the re-architecture of the animal to the adult state. The larval-derived fat cells persist in the adults and later undergo cell death.

We report here that larval-derived fat cells continue to play a nutritional role and enhance the ability of the adult animal to survive starvation. Newly eclosed flies are more starvation resistant than older flies that have lost their larval-derived fat cells. Furthermore, in animals in which larval-derived fat cells life span is extended by blocking cell death, we find an increase in starvation resistance that corresponds to the perdurance of these cells. These results provide the first experimental data to describe the role of the larval fat body in the adult fly.

1029C

The MAPKKK D-Mekk1 regulates the expression of turandot stress genes in response to injury in *Drosophila*. Sylvain Brun¹, Sheila Vidal¹, Paul Spellmann², Kuniaki Takahashi³, Herve Tricoire⁴, Bruno Lemaitre¹. 1) Centre de Genetique Moleculaire, CNRS, 91198 Gif-sur-Yvette, France; 2) Department of Molecular and Cell Biology and Howard Hughes Medical Institute, University of California at Berkeley,

Berkeley, CA 94720-3200, USA; 3) National Institute of Genetics, Mishima, Shizuoka 411-8540, Japan; 4) Institut Jacques Monod, 2 place Jussieu, 75251 Paris, France.

Septic injury triggers a rapid and widespread response in *Drosophila* adults that involves the up-regulation of many genes required to combat infection and for wound healing. Genome wide expression profiling has already demonstrated that this response is controlled by signalling through the Toll, Imd, JAK-STAT and JNK pathways. Using oligonucleotide microarrays, we now demonstrate that the MAPKKK D-Mekk1 regulates turandot (*tot*) stress genes as well as a small subset of genes induced by septic injury. Interestingly, *tot* genes are tightly regulated by D-Mekk1 and epistatic analysis suggests that D-Mekk1 acts upstream or independently of the JAK-STAT pathway. Our study also reveals that *tot* genes are also targets of the Imd pathway. Interestingly, D-Mekk1 flies are resistant to microbial infection but susceptible to paraquat, an inducer of oxidative stress. These results point to a role of D-Mekk1 in the protection against tissue damage and/or protein degradation and indicate complex interactions between stress and immune pathways in *Drosophila*.

1030A

Generation and Characterization of *Debcl* null animals, a *Drosophila* Pro-apoptotic Bcl-2 Family Member. Kathleen A. Galindo, Arisha Patel, John M. Abrams. Cell Biology, UT Southwestern Medical Center, Dallas, TX.

Apoptosis is a process required for proper development, adult tissue homeostasis, and the removal of damaged cells in response to stress stimuli. The core apoptotic machinery is highly conserved from *C. elegans* to mammals. Bcl-2 proteins are central regulators of the mitochondrial signal in mammals, which is thought to function upstream of the caspase cascade. Currently, there is no evidence for mitochondrial release of cytochrome c in *C. elegans*, and evidence regarding an apoptogenic role for cytochrome c in the activation of programmed cell death (PCD) in the fly cell-death is ambiguous. Flies have two Bcl-2 family members: *Debcl/dBorg-1/dBok/Drob-1* and *Buffy/dBorg-2*. The former is thought to exert pro-apoptotic functions, whereas the latter seems to encode anti-apoptotic action. Limited studies on *Debcl* function implicate a role for this gene in developmental PCD. Null mutations in *Debcl* have yet to be studied, and these analyses are necessary to properly dissect the role and impact this gene plays in *Drosophila* apoptosis. We used ends-out homologous recombination to generate a null mutation of the *Debcl* gene. We screened a total of 56,000 flies for targeted homologous recombination. A total of 64 candidate recombination events were obtained, 10 of which mapped onto the *Debcl* native chromosome (chromosome 2), and seven independent targeted events were verified by PCR analysis. RT-PCR analysis confirms that *Debcl* RNA levels are abolished in *debcl*^{KO} animals and the expression levels of neighboring transcripts are unaffected when compared to WT animals. *debcl*^{KO} animals are viable and fertile. A series of molecular and genetic experiments will be performed to examine the role *Debcl* plays in *Drosophila* apoptosis. Results from these analyses will be presented.

1031B

Dual role of *Drosophila* caspases in execution of apoptosis and resumption of mitosis after DNA damage-induced cell cycle arrest. Shu Kondo^{1,2,3}, Yasushi Hiromi^{2,3}, Masayuki Miura^{1,3}. 1) Department of Genetics, Grad. Sch. Pharm. Sci., University of Tokyo, Japan; 2) Division of Developmental Genetics, NIG, Mishima, Japan; 3) CREST, JST, Japan.

Cell death and proliferation are two biological processes that have opposite consequences in organ development. Coordination of these two processes is critical for ensuring the correct shape and size of organs. *Drosophila* imaginal discs are an ideal model system to study their relationship. High-dose γ -irradiation rapidly induces G₂ arrest and causes more than 50% of the imaginal disc cells to undergo apoptosis, which is followed by elevated cell proliferation that compensates for the cell loss. This observation suggests a link between cell death and proliferation. Recent studies have also shown that forced inhibition of apoptosis by the caspase inhibitor p35 induces hyperproliferation, suggesting that apoptosis may somehow promote cell proliferation. As a first step to investigate how cell death and proliferation are coordinated, we created null mutants of caspases, the most downstream components of the apoptotic signaling cascade. Of the seven caspases in the *Drosophila* genome, we found that the apical caspase *dronc*, the effector caspases *drice* and *dcp-1* are required for all developmental apoptosis from embryo to adult. Then, we examined the DNA damage response of the caspase mutants. While *dronc* and *drice* mutant wing discs undergo G₂ arrest normally after γ -irradiation, apoptosis is completely blocked in both mutants. Interestingly, mitosis resumes significantly earlier in *drice* mutants than in wild-type animals. In contrast, resumption of mitosis is somewhat delayed in *dronc* mutants. These results contradict with the idea that *drice* activation depends on *dronc*. One possibility is that *dronc* is hyperactivated in the *drice* mutant cells that normally undergo apoptosis, since the apoptotic signaling cascade up to *dronc* is intact. To directly address this issue, we are in the process of analyzing *dronc/drice* double mutants. We tentatively propose that the apical caspase *dronc* not only triggers cell death but simultaneously promotes recovery from G₂ arrest.

TECHNIQUES AND GENOMICS

1032C

Annotating the newly sequenced *Drosophila* genomes using *Drosophila melanogaster*. Venky N Iyer¹, Daniel A Pollard², Michael B Eisen^{1,2,3}. 1) Dept. of Molecular and Cell Biology, UC Berkeley, Berkeley, CA; 2) Biophysics Graduate Group, UC Berkeley, Berkeley, CA; 3) Genome Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA.

We have developed and implemented a strategy to comparatively annotate protein coding genes in the newly sequenced *Drosophila* genomes using the Flybase Release 4.0 annotations for *Drosophila melanogaster*. The strategy is a modification of the standard reciprocal BLAST approach, allowing for flexibility in predicting orthology/paralogy relationships. Briefly, we used TBLASTN to infer regions in the assembled genomes that could potentially contain a query gene, built gene models using the comparative gene structure prediction algorithm GeneWise (Birney et. al 2004), and then predicted one-to-one orthology, or paralogy relationships in either or both species based on the results of a BLASTP search of the predicted translation against the *D.melanogaster* translations. We present details of our methodology as well as statistics of the annotation data sets and genome-wide phylogenetic trees for the following species: *D.simulans*, *D.yakuba*, *D.erecta*, *D.ananassae*, *D.pseudoobscura*, *D.mojavensis* and *D.virilis*. The annotations will be made available to all interested researchers.

1033A

Genome-wide analysis of gene expression in adult *Anopheles gambiae*. Osvaldo Marinotti¹, Eric Calvo², Nguyen Quang K.¹, Dissanayake Sumudu¹, Nirmala Xavier¹, Ribeiro Jose M. C.², James Anthony A.¹. 1) Molecular Biology and Biochemistry, Univ. California Irvine, Irvine, CA; 2) Laboratory of Malaria and Vector Research, National Institutes of Health, Rockville, MD.

Mosquitoes have considerable public health significance as vectors of parasitic and viral pathogens. With its genome sequenced, *Anopheles gambiae*, a major vector of human malaria in sub-Saharan Africa, provides an opportunity to research the genetic bases of key physiological and behavioral traits that make possible pathogen transmission as well as explore fundamental aspects of comparative, evolutionary and developmental biology. A whole-genome microarray was used to investigate changes in gene expression associated with a crucial capacity, hematophagy, of the adult female mosquito as well as look at differences between males and females and newly-emerged and aged animals. Twenty percent of the genes analyzed display sex-restricted expression. Remarkably, as many as 50% of all genes vary significantly in transcript abundance in females during the three days after feeding. These changes reflect metabolic and behavioral commitments to blood digestion and egg formation at the cost of activities such as flight and response to environmental stimuli. Multiple blood meals prior to completion of a gonotrophic cycle elicit similar transcription profiles of specific genes. Aging is accompanied by differential regulation of ~5% of the genome. Fourteen of the 200 most-abundant, constitutively-expressed genes in the mosquito have no known homologues in any other species. All primary data and analyses are available on a public accessible website at: <http://www.angogepuci.bio.uci.edu>. Due to the disparity in food sources, digestive processes, and reproductive physiology, gene expression in *Anopheles gambiae* and *Drosophila melanogaster* show several differences, however many common traits could be identified.

1034B

Development of Insect Retroviral Vectors for Somatic and Field Transformation. James P. Mohler, Mansi Mehta, Rosio Ramos, Sana Ali. Biological Sciences, Barnard College, New York, NY.

Redesigned mammalian retroviruses have proven highly useful transformation vectors for vertebrate and human somatic recombination. We are investigating whether endogenous insect retroviruses can also be utilized for somatic transformation and potentially for non-laboratory, field transformation. Our pilot study involves reconfiguring *Drosophila* retrotransposons gypsy and springer to characterize their ability to transfer non-viral genes between individuals (either as defective viruses, using the endogenous retrotransposon sequences as helpers, or as intact viruses). We are also trying to manipulate the tissue expression of these retroviral transposons by addition of specific-tissue enhancers in order to target expression of the introduced genes. We have also identified five endogenous retroviral transposons in the *Anopheles gambiae* genome (four gypsy-class retrotransposons and one BEL/roo-class) and are characterizing these endogenous retroviral transposons to ascertain whether any of these may be suitable to develop into a mosquito transformation vector.

1035C

A new family of direct-drive dfd::YFP balancers. Heeren Patel, Tien Le, ZhiGuo Liang, Stephanie Ray, Matthew Slovitt, Gita Sivasubramaniam, Marcus Yu, Greg Beitel. BMBCB, Northwestern University, Evanston, IL., United States.

In working with fluorescent balancers for analyzing late stage embryos, we found existing balancers had a number of limitations including poor visibility, not being direct drive (i.e gal4/UAS based), and being w+. We

therefore created a marker consisting of the *dfd* HZ2.7 enhancer (Bergson and McGinnis, EMBO vol. 9 1990) directly driving eYFP in a Pelican vector (Barolo et al., Biotechniques vol. 29, 2000). The *dfd* enhancer drives strong expression in spots in the head from about stage 14 onwards when scoring fluorescence, or stage 12 if using anti-GFP antibodies. Expression continues in larva and adults. We find balancers bearing this element much easier to score in both compound and dissecting microscopes than balancers such as *Actin::GFP*. To increase the utility of these balancers, we are using mutagenesis to eliminate the *w+* marker. Our goal is to create a family of balancers that include chromosomes X, II and III. Our progress towards this set will be presented on the poster, and the stocks are being contributed to the Bloomington stock center as the lines are created. A list can be found on the Beitel lab website <http://www.biochem.northwestern.edu/beitel/home2.htm>

One issue we discovered in making the balancers is that the *dfd::YFP* Pelican insertions were very difficult to hop compared to standard insertions. We estimate the frequency of hopping in a *delta 2-3* background to be approximately 1 in 3000 - 5000. Previously we had found that the *dfd::lacZ* element hopped at a frequency of 1 in 3. Whether this reflects something odd about the *dfd::eYFP* or the insulator elements in the Pelican vector is unclear. We would appreciate feedback if anyone else has tried to hop a Pelican based insert.

1036A

A prototype of Laboratory Information Management System for processing and analysis of confocal images of gene expression patterns. Maria G Samsonova¹, Andrei S Pisarev¹, Ekaterina G Poustelnikova¹, Konstantin N Kozlov¹, Ekaterina M Myasnikova¹, John Reinitz². 1) Dept. of Computational Biology, State Polytechnical University, St. Petersburg, Russia; 2) Stony Brook University, NY, USA.

We have devised a six step data pipeline for acquisition of quantitative gene expression data from confocal images of segmentation gene expression patterns to understand the dynamical regulatory mechanisms controlling the expression of segmentation genes (Jaeger et al, (2004), Nature, 430).

Because of ongoing data acquisition as well as the development of new processing and analysis methods, serious problems arise with respect to data storage and the management of application programs. To solve these problems we have applied the technology of multiagent systems to develop a prototype of a Laboratory Information Management System (LIMS) known as PIPE. In this system each image or data processing step is implemented as a separate application program module (APM). Complex scenarios of image processing and analysis are executed by constructing different application programs from these modules. Among APM already implemented are summation and subtraction of images, thresholding, dilation, erosion, and a median filter. These operations can be applied to construct the whole embryo mask, crop an embryo image to the size of the mask, rotate and flip embryos to obtain the embryo image in standard orientation, remove background from images, etc. A user interface empowers a user to visually construct an application program from APM, interactively execute a program and visualize both intermediate and final results.

PIPE can be used for the automatic analysis, modeling and mining of large image sets to test specific biological hypothesis.

1037B

Insertional mutagenesis systems in the red flour beetle, *Tribolium castaneum*. K.S. Siebert¹, M.D. Lorenzen², Y. Park¹, S.J. Brown³, R.W. Beeman². 1) Dept. of Entomology, Kansas State University, Manhattan, KS; 2) USDA-ARS-GMPPRC, Manhattan, KS; 3) Div. of Biology, Kansas State University, Manhattan, KS.

The genome of *Tribolium castaneum* has been sequenced, and a preliminary assembly released (HGSC, Baylor College of Medicine). In order to progress toward a systematic, functional analysis of the genome we have developed a highly efficient, hybridization-based method of generating new insertions using transgenic helper and donor strains. The helper line harbors an *hsp70*-driven *piggyBac* transposase flanked by *Minos* termini. This line is crossed to the previously available *piggyBac* donor line Pig-19, which carries the 3xP3-EGFP reporter flanked by *piggyBac* termini, and shows muscle-specific expression of EGFP due to its insertion near a muscle-specific enhancer. This system generates a high rate of remobilization, but many lines bear multiple insertions. While very high numbers of multiple insertions are advantageous for some types of F₁ screens, generating a large collection of insertion lines with single-gene disruptions is also an important goal. We are testing alternative promoter-helper constructs and alternative donor lines in an effort to achieve this goal.

The *Drosophila alpha1-tubulin* promoter driving *piggyBac* transposase results in a high rate of remobilization with single-insertion events in *Drosophila*. Thus, we have cloned a *Tribolium alpha-tubulin* gene and its promoter. Evidence from transient assays demonstrates that this promoter is functional when used to drive *vermillion* (*Tcv*), an eye-color gene. In addition, we have tested the remobilization rate of a Y-linked, *Tcv*-marked *piggyBac* insertion. A single copy of *Tcv* in *Tribolium* is usually sufficient to confer black eye pigmentation on *Tcv*-deficient mutants, but due to the position of Y18, this line has reduced expression, resulting in a red-eye phenotype. The remobilization of Y18 yielded only single insertions, easily recognized by their associated black-eye phenotype. Development of *Polyubiquitin*-driven helpers will also be presented.

1038C

High throughput collection of *Drosophila* embryos for homozygous lethal mutants based on *deformed* driven YFP expression. Bo Wang¹, Julia Thompson¹, Greg Beitel², Rock Pulak¹. 1) Union Biometrica, 35 Medford Street, Suite 101, Somerville, MA 02143; 2) Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208.

COPAS instruments automate the analysis, sorting, and dispensing of *Drosophila* embryos, measuring the embryo size and the intensity of fluorescent markers. Once analyzed, embryos can be selected according to user defined criteria, and then dispensed into multi-well microtiter plates for high throughput screening or collected in bulk for further biochemical analysis. With this technology, time required for large scale embryo collections can be dramatically reduced and human error is eliminated. Here we show that the COPAS instrument is able to distinguish and sort homozygous mutant embryos from their heterozygous siblings based on *deformed* promoter driven YFP expression. Two different transgenic strains with the transgene integrated on the TM3 balancer chromosome and one strain with the integration on the CyO balancer chromosome were tested. Our results showed that high purities (>98%) were able to be achieved for the collected embryos. Therefore, these transgenic strains can be used as alternatives to current GFP tagged balancer strains (e.g. *actin* or *GAL4-UAS* driven) for large scale homozygous lethal mutant embryo collection.

DROSOPHILA MODELS OF HUMAN DISEASES

1039A

MMPs alter the invasive ability of *lethal giant larvae* mutant tumors. Michelle L. Beaucher¹, Evelyn Hersperger¹, Andrea Page-McCaw², Allen Shearn¹. 1) Dept Biol, Johns Hopkins Univ, Baltimore, MD; 2) Dept Biol, Rensselaer Polytechnic Inst, Troy, NY.

A critical factor in metastasis is the ability of the tumor cells to degrade the surrounding extracellular matrix, allowing for migration to distant sites in the body. Work in vertebrate systems has found correlations between metastatic ability and expression levels of a variety of extracellular proteases in tumor lines. Our lab is using a *Drosophila* tumor invasion assay to directly examine the involvement of specific proteases in metastasis.

To gauge the metastatic abilities of *Drosophila* tumors, donor tissue is injected into the abdomen of adult female hosts. Metastasis is measured by the presence of donor tissue within the ovary of the host after incubation. Preliminary work in the lab determined that tumor cells from *lethal giant larvae* (*lgl*) mutants are capable of invading the ovary while tumor cells from *brain tumor* (*brat*) mutants are not. To understand the mechanisms involved in metastasis, we are attempting to alter the metastatic abilities of these tumors by manipulating extracellular protease expression levels. We are focusing on the two MMPs identified in *Drosophila*. MMP1 is a soluble extracellular protease and MMP2 is membrane bound by a GPI anchor. Current work has shown that *mmp* gene expression affects *lgl* tumor invasive ability. Tumor invasion is significantly reduced when *mmp2* gene expression is lowered. Conversely, removal of *mmp1* gene expression from tumor cells causes an increase in metastasis.

1040B

The role of the *Drosophila* Nbs protein in telomere protection. Laura Ciapponi, Giovanni Cenci, Claudia Berdini, Maurizio Gatti. University of Rome "La Sapienza", Department of Genetics and Molecular Biology, Rome, Italy.

The MRN complex consists of the two evolutionarily conserved components Mre11 and Rad50 and the third less conserved component Nbs1. This complex mediates telomere maintenance plus a variety of functions in response to DNA double strand breaks, including homologous recombination, non-homologous end joining (NHEJ) and activation of DNA damage checkpoints. Mutations in the *Nbs1* and *Mre11* genes cause the Nijmegen breakage syndrome and the human ataxia-telangiectasia like disorder (ATDL), respectively. We have previously shown that null mutations in the *Drosophila mre11* and *rad50* genes cause both telomeric fusion and chromosome breakage. We have identified a mutation in the *Drosophila nbs* gene. In *nbs* mutants, brain cell chromosomes also display both end-to-end fusions and breakage. To define the role of *nbs* in telomere protection, mutant chromosome preparations were immunostained for both HP1 and HOAP, two proteins that protect *Drosophila* telomeres from fusion. Cytological analysis revealed that mutations in *nbs* drastically reduce accumulation of HOAP and HP1 at telomeres. This indicates that all components of the MRN complex are required for protection of *Drosophila* telomeres by mediating recruitment of HOAP and HP1 at chromosome ends.

1041C

An enhancer/suppressor screen for genes that function in the Lkb1 pathway, and characterisation of the Lkb1 phenotype in *Drosophila Melanogaster*. Afifa Khan, Ruth Wheeler, Helen McNeill. Developmental Patterning, Cancer Research UK, London, UNITED KINGDOM.

Lkb1 is a highly conserved serine-threonine kinase, which has been shown to have roles in cell polarity, proliferation, and apoptosis. Loss of Lkb1 function in humans leads to Peutz-Jegher syndrome (PJS), an inherited cancer syndrome. PJS is a rare autosomal-dominant polyposis disorder that clinically manifests as multiple benign hamartomatous polyps throughout the GI tract, alongside melanin 'freckles' of the buccal mucosa. PJS individuals are also further predisposed to tumours at other sites.

Both the function and regulation of Lkb1 are poorly understood: our objective therefore is to uncover novel substrates of Lkb1, and to understand some of the molecular mechanisms by which Lkb1 is regulated.

We have conducted a small-scale dominant modifier screen in *Drosophila Melanogaster* to isolate components of the Lkb1 pathway. We have screened 10,000 flies, and recovered a number of mutants, which we are currently mapping using a multi-tiered approach. Our mapping strategy is to use a set of fluorescently labelled PCR product-length polymorphisms (PLPs). We are also using P-element based mapping in conjunction with SNP markers to enable mapping to a high resolution.

We are also interested in the role of Lkb1 in regulating cell polarity. Using the Flp-FRT system to create mosaic clones, we have been studying the adult phenotype of Lkb1 in *Drosophila* mosaic eyes. Optical and EM sections of Lkb1 mutant tissue reveal increased cell death, disorganised ommatidia and defects in morphology. We are continuing to characterise this phenotype.

1042A

***kal-1*, the *Drosophila* orthologue of human Kallmann syndrome gene KAL1, is required for the neurite branching of embryonic motorneurons.** Young-II Kim, Wonseok Son, Sun-Mi Woo, Ook Joon Yoo. Dept Life Sci, KAIST, Deajeon, South Korea.

Kallmann syndrome is an inherited disorder of hypogonadotropic hypogonadism and anosmia owing to lesions in axonal organization and disrupted cell migration of neuroblasts from developing olfactory epithelium. The gene responsible for the X-linked type of Kallmann syndrome, KAL1, encodes an extracellular matrix protein, Anosmin1 that is required for axonal outgrowth in developing embryos. For the detailed biological analysis for the KAL1, we isolated the *Drosophila* KAL1 homologue, *kal-1*, and generated its mutants by P-element mobilization. The *kal-1* embryos showed impaired axonal branching: SNb motor neurons could not extend their axons to proper muscle targets, and the exterior longitudinal FasII-expressing axon bundles were openly misconnected. Specific α -Kal-1 sera were generated, and revealed dynamic expressions during various morphogenetic processes such as the germ band retraction as well as head sensory organs and segmentally repeated expressions in several subsets of motor neurons. These results suggest that the *Drosophila* Kal-1 has roles in neurite branching and cell adhesion during the embryogenesis as a functional homologue of vertebrate KAL1s.

1043B

A *Drosophila* model recapitulating key clinical features of spinal and bulbar muscular atrophy.

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Spinobulbar muscular atrophy (SBMA) is an X-linked disorder characterized by slowly progressive weakness resulting from degeneration of motor neurons of the brainstem and spinal cord. SBMA is caused by trinucleotide (CAG) repeat expansion in the first exon of the androgen receptor (AR) gene. Only males are affected by SBMA. The AR is a ligand-dependent transcription factor belonging to the nuclear hormone receptor superfamily. Similar to other members of this family, the AR becomes activated after ligand binding and translocates to the nucleus to alter the transcription of target genes. The goal of our work is to determine to molecular basis of neurotoxicity of polyglutamine-expanded AR. Toward that end, we have established a conditional *Drosophila* model of SBMA. We generated transgenic *Drosophila* expressing human AR (hAR) with polyglutamine lengths of 12, 20, 45, 52, 77, or 121 in the highly versatile UAS/Gal4 system. We detected no evidence of degeneration in adult flies expressing hAR of any polyQ repeat length in multiple tissues. However, exposure of adult flies to dihydrotestosterone (DHT) resulted in marked neurodegeneration in flies expressing hAR with repeat lengths of $\geq 45Q$. Neurodegeneration was manifested as retinal degeneration, locomotor defects, or reduced longevity when expression was directed to the eye, motor neurons, or whole brain, respectively. Moreover, there was an inverse correlation between polyglutamine repeat length and age of onset. Our fly model of SBMA supports the hypothesis that interaction of polyglutamine-expanded hAR with its natural ligand is a critical determinant of neurotoxicity. It is well established that ligand-AR interaction results in a substantial conformational change leading to post-translational modifications (including acetylation, sumoylation and phosphorylation), substitution of binding partners, and nuclear translocation. The role of these ligand-dependent events in polyglutamine toxicity will be explored.

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***datilógrafo (dati)* encodes a zinc finger transcription factor required for proper locomotor activity in *Drosophila*.** Rui Sousa-Neves¹, Claudia Mieko Mizutani², Tamas Lukacsovich¹, Judith Purcell¹, J.L. Marsh¹.
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Here we report the identification of *Dati*, a poly-Q containing transcription factor. The founder embryonic lethal allele *dati*¹ was mapped on the *Drosophila* fourth chromosome using a series of nested terminal deletions next to the breakpoint of Df(4)B6-2D (102B6). Allelic and reversion tests between the progenitor P insertion of Df(4)B6-2D demonstrated that *dati*¹ is allelic to this single fourth chromosome insertion. Sequence searches show that *dati* belongs to a group of conserved genes that include the *C.elegans* LIN29, the *Drosophila* *rotund/roughened eye*, *squeeze* and the human NMP4/ZNF384, the gene of the Acute Lymphoblastic Leukemia (ALL). Like *squeeze*, *dati* is expressed in the ventral cord, the larval and adult brain. Complete loss of function of *dati* leads to embryonic lethality while partial loss of function causes a severe locomotor impairment and sterility in adult flies.
