52nd Annual
Drosophila Research Conference

Program Addendum

Town & Country Hotel & Conference Center
San Diego, CA
March 30-April 3, 2011

Sponsored by The Genetics Society of America
9650 Rockville Pike
Bethesda, MD 20814-3998
301/634-7300
301/634-7079 fax
Society@genetics-gsa.org
http://www.drosophila-conf.org
PROGRAM CHANGES

Friday, April 1

- Additional Workshop 1:45 pm Undergraduate Plenary Session Pacific Ballroom Salon 1

Saturday, April 2

- Platform – Stem Cells 4:30 pm Abstract #143 will be presented by Aditya Sen

ADDITIONAL EXHIBITOR

Leica Microsystems
1700 Leider Lane
Buffalo Grove, IL 60089
Phone: 847/405-0123 800/248-0123
Fax: 847/405-0164
E-mail: info@leica-microsystems.com
URL: www.leica-microsystems.com

Leica Microsystems will display imaging solutions designed to improve research. The world's most powerful stereofluorescence microscope will be on display, the Leica M205 FA, which conducts time lapse, image overlay, and movie making. The Leica M205 is uniquely designed with Fusion Optics, which allows high resolution and high depth of field imaging simultaneously, over the entire zoom range. Leica will also show the Leica SP E 'personal' confocal microscope.

POSTER CHANGES

- Presentations Cancelled:
  Abstract #251B (Chaudhary)
  Abstract #261C (Silva)
  Abstract #449B (Dean)
  Abstract #711C (Volkan)
  Abstract #941B (Kagesawa)

- Late Poster Abstracts (see complete text of abstracts at www.drosophila-conf.org):

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<th>Poster #</th>
<th>First Author/Presenter</th>
<th>Abstract Title and Co-Authors</th>
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<tr>
<td>959B</td>
<td>Jens Januschke</td>
<td>Drosophila neuroblasts retain the daughter centrosome. Jens Januschke1, Salud Llamazares1, Jose Reina2, Cayetano Gonzalez1,2. 1) Cell Division Group, IRB-Barcelona, PCB c/ Baldiri Reixac 10-12, Barcelona, Spain; 2) Institut Catalana de Recerca i Estudis Avancats (ICREA). Passel Lluís Companys 23, Barcelona, Spain.</td>
</tr>
<tr>
<td>960C</td>
<td>Bo Zhou</td>
<td>Retromer regulates apical-basal polarity through recycling Crumbs. Bo Zhou1, Xinhua Lin1,2. 1) Dev Biol, Cincinnati Children’s Hosp, Cincinnati, OH; 2) Institute of Zoology, Chinese Academy of Sciences, Beijing, China.</td>
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<tr>
<td>962B</td>
<td>Imre Molnar</td>
<td>The role of Drosophila DAAM in the development of the Indirect Flight Muscle. Imre Molnar1, Ede Migh1, Tibor Kalmar1, Zacharias Orfanos2, John Sparrow2, Szilvia Barko3, Miklos Nyitrai3, Jozsef Mihaly1. 1) Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary; 2) Department of Biology, University of York, York, UK; 3) Faculty of Medicine, Department of Biophysics, University of Pecs, Pecs, Hungary.</td>
</tr>
<tr>
<td>963C</td>
<td>Alexi Brooks</td>
<td>Towards a quantitative analysis of Ras pathway effects on BMP responses in the wing. Alexi Brooks1,2, Jing Cao3, Mohit Bahel1,2,3, Michal Jager1, Tara Brosnan1, David Umulis4, Laurel Raftery1,2,3. 1) School of Life Sci, Univ Nevada, Las Vegas, NV; 2) CBRC, MGH/Harvard Med Sch, Charlestown, MA; 3) New York Univ, New York, NY; 4) Dept. of Ag &amp; Biol Engineering, Purdue</td>
</tr>
<tr>
<td>964A</td>
<td>Abbie Saunders</td>
<td>Dynamic Regulation of the Dpp Signalling-Responsive Transcriptional Network in the Drosophila Embryo. Abbie Saunders, Catherine Sutcliffe, Hilary Ashe. Faculty of Life Sciences, University of Manchester, UK.</td>
</tr>
<tr>
<td>965B</td>
<td>Annick Sawala</td>
<td>Integrin signalling enhances BMP activity in the early Drosophila embryo. Annick Sawala, Hilary L. Ashe. Faculty of Life Sciences, University of Manchester, Manchester, UK</td>
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</table>

967A  Martin S. Buckley  Recruitment Timing and Dynamics of Transcription Factors at the Hsp70 Loci in Living Cells. Martin S. Buckley1, Katie L. Zobeck2, Warren R. Zipfel2, Lis J. Lis1. 1) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) Department of Biomedical Engineering, Cornell University, Ithaca, NY.


969C  Lucie Kucerova  Function of Drosophila AdaR in tissue culture and in vivo. Lucie Kucerova1,2, Vaclav Broz1,2, Jana Fleischmannova2, Roman Sidorov2, Vladimir Dolezal2, Michal Zurovec1,2, 1) Department of Biochemistry and Biophysics, Czech Academy of Sciences, Bohemia, Czech Republic; 2) Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic.

970A  Weizhe Li  The Drosophila TRPP2 homolog, Amo, is required for sperm activation in the female uterus. Weizhe Li1,2, Alexis Hofherr2, Kristy Chu1,3, Steve Matson1, Jeffrey Sekelsky, 1) Department of Genetics, University of Wisconsin, Madison, WI; 2) Renal Division, University Hospital Freiburg, Freiburg, Germany.

971B  Adam Kleinschmit  Drosophila heparan sulfate 6-O endosulfatase regulates Wingless morphogen gradient formation. Adam Kleinschmit, Takashi Koyama, Katsufumi Dejima, Yoshiki Hayashi, Keisuke Kamimura, Hiroshi Nakato. Genetics, Cell Biology, & Development, University of Minnesota, Minneapolis, MN.

972C  Shin-Hong Shiao  Functional analysis of Wnt signaling pathway in the regulation of mosquito vitellogenesis. Shin-Hong Shiao. Department of Parasitology, National Taiwan University, Taipei, Taiwan.

973A  Xiaofang Tang  Roles of ubiquitination in Wnt signaling pathway in the regulation of mosquito vitellogenesis. Xiaofang Tang1, Xinhua Lin1,2. 1) Developmental Biol, Cincinnati Children's Hosp, Cincinnati, OH; 2) State Key Laboratory of Biomembrane and Membrane Biotechnology, and Key Laboratory of Stem Cell and Developmental Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing.

Cell cycle and checkpoints


975C  Jeffrey P. Chmielewski  Characterizing the role of Psf2 in maintaining genomic integrity. Jeffrey P. Chmielewski, Tim W. Christensen. Biology, East Carolina University: Biology Department, Greenville, NC.

976A  Stephanie Bellendir  Investigating the Role of GEN in Drosophila. Stephanie Bellendir, Sabrina Andersen, Jeff Sekelsky. Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Cell death


978C  Antonina Pechkovsky  The adenovirus E4orf4 protein promotes a low level of cell death in Drosophila normal tissues and inhibits Reaper-, HID-, and Eiger-induced apoptosis. Antonina Pechkovsky1,2, Maoz Lahav1,2, Adi Salzberg1, Tamar Kleinberger2. 1) Department of Genetics, Rappaport Faculty of Medicine, Technion, Haifa, Israel; 2) Department of Microbiology, Rappaport Faculty of Medicine, Technion, Haifa, Israel.


980B  Skye Souter  Use of integrase-mediated BAC transgenesis to identify p53 alleles that separate regulation of DNA repair from apoptosis. Skye Souter, Sarah Oikemus, Naoto Ito, Michael Brodsky. Gene Function & Expression, UMass Medical School, Worcester, MA.

Cell division and growth control


982A  Andrew Guzman  A novel role for folate metabolism during cellularization in Drosophila embryos. Andrew Guzman1, Justin Crest2, Jian Cao2, William Sullivan1. 1) MCD Biology, University of California, Santa Cruz, CA; 2) Stanford University School of Medicine, Stanford, CA.

983B  Stefan K. Heidmann  Assessing a potential cohesive role for the mitotic cohesin Scc1/Rad21 during female meiotic divisions. Stefan K. Heidmann1, Evelin Urban1, Christian Lehner2, Sonal Nagarkar Jaiswal1. 1) Dept of Genetics, University of Bayreuth, Bayreuth, Germany; 2) Institute of Zoology, University of Zurich, Zurich, Switzerland.
Interaction of Drosophila importin-a2 and kelch during early embryogenesis. Sowjanya Kallakuri1, Bernard Mechner2, Lynn Cooley1. 1) Genetics, SHM I-339, Yale University, New Haven, CT, USA; 2) Developmental Genetics, AO40, German Cancer Research Center, INF-S81, D-69120.

Control of Mitochondrial Structure and Function by the Yorkie Oncogenic pathway. Kevin T. Jones3, Raghavendra Nagaraj1, Shubha Gururaja-Rao1, Matthew Slattery2, Nicolas Negre3, Daniel Braas4, Heather Christolf2, Kevin White3, Richard Mann2, Utpal Banerjee1. 1) MCDB, UCLA, Los Angeles, CA; 2) Dept. of Biochemistry and Biophysics, Columbia University, New York, NY; 3) Institute for Genomics and Systems Biology, University of Chicago, Chicago, IL; 4) Institute for Molecular Medicine, David Geffen School of Medicine, UCLA, Los Angeles, CA.

Patch ed is a conditional, non-autonomous, growth suppressor identified in a mosaic screen for growth suppressors in the background of blocked cell death. Jacob Kagey, Jordan Brown, Kenneth Moberg. Cell Biology, Emory University, Atlanta, GA.

Transcription in the Absence of H3.3. Sasha A. Langley1, Jacob Kagey1, Gary H. Karpen2,3. 1) Life Sciences Division, LBNL, Berkeley, CA; 2) UC Berkeley, Department of Molecular and Cell Biology.

Epigenetic regulation of ERK pathway activity. Julien Rougeot, Frédérique Peronnet, Emmanuèle Mouchel-Vielh. UMR7622, UPMC-CNRS, PARIS, France.


Ectopic expression of germline genes drives malignant brain tumor growth in Drosophila. Ana Janic1, Leire Mendizabal1, Salud Llamazaressal, David Rosel1, Cayetano Gonzalez1,2. 1) IRB Barcelona, Barcelona; 2) Instituto Catalana de Recerca i Estudis Avançats (ICREA), Barcelona.

Hypoxia TOLERANCE IN DROSOPHILA: DEVELOPMENT, TISSUE SPECIFICITY, AND HEART FUNCTION IN RESPONSE TO ACUTE, LOW OXYGEN EXPOSURE. Rachel Zarndt Ellison1, P. Azad2, Gabrielle Haddad1, Karen Ocorr1, Rolf Bodmer1. 1) Sanford-Burnham Medical Research Institute, La Jolla, CA; 2) University of California, San Diego, La Jolla, CA.

Modification of Serines in Httex1p suppresses Huntington’s disease (HD) pathogenesis in Drosophila. Namita Agrawal1,2, Tamas Lukacsovich1, Charity Aiken1, Joan Steffan2, Leslie Michels Thompson1,2, J. Lawrence Marsh1. 1) Department of Developmental and Cell Biology, 4444 McGaugh Hall, University of California, Irvine, California 92697, USA; 2) Department of Biological Chemistry, D240 Medical Sciences I, University of California, Irvine, California 92697, USA; 3) Department of Psychiatry and Human Behavior, Gillespie 2121, University of California, Irvine, California 92697, USA; 4) Department of Zoology, University of Delhi 110007, India.

Drosophila Model of Parkinson’s Disease: In search for Genetic Interactors of Leucine-Rich Repeat Kinase 2. Katerina Venderova1, Sean Kabbach1, Paul Macrogliese2, Elizabeth Abdel-Messih3, Gary Li1, Sameera Abuash2, Emdadul Haque2, Ruth Slack2, David Park2. 1) Department of Physiology and Pharmacology, Thomas J Long School of Pharmacy and Health Sciences, University of the Pacific, Stockton, CA; 2) Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, ON.

P3K signaling modulates the bang sensitivity of slamdance mutants. Derek M. Dean, Ma Kin Pyi Son, Cynthia Cortes, Daniel Nachun, Jingyi Liu. Biology, Williams Col, Williamstown, MA.

Influenza A NS1 may provide protection to the host by altering Hedgehog signaling. Margery G. Smelkinson1, Meghana Malum2, John Teijaro3, Robert Krug2, Michael Oldstone2, Ethan Bier1. 1) Cell & Developmental Biol, Univ California, San Diego, La Jolla, CA; 2) University of Texas at Austin 2500 Speedway Austin, Texas 78712; 3) The Scripps Research Institute 10550 N. Torrey Pines Road, IMM-6 La Jolla, CA 92037.
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<td>1000A</td>
<td>Julián Mensch</td>
<td>Evolution of embryonic pathways in <em>Drosophila</em>: patterns of constraint and positive selection support the <em>hourglass</em> model. Julián Mensch¹, François Serra², Nicolás Lavagnino¹, Hernán Dopazo³, Esteban Hasson³. 1) Departamento de Ecología, Genética &amp; Evolución, Universidad de Buenos Aires, Argentina; 2) Centro de Investigaciones Príncipe Felipe, Valencia, España.</td>
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<td>1001B</td>
<td>Alejandra Petino Zappala</td>
<td>The gene <em>inverted</em> has a pleiotropic effect on developmental adaptive traits in <em>D. melanogaster</em>. Alejandra Petino Zappala, Julián Mensch, Valeria Carreira, Juan José Fanara. Ecología y Evolución, Universidad de Buenos Aires, Buenos Aires, Buenos Aires, Argentina.</td>
</tr>
<tr>
<td>1001C</td>
<td>Li Zhao</td>
<td>Screening, Targeting and Phenotyping of New Genes in <em>Drosophila</em>. Li Zhao¹, Qiye Li², Guojie Zhang¹², Deying Yang¹, Yun Ding¹, Lijun Jin¹, Xin Li¹, Qi Zhou¹, Wen Wang¹. 1) CAS-Max Planck Junior Research Group, State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, The Chinese Academy of Sciences, Kunming, China; 2) BGI-Shenzhen, Shenzhen, China.</td>
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<td>1002C</td>
<td>Mira Han</td>
<td>Gene transpositions in the <em>Drosophila</em> genomes. Mira Han¹, Matthew Hahn¹². 1) School of Informatics and Computing, Indiana University, Bloomington, IN; 2) Department of Biology, Indiana University, Bloomington, IN.</td>
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<tr>
<td>1003A</td>
<td>James B. Pease</td>
<td>Independent Origin of Sex Chromosomes in Winged Insects. James B. Pease¹, Matthew W. Hahn¹². 1) Department of Biology, Indiana University, Bloomington, IN 47405, USA; 2) School of Informatics and Computing, Indiana University, Bloomington, IN 47405, USA.</td>
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<td>1004B</td>
<td>Alfred Simkin</td>
<td>Searching for Signatures of an <em>evolutionary arms race</em> between transposons and piRNAs. Alfred Simkin, William Theurkauf, Jeffrey Jensen. University of Massachusetts Medical School, Shrewsbury, MA.</td>
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<td>1005C</td>
<td>PATRICIA RAMOS</td>
<td>Glyphosate affects the reprotoxic performance in <em>Drosophila melanogaster</em>. Resistance at sight. PATRICIA RAMOS¹², ADRIANA MUÑOZ¹, HUGO RIVAS², BLANCA R. HERNANDEZ², J. ARMANDO MUÑOZ². 1) Lab Genética y Toxicología Ambiental, Depto. Biología, Facultad de Ciencias, CU, Universidad Nacional Autónoma de Mexico, D.F., Coyoacan, Mexico; 2) Drosophila Stock Center Mexico, Facultad de Ciencias, UNAM; 3) CCH-Sur, UNAM.</td>
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<tr>
<td>1006A</td>
<td>Anna‐Sophie Fiston‐Lavier</td>
<td>Screening for transposable element‐induced adaptations in <em>Drosophila melanogaster</em> using next‐gen sequencing data. Anna‐Sophie Fiston‐Lavier, Dmitri A. Petrov, Josefa González. Biology, Stanford University, Stanford, CA.</td>
</tr>
<tr>
<td>1007B</td>
<td>Pankaj K. Tyagi</td>
<td>Effect of food preference on oviposition behavior in immigrans subgroup of <em>Drosophila</em>. Pankaj K. Tyagi, Shruti Tyagi. Department of Biotechnology, Meerut Institute of Engineering and Technology, Meerut, India.</td>
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<td>1008C</td>
<td>Amanda Norvell</td>
<td>Regulation of <em>gurken</em> (<em>grk</em>) mRNA translation by polyadenylation. Amanda Norvell, Anita Rao, Ashley Silakoski, Sakina Attar, Jason Wong. Dept Biol, Col of New Jersey, Ewing, NJ.</td>
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<tr>
<td>1009A</td>
<td>Deanne M. Francis</td>
<td>Characterization of Two Novel EMS Induced Mutants in the Drosophila Trachea. Deanne M. Francis. Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA.</td>
</tr>
<tr>
<td>1010B</td>
<td>Caitlin D. Hanlon</td>
<td>The role of the Fox transcription factor fd64a in embryonic salivary gland migration. Caitlin D. Hanlon, Deborah J. Andrew. Cell Biology, Johns Hopkins Med Inst, Baltimore, MD.</td>
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<td>1012A</td>
<td>Pao‐Ju Chang</td>
<td>Control of transit‐amplifying divisions and organ size by Smurf in <em>Drosophila</em> male germ line development. Pao‐Ju Chang¹, Chang‐Che Hsieh¹, Margaret T. Fuller², Haiwei Pi¹. 1) Department of Biomedical Sciences, Chang‐Gung University, Tao‐Yuan, Taiwan, Taiwan 333; 2) Department of Developmental Biology, Stanford University School of Medicine, Beckman Center B300, 279 Campus Drive, Stanford, CA 94305‐5329, USA.</td>
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<td>1013B</td>
<td>Miriam MS Costa</td>
<td>Regulation of innate immune response and apoptosis by synthetic microRNAs. Chun‐Hong Chen¹², Wan‐Hsun Lin¹, Haixia Huang², Bruce A. Hay². 1) Division of Molecular and Genomic Medicine, National Health Research Institutes, Zhunan Mioali, Taiwan, Taiwan; 2) Division Of Biology, Mc 156‐29, California Institute Of Technology, Pasadena, CA.</td>
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<td>1014C</td>
<td>Laping Liu</td>
<td>Gut immunity. Laping Liu¹², Jianquan Ni¹². 1) Tsinghua Medical School, Tsinghua University, Beijing, Beijing, China; 2) Harvard Medical School, Boston, MA 02115.</td>
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### Neural physiology and behavior

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<td>1018A</td>
<td>Weiwei Liu</td>
<td>Social Regulation of Aggression: A Single Pheromone Functions through Two Olfactory Receptor Neurons in a Temporally Differential Manner to Oppositely Regulate the Same Behavior in Drosophila. <strong>Weiwei Liu</strong>1,2, Xinhua Liang1,3, Yi Ran1,4. 1) National Institute of Biological Sciences, Beijing, China; 2) Beijing Normal University College of Life Sciences, Beijing, China; 3) Institute of Neuroscience, Shanghai, China; 4) Peking University College of Life Sciences, Beijing, China.</td>
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<tr>
<td>1019B</td>
<td>Tomoko Sunayama-Morita</td>
<td>Genetic basis of sine song evolution in <strong>Drosophila</strong>. <strong>Tomoko Sunayama-Morita</strong>1,2, Peter Andolfatto1, David L. Stern1,2. 1) Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ; 2) HHMI.</td>
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<tr>
<td>1020C</td>
<td>Joyce Yushi Kao</td>
<td>Natural variation of post-mating behavior in <strong>Drosophila melanogaster</strong>. <strong>Joyce Yushi Kao</strong>, Sergey Nuzhdin. Computational Molecular Biology, University of Southern California, Los Angeles, CA.</td>
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<tr>
<td>1021A</td>
<td>Subhashree Ganesan</td>
<td>Regulation of Drosophila Glutamate Receptors by novel genes: optimus-prime (opr) and bumblebee (bmb). <strong>Subhashree Ganesan</strong>, David Featherstone. Univ Illinois at Chicago, Chicago, IL.</td>
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### Neurogenetics and neural development

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<td>1024A</td>
<td>Deepak Kumar</td>
<td>A Genetic Modifier Screen of midline to Identify Candidate Enhancer and Suppressor Genes that Regulate Interommatidial Bristle Formation in the Adult <strong>Drosophila</strong> Eye. <strong>Deepak Kumar</strong>, Sandra Leal. Biological Sciences, University Of Southern Mississippi, Hattiesburg MS.</td>
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<tr>
<td>1025B</td>
<td>Tongchao Li</td>
<td>A genetic screen for novel genes in the auditory system. <strong>Tongchao Li</strong>, Andrew Groves1,2,3,4, Hugo Bellén1,2,3,4. 1) Program in Developmental Biology, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute, BCM, Houston, TX; 3) Department of Molecular and Human Genetics, BCM, Houston, TX; 4) Department of Neuroscience, BCM, Houston, TX.</td>
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<td>1026C</td>
<td>Georgina Portillo-Aguilar</td>
<td>An analysis of interactions between alleles of discs large, strawberry notch and changing a cyclin level. <strong>Georgina Portillo-Aguilar</strong>, Flora Retano, Josh Duah, Jennifer Zelaya, Catherine Coyle-Thompson. Biology Department, CSU, Northridge, 18111 Nordhoff St. Northridge, CA.</td>
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<td>1027A</td>
<td>Fabien Soulavie</td>
<td>Characterization of RFX target genes required for ciliogenesis in Drosophila. <strong>Fabien Soulavie</strong>, Anne Laurencin, Joëlle Thomas, Brigitte Chinn, Camille Enjolras, Bénédicte Durand. CGMC, Université Lyon 1, Villeurbanne, France.</td>
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<td>1028B</td>
<td>Shan Jin</td>
<td><strong>Drosophila</strong> katamin 60 regulates microtubule network formation and neuromuscular synapse development. <strong>Shan Jin</strong>, Cuan X. Mao1, Zhao H. Xiong2, Young Q. Zhang2. 1) College of Life Sciences, Hubei University, Wuhan, Hubei 430062, China; 2) Key Laboratory of Molecular and Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China.</td>
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### Pattern Formation

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<td>1030A</td>
<td>Christian Dahmann</td>
<td>Mechanical tension and the influence of cell proliferation on the maintenance of the <strong>Drosophila</strong> dorsoventral compartment boundary. <strong>Christian Dahmann</strong>, Jens-Christian Röper1, Maryam Aliee2, Katharina Landsberg1, Constanze Pentzold1, Thomas Widmann2, Frank Jülicher2. 1) Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; 2) Max Planck Institute for the Physics of Complex Systems, Dresden, Germany.</td>
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### Physiology and aging

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<td>1031B</td>
<td>Hadise Kabil</td>
<td>Modulation of lifespan and healthspan by the transsulfuration pathway. <strong>Hadise Kabil</strong>1,2, Omer Kabil1, Robert Wessels2, Lawrence Harshman3, Scott Pletcher3. 1) Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI; 2) Department of Internal Medicine, University of Michigan, Ann Arbor, MI; 3) School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE.</td>
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<td>1033A</td>
<td>Joy Alcedo</td>
<td>Positive and negative gustatory inputs affect <strong>Drosophila</strong> lifespan partly in parallel to dFOXO signaling. <strong>Joy Alcedo</strong>, Werner Boll, Ivan Ostojeic. 1) Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland; 2) Institute of Molecular Life Sciences, University of Zürich, Switzerland.</td>
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### Regulation of gene expression

**1036A Jessica Chery**
The Role of CLAMP in Drosophila Dosage Compensation. *Jessica Chery, Erica Larschan*. MCB, Brown University, Providence, RI.

**1037B Myungjin Kim**
Grainy head phosphorylation is essential for wound-dependent regeneration of an epidermal barrier. *Myungjin Kim, William McGinnis*. University of California, San Diego, 9500 Gilman Dr. #0349, La Jolla, CA 92039-0349.

**1038C Mark A. Hiller**
The *tfiia-s2* gene is a germ-line-specific homolog of the small subunit of TFIID. *Mark A. Hiller, Alexander Daniel, Margaret Wood, Cynthia Cain*. Dept of Biological Science, Goucher College, Baltimore, MD.

**1039B Marcela Soruco**
A positive feedback mechanism contributes to X chromosome identification during Drosophila dosage compensation. *Marcela Soruco,1 Shouyong Peng,2 Lingsheng Dong,1 Erica Larschan1*. 1) MCB, Brown University, Providence, RI; 2) Harvard Medical School, Boston, MA.

**1040B Nicole C. Evans**

**1041C Hitoshi Ueda**

### RNA biology

**1042A Evan J. Waldron**
Identifying relevant targets of *miR-8* responsible for the pigmentation defect in mutants. *Evan J. Waldron, Jennifer A. Kennell*. Vassar College, Poughkeepsie, NY.

**1043B Rippe Hayashi**

### Stem cells

**1044C Shinya Matsuoka**
Maintenance of undifferentiated state of stem cell precursors in the *Drosophila* ovary. *Shinya Matsuoka1,2, Miho Asaoka1,2, Yasushi Hiromi1,2*. 1) National Institute of Genetics, Mishima, Japan; 2) SOKENDAI, Kanagawa, Japan.

**1045A Nicola Ford**
Early oogenesis phenotypes associated with disrupted function of DPR9, a brain expressed Ig domain protein. *Nicola Ford, Laura Ponting, Martin Baron*. Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom.

**1046B Jiwon Shim**

**1047C Guonan Lin**

**1048A Na Xu**
EGFR, Wingless and JAK/STAT signaling cooperatively maintain Drosophila intestinal stem cells. *Na Xu, Siqi Wang, Guonan Lin, Rongwen Xi*. National Institute of Biological Sciences, Beijing, China.

### Techniques and functional genomics

**1049B Rachel Patterson**
Serine protease activation of the epidermal wound response in *Drosophila*. *Rachel Patterson, William McGinnis*. Biology, UCSD, La Jolla, CA.

**1050C Peter Andolfatto**
Multiplexed Shotgun Genotyping for Rapid and Efficient Genetic Mapping. *Peter Andolfatto1,2, Dan Davison1, Deniz Erezylmaz1,2, Tina Hu1,2, Joshua Mast1,2, Tomoko Sunayama-Morita1,2, David Stern1,2*. 1) Ecology and Evolutionary Biology, Princeton University, Princeton, NJ; 2) The Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ; 3) Howard Hughes Medical Institute, Princeton University, Princeton, NJ; 4) Dept. of Statistics, Oxford University, Oxford, UK, OX1 3TG.

**1051A Julie H. Simpson**
Drosophila Brainbow can be used to subdivide complex GAL4 patterns. *Julie H. Simpson, Stefanie Hampel, Phuong Chung, Claire McKellar, Donald Hall, Loren Looger*. HHMI, Janelia Farm Res Campus, Ashburn, VA.

### Educational Initiatives

**1052B Rosario Rodriguez-Arnaiz**
969B  
**Drosophila neuroblasts retain the daughter centrosome.** Jens Januschke1, Sudal Llaamares1, Jose Reina1, Cayetano Gonzalez2, 1. 1) Cell Division Group, IRB-Barcelona, PCB c/ Baldri Reixac 10-12, Barcelona, Spain; 2) Institucio Catalana de Recerca i Estudis Avancats (ICREA). Passeig Lluis Companys 23, Barcelona, Spain.

In asymmetric cell division, both in male *Drosophila* germ line stem cells and in mouse embryo neural progenitors, the mother centrosome is retained by the stem cell, hence suggesting that mother centrosome inheritance might contribute to stem cell self-renewal. We have tested this hypothesis in *Drosophila* neuroblasts. Tracing photo converted centrioles by live cell imaging we have found that neuroblasts invariably retain the daughter centrosome. Thus, while demonstrating maturation-dependent centrosome fate, these results show that stemness is not linked to mother centrosome inheritance in *Drosophila* neuroblasts.

969C  
**Retromer regulates apical-basal polarity through recycling Crumbs.** Bo Zhou1, Xinhua Lin1,2, 1) Dev Biol, Cincinnati Children's Hosp, Cincinnati, OH; 2) Institute of Zoology, Chinese Academy of Sciences, Beijing, China.

Epithelial cells are characterized by an “apical-basal” polarization. The transmembrane protein Crumbs (Crb) is an essential apical determinant which confers apical membrane identity. Previous studies indicate that Crb does not constantly reside on the apical membrane, but is actively recycled. However, the cellular mechanism(s) underlying this process are unknown. Here we show that in Drosophila retromer, which is a retrograde complex recycling certain transmembrane proteins from endosomes to trans-Golgi network (TGN), can regulate the intracellular recycling of Crb. In the absence of retromer activity, apical-basal polarity is disrupted in epithelial cells. We further find that without retromer activity, Crb fails to be recycled after internalization and is misdirected to lysosomes for degradation, resulting in a disruption of apical-basal polarity. In wild type epithelial cells, Crb co-localizes with retromer. Antibody uptake experiments in cell culture reveal that retromer regulates the retrograde trafficking of Crb from endosomes to TGN. Together, our data demonstrate that the intracellular recycling of Crb is regulated by retromer and this process is essential for epithelial apical-basal polarity during development.

961A  

ERM (Ezrin-Radixin-Moesin) proteins play a critical role in organizing the cortical cytoskeleton, providing both structural and regulatory functions. Moesin, the single Drosophila ERM protein, is a known to organize the actin cytoskeleton in the apical domain of epithelial cells. Furthermore, Moesin has been shown to regulate epithelial morphology by antagonizing the small GTPase Rho1. We have been identifying Moesin interacting proteins to better understand how Moesin is regulated and its role in regulating Rho1. Using a truncated form of Moesin that stabilizes interactions with its binding partners and mass spectrometry, we identified three new Moesin interactors - a putative RabGAP (CG7112), a predicted metalloendopeptidase (CG2025) and a zinc finger protein (CG1677). Preliminary co-immunoprecipitation and colocalization experiments in cultured cells confirm that these proteins form a complex with Moesin and that all three interact with the Moesin FERM domain. In addition, we are using available RNAi transgenes for each gene to ask whether they interact genetically with Moesin mutations in vivo and whether their phenotypes resemble those of Moesin mutants. Our preliminary phenotypic data suggest that reduction of CG1677 resembles loss of Moesin and overexpression of Rho1, consistent with a synergistic function with Moesin. We are currently conducting further genetic experiments to investigate whether the other genes function together with Moesin, and if so to further characterize their functions. Together these experiments should allow us to determine the role of these putative Moesin interacting proteins in cytoskeletal organization and epithelial integrity.

961B  
**The role of Drosophila DAAM in the development of the Indirect Flight Muscle.** Imre Molnar1, Ede Migh1, Tibor Kalmar3, Zacharias Orfano6, John Sparrow2, Sziilvia Bako5, Miklos Nyitrai1, Jozsef Mihaly1, 1) Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary; 2) Department of Biology, University of York, York, UK; 3) Faculty of Medicine, Department of Biophysics, University of Pecs, Pecs, Hungary.

In non-muscle cells, generally there are two major actin assembly factors, the formins and the Arp2/3 complex. Formins are producing long straight actin filaments, whereas the Arp2/3 complex promotes the formation of a branched actin network. Because the unbranched straight actin filament is the major form in striated muscle cells, it is possible that a formin family protein serves as the key regulator of actin dynamics in myofibrils. These straight actin filaments constitute one of the major filament system of the sarcomeres but little is known about the regulation of actin assembly in muscle cells. Recently, we found that the Drosophila formin DAAM (dDAAM) is highly enriched in the Indirect Flight Muscle (IFM) of both the pupae and adult flies. Subsequently, with the use of different approaches such as muscle functional tests, immunohistochemistry and biophysical assays, we demonstrated that dDAAM plays role in the filament formation and sarcomere assembly. The dDAAM loss of function mutants exhibit sarcomere length reduction and in more extreme cases, severe sarcomere assembly defects that can be fully rescued by the full length wild type protein. Moreover, the dDAAM mutants exhibit a strong dominant genetic interaction with that of Act88Fkm88, an IFM specific actin null mutant. All together, our findings suggested that sarcomeric actin assembly and thus sarcomere organization in Drosophila is critically dependent on the formin dDAAM. Our poster will provide detailed information on our major findings as to how dDAAM contributes to sarcomeric thin filament assembly and muscle development.

963C  
**Towards a quantitative analysis of Ras pathway effects on BMP responses in the wing.** Alexi Brooks1,2, Jing Cao3, Mohit Bahel2,3, Michal Jager3, Tara Brosnan1, David Umulis1, Laurel Raftery2, 1) School of Life Sci, Univ Nevada, Las Vegas, NV; 2) CBRC, MGH/Harvard Med Sch, Charlestown, MA; 3) New York Univ, New York, NY; 4) Dept. of Ag & Biol Engineering, Purdue Univ, Lafayette, IL.

Bone morphogenic proteins (BMPs) are morphogens in many tissues of vertebrates and invertebrates. Extracellular BMP activates receptor-phosphorylated Mad (C-phospho-Mad) are proportional to the level of BMP activity and resultant target gene expression. Thus, intracellular R-Smads, via receptor-kinase phosphorylation of R-Smad C-termini. For fly BMPs, Mad is the critical R-Smad. Nuclear levels of activated Ras can reduce endogenous C-phospho-Mad. Ongoing experiments will test whether linker mutation of linker phosphorylation sites creates a hyperactive form of Mad, and Mad is phosphorylated by Erk MAP kinase in vitro. Widespread expression of activated Ras can reduce endogenous C-phospho-Mad. Ongoing experiments will test whether linker phosphorylation is the relevant mechanism. To test the significance of these pathway interactions to endogenous gradient interpretation, we next plan to use an imaging method to detect quantitative differences in the presence of two sources of variable optical 1- from cell to cell across each field and 2-from field to field. We are testing algorithms to detect quantitative changes in staining intensity between experiment and control fields. Most quantitative studies of BMP gradients in the wing primordium use data from one spatial dimension. We
functions, such as bone formation, tumour angiogenesis, and chronic kidney disease. The Drosophila BMP signalling molecule, Decapentaplegic (Dpp) is part of a family of signalling molecules that is central to development and encompasses a range of human diseases. A gradient of Dpp specifies distinct cell fates in the dorsal ectoderm of the early embryo. Dpp signalling leads to activation of the Mad/Medea transcription factor complex, which in turn, orchestrates a specific, yet largely unknown gene expression programme. The transcriptional repressor, Brinker (Brk), represses Dpp target genes in cells receiving low levels of signal, thus ensuring tight spatial control of Dpp action. We have determined the genome-wide binding sites of Mad and Brk using ChIP-seq in early embryos. While Mad and Brk bind to many of the same enhancers, we have also identified enhancers that bind only one or the other. Experiments are ongoing to determine the regulatory features that specify these different types of enhancers. We are further verifying functional Mad and Brk enhancers and gene targets using candidate embryo in situ studies and cell culture assays. Overall, these results will identify fundamental principles underlying the reprogramming of gene expression by a signalling pathway in a multicellular organism.

Integrin signalling enhances BMP activity in the early Drosophila embryo. Annick Sawala, Hiliary L. Ashe. Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom.

Bone morphogenetic proteins (BMPs) form a conserved family of signalling molecules with diverse functions in development and disease. The early Drosophila embryo provides an excellent system to study BMP regulation, as a gradient of BMPs induces distinct target genes at different signalling thresholds, providing a simple read-out of BMP activity. Recently, we showed that the extracellular matrix protein collagen IV promotes BMP signalling in the Drosophila embryo. Collagen IV directly binds BMPs and the BMP antagonist Sog in vitro. Biochemical and in vivo data indicate that collagen IV promotes peak signalling both by facilitating the transport of BMPs towards the dorsal midline, where peak signalling occurs, and by enhancing BMP-receptor interactions at the cell surface.

Here, we describe an important role for integrins, which are cell surface receptors for collagen IV, in promoting BMP signalling. In embryos lacking all integrin function, peak BMP signalling is lost, resulting in disruption of BMP target gene expression and reduced accumulation of the BMP signal transducer phospho-Mad. This defect can be rescued by an integrin-construct that cannot bind extracellular ligands but is capable of signalling, demonstrating that the signalling function of integrins is sufficient for their effect on BMP pathway activation. Furthermore, constitutively active integrin signalling can also partially rescue the BMP defects of collagen IV mutant embryos. We therefore propose that, in addition to its role in regulating extracellular BMP distribution, collagen IV may promote BMP signalling by binding to and activating integrin receptors.

Current studies are aimed at identifying the downstream components that link integrin signalling to BMP pathway activation. These findings may have important implications for our molecular understanding of processes which involve BMP, collagen IV and integrin functions, such as bone formation, tumour angiogenesis, and chronic kidney disease.


Hedgehog (Hh) signaling is important in stem cell biology, embryonic development, and disease, including cancer. Hh acts as a morphogen to regulate growth and development of distant cells in a variety of tissues. Hh spreading is a complex process that requires the interaction of various proteins, some of which are membrane bound while others form part of the extracellular matrix network. The Ihog (interference hedgehog) family of transmembrane proteins, which in Drosophila consist of the products of the ihog and brother of ihog (boi) genes, have been described to act as Hh co-receptors both in flies and in vertebrates and required to maintain normal extracellular Hh levels in Drosophila wing discs. boi codes for a protein that bears a 45% identity with Ihog and, so far, boi has been described to be redundant with Ihog in all its functions. Here, we investigate the function of Ihog, and the related Boi proteins in Hh movement in the wing imaginal disc epithelium by gain and loss of function experiments. We also analyze the interaction of Ihog and Boi with the diffusible factor Drosophila WIF, product of the shifted gene, another extracellular matrix component shown to be important for Hh to facilitate both Hh spreading and stability. Our lab has recently proposed that cytoneme-mediated Hh transport provides a mechanistic explanation for Hh gradient formation. Here, we show that Shifted/DmWIF interacts with Ihog and Boi for Hh attachment at the plasma membrane and for proper cytoneme-mediated morphogen distribution. We have also observed that Ihog and Boi have distinct function in the formation of the short- and long-range Hh gradient as well as in their interaction with Shifted/DWIF.

Recruitment Timing and Dynamics of Transcription Factors at the Hsp70 Loci in Living Cells. Martin S. Buckley¹, Katie L. Zobeck¹, Warren R. Zipfel², Lis J. Lis¹. 1) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) Department of Biomedical Engineering, Cornell University, Ithaca, NY.

Chromatin immunoprecipitation (ChIP) studies provide snapshots of factors on chromatin in cell populations. Here, we use live cell imaging to examine at high temporal resolution the recruitment and dynamics of transcription factors to the inducible Hsp70 loci in individual Drosophila salivary gland nuclei. Recruitment of the master regulator, HSF, is first detected within 20 sec of gene activation and the timing of its recruitment resolves from RNA polymerase II and P-TEFb, and these factors resolve from Spt6 and Topo I. Remarkably, the recruitment of each factor is highly synchronous between different cells. In addition, Fluorescence Recovery after Photobleaching (FRAP) analyses show that the entry and exit of multiple factors are progressively constrained upon gene activation, suggesting the gradual formation of a transcription compartment. Furthermore, we demonstrate that PolyADP-Ribose (PAR) Polymersase activity is required to maintain the transcription compartment. We propose that PAR polymers locally retain factors in a transcription compartment.

The large Maf transcription factor Traffic Jam regulates border cell migration in the Drosophila ovary. Felix Gunawan, Milica Arandjelovic, Dorothea Godt. Cell and Systems Biology, University of Toronto, Toronto, Ontario, Canada.

The border cell cluster (BCC) in the ovarian follicle is used as a model to study cell migration. The BCC forms when the two anterior polar cells induce their neighbouring cells to become migratory rosette cells through JAK/Stat signaling. The rosette cells delaminate from the follicular epithelium and migrate with the polar cells between the nurse cells toward the oocyte. We have previously shown that the large Maf transcription factor Traffic jam (Tj) is required for differentiation of somatic gonadal cells and their interaction with the germline. Here, we show that Tj regulates migration of the BCC. Tj is expressed in the BCC, but the amount of protein gradually decreases during migration. Reduction of Tj expression to lower-than-wildtype level in the BCC led to a significant delay in migration. Tj overexpression caused an even more severe defect, with border cells usually failing to initiate migration. These data indicate that Tj needs to be expressed at its endogenous low level for proper BCC migration. Studying interactions between Tj and Slow border cells (Slbo), a transcription factor that is activated by JAK/Stat signalling and is required for BCC migration, revealed that both knockdown and overexpression of Tj cause a strong decrease in slbo expression at the RNA and protein level. Slbo overexpression significantly rescued the BCC migration defect caused by Tj.
knockdown and overexpression. Our results indicate that sibo is an important target of Tj activity in the BCC. Moreover, reduction of Sibo prevented Tj downregulation in the BCC, whereas sibo overexpression accelerated the reduction of Tj expression. These data suggest that Tj and Sibo regulate each other in a feedforward loop, with Sibo causing repression of Tj and a low concentration of Tj leading to an activation of sibo. Tj and Sibo appear to interact in a JAK/Stat-regulated transcriptional network that allows the BCC to properly undergo migration.

986C

**Function of Drosophila AdoR in tissue culture and in vivo.** Lucie Kucerova1,2,3, Vaclav Broz1,2,3, Jana Fleischmannova1, Roman Sidorov1, Vladimir Dolezal2, Michal Zurovec1,2,3. 1) BC AAVC, Ceske Budejovice, Czech Republic; 2) Department of Neurochemistry, Institute of Physiology, Czech Acad Sci., Praha, Czech Republic; 3) Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic.

Adenosine affects a number of physiological processes in Drosophila ranging from energy metabolism to complex behavior. Most of its effects are mediated by a G protein-coupled adenosine receptor (AdoR) showing significant structural similarity to mammalian adenosine receptors. We show that the adenosine responses in Drosophila neuroblast and embryonic cells with endogenous AdoR expression stimulate cAMP production but they do not influence calcium signaling. We also tested the pharmacological properties of AdoR by examining a number of commercially available human adenosine receptor agonists and antagonists. Several AdoR agonists and antagonists were detected and the most efficient ones were confirmed in Drosophila in vivo by mimicking the effects of AdoR overexpression and mutation, respectively.

972C

**Function of Drosophila TRPP2 homolog, Amo, is required for sperm activation in the female uterus.** Weiwei Li1, Alexis Hofherr2, Kristy Chu1, Stacey Cook1, Michael Kötgen2, Terry Watnick1. 1) Division of Nephrology, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Renal Division, University Hospital Freiburg, Freiburg, Germany.

In *Drosophila melanogaster*, sperm must first transit from the uterus to specialized female storage organs. Although this process is thought to involve directed sperm motility, there is little known about the characteristics of sperm movement within the mated female. Since activated flagellar beating in other species is critical for sperm motility, including sperm turning and capacitation, we investigated the beat frequency of sperm tails before and after transfer to the female reproductive tract. We found that wild type sperm released from the uterus immediately after mating have a significantly higher flagellar beat frequency when compared to sperm released from the male seminal vesicle, suggesting that *Drosophila* sperm undergo an activation step similar to what has been described for mammalian sperm. We have recently demonstrated that Amo, a homolog of TRPP2, is a sperm enriched protein that is essential for sperm storage in Drosophila. Male flies lacking Amo protein produce motile sperm that are transferred to the uterus but they are not stored in the female storage organs. A detailed analysis of motility patterns in amo- mutant sperm demonstrated that their baseline beat frequency when released from the seminal vesicle was similar to wild type. However they failed to demonstrate an increase in beat frequency when released from the uterus. This defect in sperm activation was rescued by a wild type amo transgene. To test whether the decreased beat frequency of amo- mutant sperm translated into altered swimming speed of sperm in vivo, we tracked the movement of red fluorescent protein-labeled sperm heads in the uterus. Consistent with the observed decrease in beat frequency, the swimming speed of amo- sperm in the uterus was reduced significantly when compared to wild type sperm. Taken together, these defects in sperm function are likely to explain the inability of amo- mutant sperm to reach the female sperm storage organs.

971B

**Drosophila heparan sulfate 6-O endosulfatase regulates Wingless morphogen gradient formation.** Adam Kleinschmit, Takashi Koyama, Katsufumi Dejima, Yoshiaki Hayashi, Keisuke Kamimura, Hiroshi Nakato. Genetics, Cell Biology, & Development, University of Minnesota, Minneapolis, MN.

Heparan sulfate proteoglycans (HSPGs) play critical roles in the distribution and signaling of growth factors, but the molecular mechanisms regulating HSPG function are poorly understood. Here, we characterized Sulfl, which is a Drosophila member of the HS 6-O endosulfatase class of HS modifying enzymes. Our genetic and biochemical analyses show that Sulfl acts as a novel regulator of the Wg morphogen gradient by modulating the sulfation status of HS on the cell surface in the developing wing. Sulfl affects gradient formation by influencing the stability and distribution of Wg. We also demonstrate that expression of Sulfl is induced by Wg signaling itself. Thus, Sulfl functions in a feedback loop, potentially stabilizing the shape of the Wg gradient. Our study shows that the modification of HS fine structure provides a novel mechanism for the regulation of morphogen gradients.

972C

**Functional analysis of Wnt signaling pathway in the regulation of mosquito vitellogenesis.** Shin-Hong Shiao. Department of Parasitology, National Taiwan University, Taipei, Taiwan.

Mosquito-borne diseases are the most devastating agents for human being, due to its high diversity of transmissible pathogens like *plasmodium* and viruses. Despite the efforts from government agencies that have contributed the eradication of the mosquito-borne diseases for several decades, the goal has not been achieved yet. Therefore, many research institutes turn their attentions toward the mosquito life cycle and immune system to halt the disease transmission. Previous studies have already demonstrated that Target of Rapamycin (TOR) pathway plays an important role in mosquito reproductive development as it is involved in mosquito vitellogenesis whereas Wnt pathway participates in the embryonic development and cell polarity. Besides, it has long been accepted that Imd and Toll pathway regulate the production of antimicrobial peptides. However, the interactions between these pathways are poorly understood. In this study, we propose a hypothesis that factors of TOR and Wnt signaling pathway play synergistically in the mosquito vitellogenesis. We attempt to characterize Wnt signaling components in the mosquito, Aedes aegypti. Our results showed that silencing of *Fz2*, a homolog of Wnt receptor, and *Tj* resulted in a decrease of Aedes aegypti survival fitness against *S. aureus* and *E. coli* infection. Interestingly, the oviposition ability has been altered in the Aedes aegypti. Our results showed that silencing of Frizzled2 (*Fz2*), a transmembrane receptor of WNT signaling pathway, and TOR resulted in the decrease of Wnt signaling components in the mosquito, Aedes aegypti. We attempt to characterize WNT signaling components in the mosquito, Aedes aegypti. We attempt to characterize WNT signaling components in the mosquito, Aedes aegypti.
pathways are part of the Wls recycling process and are therefore required for efficient Wnt secretion.


The DNA damage response in eukaryotes involves multiple, complex, and often redundant pathways. One mechanism in which genomic stability and cell proliferation is maintained is through homologous recombination (HR). This mechanism is essential in repairing DNA doublestrand breaks (DSBs) and interstrand crosslinks (ICLs), and is involved in recovering stalled or broken replication forks. In some HR pathways, a four-stranded DNA structure known as a Holliday junction (HJ) is formed. The movement, or branch migration, of HJs is mediated through specialized helicases and cut by specialized nucleases. While extensive work on Holliday junction processing has been done in prokaryotes, much less is known about HJ processing in eukaryotes. The Bloom helicase (BLM) is a protein that acts on HJs during DSB repair to prevent detrimental crossover (CO) events. Another protein of interest involved in DNA repair is FANCM. FANCM is encoded by the Fanconi anemia (FA) complementation group M gene, and is a Hel family helicase responsible for recruiting multiple FA proteins to ICLs, and recruitment of BLM to sites of DNA damage. FANCM has been observed in vertebrates and orthologous proteins of FANCM are found in some invertebrates, such as Drosophila melanogaster DmFANC-M, which shows high sequence similarity to human. Interestingly, both FANC-M and BLM are helicases that migrate HJs although each act in response to a different type of damage. To test how DmFANC-M is involved in DNA repair, we obtained missense and nonsense fanc-M alleles through TILLING. We found that fanc-M mutants show increased sensitivity to ionizing radiation, suggesting a role for fanc-M during HR. We also found that fanc-M mutants show elevated mitotic COs indicating a possible role for fanc-M in a pathway termed synthesis dependent strand annealing (SDSA). Currently, we are investigating how reduced expression of fnc-M increases the incidences of COs in a manner similar to the canonical bacterial HJ resolvase, RuvC. However, S. cerevisiae yen1 mutants show no defects in cell growth, viability, or resistance to a variety of DNA damaging agents. Interestingly, mus81 yen1 double mutants are more hypersensitive to DNA damaging agents than mus81 single mutants, suggesting partial redundancy between the two endonucleases during both meiosis and the repair of DNA damage. We are currently investigating the role of FANC-M in meiotic recombination by measuring the level of crossing over in defined intervals on the X chromosome in Gen mutants. In addition to genetic tests, we have expressed and purified FANC-M and are currently determining FANC-M’s in vitro substrate specificity.

975C  Characterizing the role of Psf2 in maintaining genomic integrity. Jeffrey P. Chmielewski, Tim W. Christensen, Biology, East Carolina University: Department, Greenville, NC.

In D. melanogaster, the CMG complex is a group of proteins that function as the DNA helicase during replication. The CMG is composed of cdcc45, MCM2-7, and the GINS complex. The GINS complex is a heterotetrameric complex composed of the protein subunits Psf1, Psf2, Psf3, and Sld5. Recent research in human dermal fibroblasts shows GINS is essential for the initiation and elongation stages of chromosomal replication. My primary hypothesis is that proper dosage of Psf2 is necessary to maintain genomic integrity, and there may be an interaction between Psf2 and Chk2 (loki) modulating DNA replication. Working with null mutations in Psf2 and Chk2, both separately and in combination, I have designed a series of experiments aimed at elucidating the function of Psf2. We are using DNA fiber analysis to determine if reduced expression of Psf2 alters the rate of the replication fork. In addition to DNA fiber analysis, we are investigating if reduced expression of Psf2 increases the incidences of h2AX foci, which could be indicative of Chk2 activation. Our lab has previously shown that the null mutation of Psf2 is homozygous lethal, we also have evidence of reduced viability in flies heterozygous for the null mutation. We are using acridine orange staining to determine if this reduced viability in flies heterozygous for the null mutation is a consequence of an increase in apoptosis.

976A  Investigating the Role of GEN in Drosophila. Stephanie Bellendir, Sabrina Andersen, Jeff Sekelsky. Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Homologous recombination is required for the accurate maintenance of the genome during meiosis and DNA repair; inaccurate repair and recombination often lead to cancer. Many parts of these two pathways are still unknown. It is thought that the correct resolution of Holliday Junctions, key intermediates of these pathways, is important for maintenance of the genetic material. Recent studies in budding yeast reveal that a Rad2/XPG-family structure-specific endonuclease, Yen1 cuts Holliday Junctions in vitro in a manner similar to the canonical bacterial HJ resolvase, RuvC. However, S. cerevisiae yen1 mutants show no defects in cell growth, viability, or resistance to a variety of DNA damaging agents. Interestingly, mus81 yen1 double mutants are more hypersensitive to DNA damaging agents than mus81 single mutants, suggesting partial redundancy between the two endonucleases during both meiosis and the repair of DNA damage. We are currently performing the first in vivo studies of Gen, the Drosophila ortholog of Yen1, to learn more about the encoded protein and its associated pathways. mus81; Gen double mutants exhibit elevated levels of apoptosis and have small, rough eyes, indicative of overlapping functions between the genes as in yeast. Surprisingly, flies harboring a mutation in the GEN ortholog DmFANC-M show elevated mitotic COs indicating a possible role for fanc-M in a pathway termed synthesis dependent strand annealing (SDSA). Currently, we are investigating how reduced expression of fnc-M increases the incidences of h2AX foci, which could be indicative of Chk2 activation. Our lab has previously shown that fanc-M is involved in DNA repair, we obtained missense and nonsense fnc-M alleles through TILLING. We found that fanc-M mutants show increased sensitivity to ionizing radiation, suggesting a role for fanc-M during HR. We also found that fanc-M mutants show elevated mitotic COs indicating a possible role for fanc-M in a pathway termed synthesis dependent strand annealing (SDSA). Currently, we are investigating how reduced expression of fnc-M increases the incidences of COs in a manner similar to the canonical bacterial HJ resolvase, RuvC. However, S. cerevisiae yen1 mutants show no defects in cell growth, viability, or resistance to a variety of DNA damaging agents. Interestingly, mus81 yen1 double mutants are more hypersensitive to DNA damaging agents than mus81 single mutants, suggesting partial redundancy between the two endonucleases during both meiosis and the repair of DNA damage. We are currently investigating the role of FANC-M in meiotic recombination by measuring the level of crossing over in defined intervals on the X chromosome in Gen mutants. In addition to genetic tests, we have expressed and purified GEN and are currently determining GEN’s in vitro substrate specificity.


Caspases are executioners of apoptosis, but also participate in a variety of vital cellular processes. Recent studies suggest that the nonlethal roles of caspases are often associated with low, compartmentalized, and/or transient caspase activity, which may be insufficient to induce apoptosis. To directly test this possibility, we compared the activity properties in vivo of the two main effector caspases in Drosophila Drice and Dcp-1. Using a genetic reporter of caspase-3-like activity, we show that following irradiation-induced apoptosis, both Drice and Dcp-1 become activated. However, consistent with the idea that Drice is the major effector caspase, the activity of Dcp-1 is moderately but significantly lower than that of Drice. The apoptosome components Ark and Dronc are both required for this activity, which can be largely, but not completely blocked by coexpressing the caspase inhibitor proteins Diap1 and p35, respectively. Interestingly, whereas loss of drice halts cell death completely, transgenic expression of Dcp-1 from the regulatory regions of drice is sufficient to induce apoptosis in irradiated flies. Likewise, this Dcp-1 transgene restores the survival rate of drice mutants to wild-type levels. We further show that while apoptosis can proceed in the absence of functional Dcp-1, these flies display a significant delay in the induction/velocity of cell death following irradiation. Finally, transgenic expression of Drice, but not Dcp-1, restores cell death and survival rate in the double mutant flies, indicating that although Dcp-1 has the potential to induce apoptosis, Drice is a more potent death effector than Dcp-1. Collectively, these results demonstrate that effector caspases must reach a threshold level to induce apoptosis and that the differences in their functions are the consequence of both distinct expression levels and execution potencies.

978C  The adenovirus E4orf4 protein promotes a low level of cell death in Drosophila normal tissues and inhibits Reaper-, HID-, and Eiger-induced apoptosis. Antonina Pechkovsky*, Maoz Lahav*, Adi Salzberg*, Tamar Kleinberger*. 1) Department of Genetics, Rappaport Faculty of Medicine, Technion, Haifa, Israel; 2) Department of Microbiology, Rappaport Faculty of Medicine, Technion, Haifa, Israel.
The adenovirus E4orf4 protein induces caspase-independent, p53-independent cell death of transformed but not normal human cells, suggesting that elucidation of the mechanisms underlying its action may provide important new opportunities for cancer therapy. We investigated the E4orf4 expression in *Drosophila*. Expression of E4orf4 in the eye imaginal disc under the regulation of the GMRGal4 driver, induced a dose-dependent small and rough eye phenotype. Expression of E4orf4 in the wing imaginal disc, using the engrailedGal4 driver, caused a dose-dependent cell death in the wing. E4orf4 induced caspase activation in *Drosophila* eye and wing discs. E4orf4-induced cell death was caspase-independent at least in part, as overexpression of the caspase inhibitors DIAP or p35 only partially rescued the E4orf4-induced cell death. We found a high degree of conservation in the mechanisms underlying E4orf4 effects in mammalian cells and in the flies, including the requirement for PP2A and Src. E4orf4 mutants that did not bind PP2A and Src induced less pronounced cell death than wild type E4orf4, and PP2A and Src binding to E4orf4 appeared to contribute in an additive manner to E4orf4-induced phenotypes. When E4orf4 was expressed in eye imaginal discs of mutant flies lacking PP2A-B55 regulatory subunit, or when Src or PP2A activity was knocked down by RNAi, less E4orf4-induced cell death was exhibited. When E4orf4 was coexpressed with the Reaper and HID in the eye disc, it partially rescued Reaper- and HID-induced cell death. When E4orf4 was coexpressed with Eiger, a *Drosophila* TNFα orthologue that has been shown to induce cell death by triggering JNK signaling, it rescued Eiger-induced tissue damage. Thus we conclude that E4orf4 can act both as a cell death-inducer and as an anti-apoptotic protein when expressed in non-transformed Drosophila tissues.

979A

Redundancy and specificity in the function of *drICE* and *dcp-1* in the cell death and development of the *Drosophila* optic lobe. Hidenobu Tsujimura, Hiromi Akagawa, Yu Togane, Yusuke Hara, Takashi Takahashi, Tatsuya Sudo, Rie Ayukawa, Keichiro Hirai, Kengo Beppu. Dept Dev Biol, Tokyo Univ Agric & Tech, Fuchu-si, Tokyo, Japan.

*drICE* and *dcp-1* are major effector caspases among four candidates in *Drosophila*. They work redundantly in some events, but specifically or cooperatively in others. In the death of the optic lobe. Great number of dying cells begin to increase after pupation and peaks at 24 hours APF. The timing is controlled by edcsides. This cell death involves the function of *hid* and *dronc*. Here we defined the type and role of effector caspases for this cell death. *drICE* and *dcp-1* are both important but play different roles in the lamina and medulla/lobula. In the lamina, *drICE* is exclusively required for most of the dying cells. In contrast, *drICE* and *dcp-1* work redundantly in the medulla/lobula and either one is required for the cell death. Amasingly specific function of *drICE* is suggested for the corpus clearance in the cell death. Moreover, we examined requirement of *drICE* and *dcp-1* for the morphogenesis and wiring of the optic lobe. Mutants caused defects in the gross anatomy of the optic lobe and the development and wiring of the lamina, medulla and lobula neuropile. Some events needs cooperative function of both caspases, but others needs either one.

980B

Use of integrase-mediated BAC transgenesis to identify p53 alleles that separate regulation of DNA repair from apoptosis. Skye Souter, Sarah Oikemus, Naoto Ito, Michael Brodsky. Gene Function & Expression, UMass Medical School, Worcester, MA.

The p53 transcription factor plays an evolutionarily-conserved role activating apoptosis in response to DNA damage and unprotected telomeres. It is commonly hypothesized that p53-mediated apoptosis helps prevent accumulation of cells with chromosome damage. Surprisingly, we found that Drosophila p53 promotes rather than suppresses genomic instability in animals with defective telomere protection. Mutations in p53 reduce the fusion frequency of unprotected telomeres and the rate of non-homologous end-joining (NHEJ) of double strand DNA breaks. Loss of the upstream kinase Chk2/MNK does not alter telomere fusion rates, suggesting that p53 plays a direct role in NHEJ independent of its ability to activate transcription. If this model is correct, it should be possible to identify p53 alleles that separate the DNA repair and apoptosis functions. Phich31 integrase-mediated transgenesis allowed us to target a bacterial artificial chromosome (BAC) containing p53 and flanking genomic sequences to a specific chromosomal landing site as described by Bellen et al. This BAC rescues both the apoptosis and telomere fusion phenotypes associated with loss of p53 function. Using a phage recombineering to modify this BAC, we altered single p53 amino acids associated with damage-induced phosphorylation or site-specific DNA binding. In vivo analysis identified separation-of-function p53 alleles that disrupt damage-induced apoptosis without affecting telomere fusion rates. These results indicate that p53 can independently regulate transcriptional induction of apoptosis and fusion of chromosome ends by NHEJ; the critical function of p53 for genomic stability appears to be repair, not apoptosis, in the presence of unprotected telomeres. More broadly, this study shows that recombineering and targeted BAC integration provide a general and rigorous method to probe the contribution of individual amino acids on in vivo gene function in a context that mirrors endogenous gene expression.

981C


The pro-apoptotic Hippo (Hpo) pathway negatively regulates tissue growth by phosphorylating Yorkie (Yki), a key growth-promoting transcriptional coactivator. This results in cytoplasmic retention of Yki due to 14-3-3 binding to phosphorylated Yki-Ser168 (1). Phosphorylation of Yki is mediated by the NDR-related kinase Warts (Wts), which in turn is activated by the Ste20-like kinase Hippo (Hpo). The core components of the Hpo-pathway are conserved in mammals and have been implicated in tumor development (2, 3). Though phosphorylation on S168 is clearly important, our preliminary data indicated that Yki is phosphorylated at a number of sites, indicating that its regulation is likely to be more complex and to involve multiple pathways. The primary aim of this project is to uncover signalling pathways that influence tissue growth by controlling Yki activity independently or via crosstalk with the Hpo pathway. Thus, a major goal is to identify kinases that modify Yki and link their activity to specific phosphorylation sites. The basic approach is to discover novel phosphorylation sites on Yki, which play a role in various aspects of its oncogenic potential e.g. transcriptional activity, binding to other transcription factors or general protein turnover rate. By means of a combination of various techniques including genetics, Mass Spectrometry (MS), computational approaches and biochemistry, novel phosphorylation-dependent mechanisms of Yki-driven tissue growth will be investigated.


982A

A novel role for folate metabolism during cellularization in *Drosophila* embryos. Andrew Guzman1, Justin Crest1, Jian Cao2, William Sullivan1. 1) MCD Biology, University of California, Santa Cruz, CA; 2) Stanford University School of Medicine, Stanford, CA.

In Drosophila embryos, the first thirteen divisions after fertilization occur synchronously every 10-25 minutes without cytokinesis. This gives rise to a syncytium containing about 6000 nuclei. Despite the lack of cytokinesis, acto-myosin based furrows form during divisions 11-13 in order to separate dividing nuclei, and the associated spindle, from neighboring nuclei. Regulation of these “metaphase furrows” has been shown to be similar to that of cytokinetic furrows. In order to find novel components or regulators of these structures, our lab has generated a class of temperature sensitive mutations that affect their formation and maintenance. One of the genes generated, ts161, fails to form furrows during cycle 14 and therefore never cellularizes. Ts161 is a homologue of the mammalian folypolyglutamate synthase (FPGS) which is an enzyme occurring early in the folate pathway. The folate pathway is required for purine synthesis and methylation of both DNA and folate metabolism during cellularization in *Drosophila* embryos.
Assessing a potential cohesive role for the mitotic cohesin Scc1/Rad21 during female meiotic divisions. Stefan K. Heidmann¹, Evelin Urban², Christian Lehner², Sonal Nagarkar Jaiswal³. 1) Dept of Genetics, University of Bayreuth, Bayreuth, Germany; 2) Institute of Zoology, University of Zurich, Zurich, Switzerland.

A role for cohesive sister chromatid segregation is essential for passing all genetic information to each daughter cell after cell division. The fidelity of this process critically depends on the fact that sister chromatids remain physically connected with each other from the time of their synthesis until the transition from metaphase to anaphase. Cohesin, a chromatin-bound heterohexameric protein complex, is required for the physical association of sister chromatids from early S phase until anaphase. The mitotic cohesin complex consists of two SMC (structural maintenance of chromosome) subunits, Smc1 and Smc3, and two non-SMC subunits, Scc1/Rad21 and Scc3. The resolution of sister chromatid cohesion is triggered by the activation the protease separase, which cleaves the Scc1/Rad21 subunit, thereby allowing sister chromatid segregation in anaphase. During meiosis, the Scc1/Rad21 subunit is replaced by the meiosis-specific subunit Rec8 in most eukaryotes, however, the Drosophila genome does not contain an obvious Rec8 orthologue. We are investigating a potential cohesive role of the mitotic cohesin Scc1/Rad21 during female meiotic divisions in Drosophila by ectopic proteolysis as a fully functional, TEV-protease cleavable Scc1/Rad21 variant in a Scc1/Rad21 mutant background. Even though this cleavage still allows normal meiotic divisions after in vitro activation, preliminary data suggest a participation of Rad21/Scc1 in meiotic cohesion. Ectopic Scc1/Rad21 cleavage results in dissolution of the synaptonemal complex, an increase in precocious chromosome/ chromatid disjunction and chromosome missegregation during meiotic divisions. Our results suggest that Scc1/Rad21 does indeed play a role in regulating cohesion during female meiotic divisions in Drosophila.

Interaction of Drosophila importin-α2 and kelch during early embryogenesis. Sowjanya Kallakuri¹, Bernard Mechter², Lynn Cooley¹. 1) Genetics, SHM I-339, Yale University, New Haven, CT, USA; 2) Developmental Genetics, A040, German Cancer Research Center, INF-581,D-69120.

During oogenesis, Imp-α2 is critically involved in ring canal (RC) assembly. In mutant imp-α2 females, the RCs are occluded and dumping of nurse cell cytoplasm into the oocyte is prevented as Kelch is synthesized but unable to bind RCs and mediate RC opening. kelch mutations produce similar RC occlusion. Further analyses (IP, pull-down or Y2H assays) reveal no direct interaction between Kelch and Imp-α2, suggesting a mechanism by which Imp-α2 binds a factor regulating Kelch function. Based on our data and previous findings, it is possible that the proteins that play a major role during oogenesis could also play an essential role during later stages of development. As Imp-α2 was recently shown to contribute to the regulation of nuclear divisions during early embryogenesis, we investigated the distribution of Kelch during mitosis. Confocal analysis showed that the Kelch protein can be detected in preblastodermic embryos. Kelch was found to decorate the centrosomes and the spindle during mitosis although its pattern of distribution is generally distinct from that of Imp-α2 but overlaps during anaphase. Both the proteins similarly decorate the mitotic spindle and more particularly the interpolar microtubules during anaphase suggesting a function at the metaphase-to-anaphase transition point. Further analysis showed that Imp-α2 is a transgene, which is unable to bind an NLS bearing cargo protein, allows oogenesis to fully proceed in imp-α2ΔNLS females expressing the P(UAS-imp-α2ΔNLS)-1 transgene but subsequently arrests nuclear division in embryos, indicating that Imp-α2 exerts specific functions in distinct processes, such as RC assembly and mitosis. Examination of these mutant embryos showed a failure of Kelch to bind to spindle microtubules suggesting that Kelch and Imp-α2 may jointly act in mitosis, in a similar way to their function during ring canal assembly.

Control of Mitochondrial Structure and Function by the Yorkie Oncogenic pathway. Kevin T. Jones¹, Raghavendra Nagaraj¹, Shubha Gururaja-Rao¹, Matthew Stattery⁷, Nicolas Negre¹, Daniel Braas³, Heath Christofk⁴, Kevin White³, Richard Mann⁷, Utpal Banerjee¹. 1) MCDB, UCLA, Los Angeles, CA; 2) Dept. of Biochemistry and Biophysics, Columbia University, New York, NY; 3) Institute for Genomics and Systems Biology, University of Chicago, Chicago, IL; 4) Institute for Molecular Medicine, David Geffen School of Medicine, UCLA, Los Angeles, CA.

Growth and proliferation of cells during oogenesis requires coordinated changes in metabolism that generate ATP and reprogram the cell for rapid biosynthesis. Reduced mitochondrial function has been shown to cause cell-cycle arrest, but a direct role of signaling pathways in controlling mitochondrial biogenesis during development and disease needs further investigation. Here, we show that the conserved Yorkie/Yap signaling pathway implicated in the control of organ size also functions in the regulation of mitochondria in Drosophila as well as in human cells. In Drosophila, activation of Yorkie causes direct transcriptional up-regulation of genes that regulate mitochondrial fusion and fusion. In Drosophila, Yorkie-mediated organ size control is conserved across evolution as activation of Yap2 in human breast cancer cell lines causes increased mitochondrial activity and fusion. Thus, mitochondrial fusion is an essential and direct target of the Yorkie/Yap pathway in the regulation organ size control during development and could play a similar role in the genesis of cancer.

Patched is a conditional, non-autonomous, growth suppressor identified in a mosaic screen for growth suppressors in the background of blocked cell death. Jacob Kagey, Jordan Brown, Kenneth Moberg. Cell Biology, Emory University, Atlanta, GA.

We have conducted a Flip/FRT-based EMS screen for mutations on chromosome 2R that confer a growth advantage in the adult eye condition on a block cell death. Previous screens of this nature have mutagenized an otherwise wild-type chromosome, and though these screens identified a number of key growth regulators, we hypothesize that a subset of growth regulators would have been missed in these screens due to the induction of apoptosis. To address this hypothesis we conducted an F2 mosaic screen on 2R in the context of blocked apoptosis. We screened more than 5,000 EMS-treated chromosomes, of which 137 mutant stocks were identified as conferring a growth advantage. A number of these mutants demonstrated a growth advantage in a wild-type background (hippo, uba1, ken). However, a subset of mutants only had an overgrowth phenotype when apoptosis was blocked. We chose to pursue the molecular mechanisms underlying the non-autonomous and conditional growth advantage conferred by mutations in the patched (ptc) gene, which encodes a negative regulator of Hedgehog signals. Loss of ptc leads to cell death in the developing head and eye (Thomas and Ingram 2003). With an autonomous block in apoptosis, ptc mutant cells induce a non-autonomous overgrowth of surrounding wild-type tissue. However, when an eye is made entirely mutant for ptc, we observe a smaller eye and pupal lethality, suggesting the ptc mutant cells require surrounding wild-type tissue for overgrowth. Given that ptc is the most frequently mutated gene in basal cell carcinoma, the understanding of the molecular mechanisms underlying this conditional and non-autonomous overgrowth phenotype may provide insight into mechanisms of human carcinogenesis.

Transcription in the Absence of H3.3. Martina Hoedl, Konrad Basler. Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland.
Chromatin structure plays a major role in activation and maintenance of gene expression in response to developmental programmes and extracellular cues. It can be influenced by post-translational modification of histones and incorporation of variant histones. Variant histone H3.3 has been shown to be primarily incorporated into nucleosomes at actively transcribed loci and has therefore been implicated in activation and maintenance of transcription. We have generated Drosophila knock outs for both His3.3 genes and, surprisingly, found double mutants to be viable but sterile. This points towards a special function for H3.3 in the germline rather than in somatic tissue and global transcription. Furthermore, H3.3 is the potential major carrier of H3K4me3, another hallmark of active chromatin. In our His3.3 double mutant animals, we found H3K4me3 levels comparable to wild type, indicating a compensatory mechanism by canonical H3. To this end, we have backsubstituted the double mutant with a H3.3K4A version, which can not be methylated. Such animals are also viable although they show a significant reduction in global H3K4me3 levels. Thus, H3.3 and its methylation at K4 are not strictly required for somatic transcriptional activity in Drosophila.

Impact of local genomic context on promoter chromatin structure and gene expression. Sasha A. Langley1, Gary H. Karpen1,2. 1) Life Sciences Division, BNL, Berkeley, CA; 2) UC Berkeley, Department of Molecular and Cell Biology, Berkeley, CA.

In eukaryotic systems, orientation of and distance between adjacent genes influences their expression and enrichment of active chromatin marks at their promoters. In Drosophila, close (<1kb) divergently transcribed genes are among the most highly expressed genes in the genome, regardless of developmental stage or cell type. Ubiquitously expressed genes are frequently found as close divergent pairs, an arrangement that likely facilitates their coexpression. Although correlated expression of gene pairs has been observed among diverse organisms, the underlying mechanisms remain unclear. We demonstrate that the frequency of coexpression, or “ON/ON” pairs, decreases as the distance between promoters increases. Conversely, the incidence of “OFF/OFF” pairs rises with intergenic distance. Promoter nucleosome occupancy and Histone H2Av enrichment patterns differ significantly for coexpressed divergent pairs, when compared to “ON/OFF” or “OFF/OFF” pairs with similar spacing. Interestingly, enrichment of active marks is often present at promoters of “OFF” members of close “ON/OFF” pairs. This suggests that genes can influence the chromatin state of their neighbors. Despite the apparent importance of gene orientation and spacing for coexpression, expressed genes of all classes are enriched for specific promoter motifs, suggesting a role for transcriptional regulation at the sequence level.

Epigenetic regulation of ERK pathway activity. Julien Rougeot, Frédérique Peronnet, Emmanuelle Mouchel-Vielh. UMR7622, UPMC-CNRS, PARIS, France.

During development, transcriptional pattern of genes must be maintained during successive cell divisions. Nevertheless, cell determination is accompanied by quick epigenetic reprogramming in response to external signals, some of which are mediated by Mitogen-Activated Protein Kinase (MAPK) signaling pathways. This gene expression reprogramming sometimes involves chromatin remodeling of target genes. It could be associated to a rapid exchange on chromatin between repressive and activating factors. Some of these are the PcG repressive and the TrxG activating complexes. PcG and TrxG complexes, which are evolutionary conserved, are involved in regulation of gene expression patterns by epigenetic mechanisms. They interact onto chromatin with the Enhancers of Trithorax and Polycomb (ETP). Furthermore, some ETPs have been shown to recruit PcG and TrxG complexes to chromatin. ETPs, as co-factors of both PcG and TrxG complexes, are therefore good candidates to organize this dynamic switch between silencing and activation in response to signaling pathway activation. We have previously shown that the ETP Corto is involved in the MAPK ERK pathway during Drosophila wing development. Corto, ERK and its scaffold protein MP1 form a complex that bind chromatin and is required for correct wing vein patterning. Scaffold proteins, such as MP1, have been shown to stimulate MAPK pathways. To highlight the mechanism by which Corto, ERK and MP1 could induce epigenetic reprogramming of PcG or TrxG targets, we focused on Corto properties. Corto is targeted to chromatin through a chromodomain, which is also a RNA binding domain. Surprisingly, we found that one of the RNA specifically bound by Corto in vivo is a long non-coding RNA antisense to the gene. We are currently testing the hypothesis that this RNA could be implicated in the generation of small interfering RNA (endo-siRNA) directed against MP1. Corto, through its binding to MP1 antisense RNA, could regulate the level of MP1 endo-siRNA. It could therefore control the amount of MP1 scaffold protein and the homeostasis of ERK signaling pathway.


Alcohol and nicotine addiction are worldwide public health problems, and both drugs are often abused together. Despite advances in the identification of the molecular mechanisms of action of both ethanol and nicotine, little is known about the interactions between these drugs. Drosophila melanogaster is a proven model system to obtain insights into the molecular, genetic and neural mechanisms of drug addiction. We set out to develop Drosophila as model system to study the interactions between alcohol and nicotine. A decrease in the effects of alcohol may develop after consumption of nicotine and/or vice-versa, a phenomenon termed cross-tolerance. Here we show that Drosophila develop cross-tolerance between nicotine and ethanol. Exposing flies to nicotine for 3 days as adults or throughout development rendered them more resistant to the sedative effects of ethanol compared to unexposed control flies. Nicotine had additional effects on development, decreasing survival and eclosion rate in a dose-dependent manner. Despite these aversive effects of nicotine, rearing flies on nicotine food could induce epigenetic reprogramming of PcG or TrxG targets, we focused on Corto properties. Corto is targeted to chromatin through a chromodomain, which is also a RNA binding domain. Surprisingly, we found that one of the RNA specifically bound by Corto in vivo is a long non-coding RNA antisense to the gene. We are currently testing the hypothesis that this RNA could be implicated in the generation of small interfering RNA (endo-siRNA) directed against MP1. Corto, through its binding to MP1 antisense RNA, could regulate the level of MP1 endo-siRNA. It could therefore control the amount of MP1 scaffold protein and the homeostasis of ERK signaling pathway.

Ectopic expression of germline genes drives malignant brain tumor growth in Drosophila. Ana Janic1, Leire Mendizabal1, Salud Liñares1, David Rosell1, Cayetano Gonzalez1,2. 1) IRB Barcelona, Barcelona; 2) Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona.

Model organisms like Drosophila can help to elucidate the human molecular basis of complex diseases such as cancer. We have found that larval brain tumors caused by loss of l(3)mbt function (1,2) express genes normally required in the germline (3). A similar soma-to-germline transformation has been described in some human somatic tumors that express orthologs of some of the germline genes upregulated in l(3)mbt tumor. We have also found that inactivation of any of the germline genes nanos, vasa, piwi, or aubergine suppresses l(3)mbt malignant growth. Human germline genes are suspected to contribute to oncogenesis traits like immortality and invasiveness, but their actual role in carcinogenesis remains unknown. Our results demonstrate that ectopic germline traits are necessary for tumor growth in Drosophila mbt tumors, suggesting that their inactivation might have tumor-suppressing effects in other species (3).

Hypoxia tolerance in Drosophila: Development, tissue specificity, and heart function in response to acute, low oxygen exposure. Rachel Zarnett Ellison1, P. Azad2, Gabrielle Haddad1, Karen Ocorr2, Rolf Bodmer. 1) Sanford-Burnham Medical Research Institute, La Jolla, CA; 2) University of California, San Diego, La Jolla, CA.
In a previous screen of 2,300 Drosophila lines with p-element insertions, a subset of novel genes was identified that increased survival in hypoxia (see Azad P, et al). We examined eclosion rates and adult survival in two of these genes tna and Alh. These genes code for transcriptional modifiers in the Polycomb group (PcG) and are conserved in humans. We also examined eclosion rates and heart function in a third gene from this subset lgf, a Notch pathway regulator. To assay hypoxia tolerance at specific life stages, we screened development (egg to pupae) and eclosion rates (pupae to adult) at an F1O2 = 0.05 and acute survival of adults at an F1O2 = 0.02. To characterize Alh, tna, and lgf gene expression for hypoxia tolerance, we used F1 progeny from either a UAS- (over-expression) or RNAi-line (knockdown) crosses with GAL4 drivers that produced ubiquitous expression of heart, muscle, hemocyte, neuronal, or glial tissue-specific expression. We found increased eclosion rates at F1O2 = 0.05 with knockdown of tna or Alh, and increased eclosion rates with over-expression of Alh. Ubiquitous knockdown of Alh and tna appeared non-advantageous to adult survival at F1O2 = 0.02, while ubiquitous or selective over-expression of Alh in brain and heart tissue increased adult survival. Further, knockdown of either gene in hemocytes greatly increased adult survival at F1O2 = 0.02. Lgf knockdown increased eclosion rates at F1O2 = 0.05 in all tissue types examined. Selective regulation by single genes, such as for Alh and tna may be advantageous under hypoxia to sustain growth or control cell proliferation, given that tissue-specific expression improves hypoxic tolerance. Further, as PcG transcriptional modifiers, these genes may act on a number of targets to modulate response to hypoxia. We are currently examining heart function and acute response to hypoxia in lgf flies.

993C

Modification of Serines in Httex1p suppresses Huntington’s disease (HD) pathogenesis in Drosophila. Namita Agrawal1,4, Tamas Lukacsovich1, Charity Aiken1, Joan Stefan2, Leslie Michaels Thompson3,2, J. Lawrence Marsh1. 1) Department of Developmental and Cell Biology, 4444 McGaugh Hall, University of California, Irvine, California 92697, USA; 2) Department of Biological Chemistry, D240 Medical Sciences I, University of California, Irvine, California 92697, USA; 3) Department of Psychiatry and Human Behavior, Gillespie 2121, University of California, Irvine, California 92697, USA; 4) Department of Zoology, University of Delhi 110007, India.

The Httex1p peptide of HD contains 2 serines, S13 and S16 located in the first 17aa of Htt, that are potential phosphorylation targets. We have tested the pathogenicity and aggregation of Htt transgene in transgenic flies expressing Httex1p Q 97 designed to mimic or prevent phosphorylation of serines. We found that mutation of serines to alanine which prevents phosphorylation (S13A, S16A), dramatically reduces neuropathology and inclusion formation as compared to native Httex1p. To further confirm if phosphorylation has direct effect on HD pathogenesis, transgenic flies with the serines mutated to aspartate (S13D, S16D) were generated which might mimic phosphorylation. All S-D mutants caused lethality and increased aggregate formation suggesting that phosphorylation of the serines may significantly enhance HD pathology. However, it has been found that mimicking phosphorylation of mutant Httex1p in an acute striatal slice culture model or in a length mutant Htt in BACHD transgenic mice reduces its toxicity due to mutant Htt clearance through a LAMP-2A dependent mechanism of Chaperone mediated autophagy (CMA). These observations support a model in which a LAMP-2A-dependent mechanism of CMA mediated autophagy selectively clears S to D mutated Htt but in the absence of this clearance, the S to D mutated Htt is not efficiently cleared leading to increased inclusion formation and toxicity. As the CMA mediated clearance mechanism declines with age, this may be a contributing factor leading to the increased pathology seen with advancing age.

994A

Drosophila Model of Parkinson’s Disease: In search for Genetic Interactors of Leucine-Rich Repeat Kinase 2. Katerina Venderova1, Sean Kabbach2, Paul Macrogliese2, Elizabeth Abdel-Messih2, Gary Li3, Sameera Abuaish2, Emdadul Haque2, Ruth Slack2, David Park2, 1) Department of Physiology and Pharmacology, Thomas J Long School of Pharmacy and Health Sciences, University of the Pacific, Stockton, CA; 2) Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, ON.

Leucine-rich repeat kinase 2 (LRRK2) is the most common Parkinson’s disease (PD)-causing gene. However the physiological function of this gene and its role in the disease pathology remains largely unknown. We therefore generated a transgenic Drosophila model of PD by overexpressing a kinase domain I2020T mutant of human LRRK2, under the control of the UAS-Gal4 system. These flies display a typical parkinsonian phenotype: loss of dopaminergic neurons, locomotor deficits, and increased susceptibility to rotenone (Venderova et al, HMG 2009). Importantly, ectopic overexpression of LRRK2 in the fly compound eye causes a rough eye phenotype. Thus, we employed this phenotype in our suppressor/enhancer screen for potential LRRK2 interactors. Our LRRK2 flies were crossed with fly lines from the Bloomington Deficiency Kit, and once we have identified the interacting regions, we used available RNAi fly lines (from Bloomington, Vienna Drosophila RNAi Center, or Drosophila Genetic Resource Center) for specific gene knock-down. Using this powerful technique, we have so far identified 16 genes that interact with human LRRK2. We believe that identification of these novel LRRK2 interactors will help to improve our understanding of LRRK2 function, uncover pathways that may be important to PD pathology, and may ultimately give us new pharmacological targets for a disease-modifying therapy of PD.

995B

Genome-Wide P-element Screen for Hypoxia Tolerance Genes in Drosophila melanogaster. Priti Azad1, Gabriel Haddad2,3, 1) Dept Pediatrics, Univ California, San Diego, La Jolla, CA; 2) Rady Children’s Hospital San Diego, San Diego, CA.

To identify the genes involved in hypoxia tolerance, we screened P-element insertion lines generated by BDGP Gene Disruption Project. For hypoxia tolerance, we screened for a) eclosion rates (% eclosion) after allowing the development of embryos placed in 5% O2 to eclosion and adulthood, b) number of adult flies that survived after eclosing hypoxia (%O2), of 2367 lines screened, 44 P-element lines had eclosion rates significantly higher (70% eclosion) than the CS or eyw controls (eclosion rate ~7.8%) under hypoxia (P<0.05, t-test). The molecular function of these resistant P-element insertion lines ranged from cell cycle regulation, DNA or protein binding, ATPase activity, GTP binding, cell-death mechanisms, and transcriptional co-regulators. In this screen, we found certain interesting candidate genes such as sec8, cpa, cyclin E, osa, l(3)mbn,alth,tna lgf which show tremendous hypoxia tolerance during all stages of one developmental cycle (egg to adult), as shown by their high eclosion rates and the number of surviving eclosed adults under 5% O2 For instance, sec 8 enriched in brain and is involved in neurodevelopmental function; its expression is trafficking of glutamate receptors, neuronal microtubular assembly, and neurotransmitter secretion. We also found that P-element lines of a number of transcriptional regulators such as osa, An and osa had a strong hypoxia resistance phenotype. These genes could be master switches and might be controlling the expression of multiple genes We verified the expression of some of these P-element lines with strong phenotype in terms of eclosion rates and number of adult eclosed flies surviving by Real-time-PCR. Furthermore, in order to understand the mechanisms of protection of these genes, we over-expressed (UAS) or knock-down (RNAi) these genes ubiquously or in various tissues such as heart, muscles, hemocytes and brain utilizing progenies of crosses with specific GAL4 drivers. Interestingly, we found regulation by single genes, such as for osa and alh can play an important role in survival during hypoxia.

996C

Correlated changes in body melanisation and desiccation resistance in Drosophila immigrans: analysis of genetics and plastic effects. Veer Bhan. Department of Biotechnology, UIET, M D University, Rohtak, Haryana, India.

Climatic stresses impose strong natural selection that may cause rapid phenotypic changes in body melanization with correlated changes in desiccation resistance and reproductive traits. In the present studies, six populations of a cosmopolitan but cold adapted species, Drosophila immigrans, were isolated for ecophysiological traits (abdominal melanisation, desiccation resistance and cuticular water loss) and reproductive fitness related traits (copulation duration and rate of fecundity) from an altitudinal gradient (600 to 2202 meters) in the
Shivalik range of Himalayan region in northern India. Changes in body melanization significantly correlated with desiccation stress (due to decrease in relative humidity along increasing altitude). Populations with increasing melanization display better reproductive success (in terms of longer duration of copulation and increased rate of egg production). Climatic conditions (temperature and humidity etc.) vary significantly along altitude and exert differential selection pressure on phenotypic traits. Multiple regression analysis of data on various traits demonstrates relative impact of altitude as well as that due to climatic factors. Tcv (seasonal thermal amplitude) of sites of origin of populations help to explain observed changes in various quantitative phenotypic traits in altitudinal populations of D. immigrans. Such observations are in agreement with thermal budget hypothesis and result in reproductive success under colder environments. Present investigations suggest role of body melanisation in maintaining thermal balance and reproductive success in altitudinal populations of D. immigrans.

997A

**PI3K signaling modulates the bang sensitivity of slamdance mutants.** Derek M. Dean, Ma Khin Pyi Son, Cythia Cortes, Daniel Nachun, Jingyi Liu. Biology, Williams Col, Williamstown, MA.

The slamdance (sda) gene encodes an aminopeptidase N (APN). Mutations in (sda) cause bang-sensitivity, a seizure-like behavior in response to mechanical shock (e.g. vortexing of the cell culture vial). Although the phenotype resembles mammalian seizure models on a physiological level, APN genes have not been implicated in these other systems, and the mechanism by which sda protects against bang-sensitivity is poorly understood. To investigate sda function further, our lab has been screening for genetic interactors with the semidominant sda 
iso7.8 allele. We have previously reported that mutations in dfoxo, a transcription factor gene that downregulates insulin signaling, strongly suppress the bang-sensitivity of sda 
iso7.8 flies. Here, we show that the lipid kinase, PI3 kinase (PI3K) lies upstream of dFOXO in this pathway, and identify several downstream targets of the dFOXO transcription factor which appear to be responsible for relaying the bang-sensitivity-modulating signal. Using the GAL4-Geneswitch system, we show that this pathway acts during adulthood in a tissue-specific manner. Aberrant insulin signaling (e.g. through hyperglycemia), has been implicated in human seizure disorders. Our studies may therefore serve as a model to study how insulin signaling modulates seizure sensitivity.

998B

**Influenza A NS1 may provide protection to the host by altering Hedgehog signaling.** Margery G. Smelkinson¹, Meghana Malur², John Teijaro³, Robert Krug⁴, Michael Oldstone⁴, Ethan Bier⁵. 1) Cell & Developmental Biol, Univ California, San Diego, La Jolla, CA; 2) University of Texas at Austin 2500 Speedway Austin, Texas 78712; 3) The Scripps Research Institute 10550 N. Torrey Pines Road, IMM-6 La Jolla, CA 92037.

The genome of influenza A encodes 11 proteins, some of which interact with host targets during viral pathogenesis. Due to the high degree of sequence conservation between disease genes in humans and flies, Drosophila can serve as a powerful yet inexpensive multicellular host model to identify novel interactions between viral proteins and host machinery. In this study, we focus on the influenza A nonstructural gene, NS1, which when expressed in the primordia of fly wings, generates phenotypes indicative of enhanced Hedgehog (Hh) signaling, a highly conserved pathway crucial in vertebrate and invertebrate development. We have observed that one consequence of this misregulation is enhanced expression of the Hh target gene, decapentaplegic (dpp) in the domain of Hh signaling at the A/P border. This produces a corresponding increase in expansion of Dpp signaling to neighboring cells. This function of NS1 requires the presence of the full-length form of the Hh transcription factor, Cubitus interruptus (Ci) which is normally only present in cells responding to Hh signaling. When full length Ci is provided ectopically via a transgene, NS1 is able to enhance its ability to activate dpp expression independent of Hh signaling. This effect does not occur by increasing the concentration or stability of full length Ci, but rather appears to alter, either directly or indirectly, the specific activity. Through EMS screening, we were able to obtain a subtle point mutant of NS1 that has reduced activity in the wing. This mutation, when incorporated into the viral genome, leads to increased virulence in mice, suggesting that enhanced Hh signaling during influenza infection may provide protection to the host. Evolutionarily, this may be a potent mechanism that some recurring viruses use to ensure that their host is not depleted.

999C

**Microevolution of larval gustatory behavior.** Joshua Mast¹,², David Stern¹,². 1) Ecology and Evolutionary Biology, Princeton University, Princeton, NJ; 2) Howard Hughes Medical Institute.

Innate behavior is often stereotyped within species, yet has quickly evolved between closely related species. How the properties and connectivity of neurons evolve to change the function of hard-wired circuits underlying these behaviors remains relatively unexplored. For example, do some aspects of neuronal physiology or connectivity evolve faster than others? Are these effects unique to specific circuits or more general? Do different neural circuits show different evolutionary constraints? To begin to address these questions, we require more examples demonstrating how variation at specific loci cause behavioral differences. Two sister species in the Drosophila subgroup, D. simulans and D. sechellia, provide an opportunity to explore these questions. First, these two species occupy different ecological niches, and have diverged for a number of behavioral traits. Second, two factors allow the mapping and identification of loci that underlie phenotypic variation: the genomes of both these species have been sequenced, and they can be interbred to make fertile hybrids. Finally, precise studies of the neurobiology of their close sister species, Drosophila melanogaster, provide a framework for ultimately understanding how loci affect behavior. Feeding behavior has evolved in these Drosophila species. D. sechellia is an extreme specialist, consuming only the fruit of Monstera deliciosa, in the wild, whereas D. simulans feeds on a variety of decaying substrates. Consistent with these changes in food preference, I have observed robust differences in the response the larval responses on these species to a variety of tastants, including two bitter compounds, denatonium and caffeine. To identify the evolve loci controlling these traits and understand how the differences in the neural circuits controlling these behaviors arise, I have begun to map the major loci controlling these traits using a new automated larval gustatory assay, and high-throughput deep-sequencing based genotyping methods developed in the Stern lab.

1000A

**Evolution of embryonic pathways in Drosophila: patterns of constraint and positive selection support the hourglass model.** Julián Mensch¹, François Serra², Nicolás Lavagnino², Hernán Dopazo², Esteban Hasson¹. 1) Departamento de Ecología, Genética & Evolución. Universidad de Buenos Aires, Argentina; 2) Centro de Investigaciones Príncipe Felipe, Valencia, España.

Several lines of reasoning lead to the hypothesis that proteins participating together in a biological function will evolve at correlated rates. Also, similar rates of evolution among interacting genes are expected since related proteins are likely to be affected by common selective pressures, which either constrain or promote their divergence. Until recently, understanding developmental change and conservation has relied on embryological comparisons and analyses of single genes. The present study has taken a genomic approach to this classical problem, providing insights into how selection operates across early stages in Drosophila development. Two main molecular evo-devo hypotheses were tested: 1) according to Von Baer's Law, early maternal expressing genes have lower rates of evolution compared to zygotic genes and 2) low incidence of positive selection will be observed since no divergence changes would occur in early stages of development. Our study of more than 2,000 embryonic genes demonstrate that genes expressed during middle embryogenesis (early zygotic genes) are more constrained (dn/ds<1) than genes expressed earlier (maternal genes) or later in the egg development (late zygotic genes). This result points that middle embryogenesis, the stage at which the identity and position of body segments begins (segmental patterning), is the most conserved along Drosophila life cycle. Nevertheless, positively selected genes were found in all
embryonic stages. Particularly, one positively selected network composed by nuclear pore proteins was identified within the maternal transcriptome. All in all, results concur with the idea that the origin of serially repeated segment formation seems to be the *Drosophila* phytophototopic stage, but at the same time, we showed that early developmental modules are not limited to evolve by stabilizing selection.

1001B

The gene *invected* has a pleiotropic effect on developmental adaptive traits in *D. melanogaster*. Alejandro Petino Zappala, Julián Menach, Valeria Carreira, Juan José Fanara. Ecología, Genética y Evolución, Universidad de Buenos Aires, Buenos Aires, Buenos Aires, Argentina.

Even though substantial progress has been made to elucidate the physiological and environmental factors underpinning differences in developmental adaptive traits, little is known about their genetic architecture. This requires identifying the genes that contribute to the expression of the trait, which can be achieved by mutagenesis screens in genetically tractable model organisms such as *D. melanogaster*. Our group found several candidate genes that orchestrate the expression of different ontogenetic traits employing a panel of P-element inserted lines. We identified that the insertion within the first intron of the gene *invected* affected the phenotypic expression of developmental time (DT), pupation height (PH) and adult body size (BS). To prove that the mutant phenotype observed in *invected* is due only to the insertion, mobilization of the P-element to restore the normal phenotype is necessary. Following a scheme of controlled crosses, several revertant lines were obtained. We tested the performance of two of these lines for the mentioned traits. Our results showed a reversion to normal DT but not to normal PH for one of these lines, and the opposite pattern for the other one. Both lines showed different levels of reversion for the characters measured to estimate BS. This confirms the role of this gene in the expression of those traits, which are affected independently, showing a pleiotropic effect for *invected*. This could be due to the presence of different DNA motifs implied in the expression of each character. Analysis of the sequences surrounding the site of the P-element insertion shows conservation of some regions in 11 species of the Drosophilidae family, including members of the *Drosophila* subgenus. The next step is to determine the function of the conserved regions, and to identify the different domains affecting adaptive traits by sequencing analysis of the gene for the revertant strains. Grant Sponsor: UBA (X076).

1002C

Screening, Targeting and Phenotyping of New Genes in *Drosophila*. Li Zhao1, Qiye Li2, Guojie Zhang12, Deying Yang1, Yun Ding1, Lijun Jin1, Qin Li1, Zhou1, Wen Wang1. 1) CAS-Max Plank Junior Research Group, State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, The Chinese Academy of Sciences, Kunming, China; 2) BGI-Shenzhen, Shenzhen, China.

Gene transpositions in the *Drosophila* genomes. Mira Han1, Matthew Hahn12. 1) School of Informatics and Computing, Indiana University, Bloomington, IN; 2) Department of Biology, Indiana University, Bloomington, IN.

We used parsimonious reconstruction on the genomic distribution of gene families to analyze gene movements in *Drosophila*. We found many inter-chromosomal gene duplications that were overlooked in previous studies, including 98 gene families with multiple independent movements on different branches of the phylogeny. Within the phylogeny of 10 *Drosophila* species, we identified 576 genes that have moved chromosomally. 349 genes were duplicated with the parental copy retained in the original locus while 527 were “relocated” and had lost the parental copy. The expanded catalog of transposed genes allowed us to detect a few new patterns in the movement of genes. The parental copy of the gene was more likely to be retained if the transposed gene was a retrogene as opposed to a DNA duplication. We detected accelerated sequence evolution following the transposition event more often in the branch leading to the transposed copy than in the branch leading to the original copy. We confirmed the excess of movements from sex chromosomes to autosomes across all retrogenes and all relocations, but did not find excess in any direction among duplicative DNA-based movements. There was a significant overrepresentation of chromosomal function within transposed genes, which included several non-SMC subunits that have moved multiple times in different lineages, and several interacting proteins involved in chromosome segregation that have moved within the same lineage. The functions of moved genes suggest a hypothesis that segregation distortion may be a driving force behind the gene movements in *Drosophila*.

1003A

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We used parsimonious reconstruction on the genomic distribution of gene families to analyze gene movements in *Drosophila*. We found many inter-chromosomal gene duplications that were overlooked in previous studies, including 98 gene families with multiple independent movements on different branches of the phylogeny. Within the phylogeny of 10 *Drosophila* species, we identified 576 genes that have moved chromosomally. 349 genes were duplicated with the parental copy retained in the original locus while 527 were “relocated” and had lost the parental copy. The expanded catalog of transposed genes allowed us to detect a few new patterns in the movement of genes. The parental copy of the gene was more likely to be retained if the transposed gene was a retrogene as opposed to a DNA duplication. We detected accelerated sequence evolution following the transposition event more often in the branch leading to the transposed copy than in the branch leading to the original copy. We confirmed the excess of movements from sex chromosomes to autosomes across all retrogenes and all relocations, but did not find excess in any direction among duplicative DNA-based movements. There was a significant overrepresentation of chromosomal function within transposed genes, which included several non-SMC subunits that have moved multiple times in different lineages, and several interacting proteins involved in chromosome segregation that have moved within the same lineage. The functions of moved genes suggest a hypothesis that segregation distortion may be a driving force behind the gene movements in *Drosophila*.

1004B

Independent Origin of Sex Chromosomes in Winged Insects. James B. Pease1, Matthew W. Hahn12. 1) Department of Biology, Indiana University, Bloomington, IN 47405, USA; 2) School of Informatics and Computing, Indiana University, Bloomington, IN 47405, USA.

Highly diverse sex-determining karyotypes have been observed in winged insects, including XY, ZW, multiple-X, and homomorphic sex chromosomes. The existence of these systems across this clade—including similar systems in different lineages—leads to the question of whether these sex chromosomes evolved from the same or different autosomal chromosomes present in the ancestor. The evolutionary relationship of sex chromosomes in insects can be tested by comparing syntenic relationships among the genomes of five species of winged insects (*Drosophila melanogaster* [XY], *Anopheles gambiae* [XY], *Bombyx mori* [ZW], *Tribolium castaneum* [XY], and *Nasonia vitripennis* [haplo-diploid]). We first determined orthologous relationships among genes in these species, and used a subset with known genomic locations to determine syntenic relationships among chromosomes; significance of synteny was determined by comparison to a random model. As previously observed, we found strong signals of synteny between the *A. gambiae* and *D. melanogaster* XY chromosomes. We found the *T. castaneum* X and the *B. mori* Z chromosomes were more related to different autosomes in the two Dipteran species, suggesting that they are not derived from a common autosomal ancestor. The two Dipteran X chromosomes, *T. castaneum* X and *B. mori* Z also showed syntenic relationships to different chromosomes in *N. vitripennis*. We observed an excess of retrogene movement off the sex chromosomes in *T. castaneum* and *B. mori*, providing new evidence for the generality of this pattern, even in a ZW system. There was no evidence for an excess of retrotransposition off any chromosome in *N. vitripennis*, reflecting its haplo-diploid sex-determination system. Our results indicate that sex chromosomes among winged insects evolved from separate ancestral autosomes, but share common features of gene movement.
1005C Searching for Signatures of an “evolutionary arms race” between transposons and piRNAs. Alfred Simkin, William Theurkauf, Jeffrey Jensen. University of Massachusetts Medical School, Shrewsbury, MA.

Uncontrolled transposable element insertions and excisions can wreak havoc on a genome, causing mutations that drastically alter the host transcriptome. Recently it has been discovered that short noncoding P-element associated RNAs (piRNAs) and the proteins that process them are essential to the silencing of transposable elements and thus to host viability. Despite this observation, many transposable elements remain active over evolutionary time. Therefore, we hypothesize that piRNAs and their processing proteins may participate in an “evolutionary arms race” with the transposable elements they regulate, in which both host and transposable element mutate rapidly to maintain a balance. This should result in signatures of positive selection among proteins that interact directly with transposable elements. Utilizing both inter- and intra-species comparisons across the Drosophila phylogeny, this study provides a comprehensive look at the evolution of piRNA pathway genes, most of which appear to be subject to positive selection. Results suggest that while some proteins including Rhino may be important for responses to novel transposable elements, others, such as Piwi, appear to be associated with adaptation to existing transposable elements within naive populations.

1006A Glyphosate affects the reprotoxic performance in Drosophila melanogaster. Resistance at sight. PATRICIA RAMOS1,2, ADRIANA MUÑOZ1, HUGO RIVAS2, BLANCA R HERNANDEZ2, J ARMANDO MUÑOZ1. 1) Lab Genetic y Toxicología Ambiental, Depto. Biología, Facultad de Ciencias, CU, Universidad Nacional Autonoma de Mexico, D.F., Coyoacan, Mexico; 2) Drosophila Stock Center Mexico, Facultad de Ciencias, UNAM; 3) CCH-Sur, UNAM.

Glyphosate (GL) is used as herbicide all over the world. In Mexico, Roundup™ (48 gr/l) or Aquamaster™ (53.5 g/L ia) (Monsanto) are the most used herbicides and surfactants. After be absorbed by Drosophila, the Senopyryrel-shikimate3phosphate synthase interfere the aromatic aa production and killing the plant. In animals, GL activity is controversial, but it is claimed as safe for humans, animals and environment health. For years, the prolific Drosophila has be used to assay for genotoxic activity; only 10 days are needed to have a new generation and hence, the in vivo effect of hazard compounds on the reproductive performance of flies, can be monitored. In Drosophila, GL induces SLRL mutations and produces somatic damage (Kaya et al, 1995, 2000). Goal: To determine whether D. melanogaster flies exposed through larval development to GL show reprotoxic damage. Methods. Wild type larvae were fed with standard food enriched with GL which, prepared for surfactant use was considered as 100%, from this, successive dilutions were done and two vials prepared. Adults were counted and sexed. The GL toxicity was determined as: experimental flies/control flies per vial (Survival Index, SI). 30 males randomly chosen were individually mated with unexposed virgin females. The male Fertility (F) was: vials with progeny/total vials. Progeny were counting and sexed. The GL toxicity was determined as: experimental flies/control flies per vial (Survival Index, SI). 30 males randomly chosen were individually mated with unexposed virgin females. The male Fertility (F) was: vials with progeny/total vials. Progeny were counting and sexed. Data represent the average of two experiments. Results. Higher concentrations were toxic (p<0.05). Middle concentrations affected the sex ratio, but low and high modified the reproductive activity. Considering the total progeny recovered no evidence of negative effects were found, however at higher concentrations assayed, the F decrease and less treated males, probably sharing some kind of genetic background that makes them resistant to the treatment form the next generation.


We previously used a PCR approach to look for transposable element (TE)-induced adaptations in Drosophila melanogaster. We showed that (i) TE-induced adaptations are readily detectable, (ii) TEs are a considerable source of recent adaptive mutations and (iii) a substantial proportion of TE-induced mutations remain to be discovered. The recent availability of next-generation sequencing (NGS) data for multiple strains should allow us to overcome the limitations of this initial screening based on the annotation of TEs on a single D. melanogaster strain. In this work, we used NGS data for 162 North Carolina strains (DGRP) and 22 Malawi strains to identify new adaptive TEs. We developed a new computational pipeline, called “T-denovo”, that uses paired-end sequencing data to identify TE insertions that were not previously annotated in the reference genome. Briefly, this pipeline screens for “one-end anchored” pairs - only one of the reads maps to the reference sequence—and check whether the unmapped read correspond to a TE sequence. “T-denovo” then selects only the pairs for which one read maps on an unique sequence while the other maps on a TE. Selected pairs are sorted based on their genomic location allowing for quick parsing and identification of TEs not annotated in the reference sequence. The pairs supporting the same TE insertion are clustered and used to build contigs. Finally, the new TE insertions are annotated using TE classifier program. We then use “T-lex”, a homemade and validated computational pipeline, to estimate the frequency of all the identified TEs. As a first step towards the identification of putatively adaptive TEs we focus on those TEs that are present in North Carolina strains and are absent or present at low frequencies in Malawi strains. Our results show that the combination of “T-denovo” and “T-lex” allows for fast, cost-effective and reliable identification of adaptive TE insertions.

1008C Effect of food preference on oviposition behavior in immigrans subgroup of Drosophila. Pankaj K. Tyagi, Shruti Tyagi. Department of Biotechnology, Meenat Institute of Engineering and Technology, Meerut, India.

Two related species of drosophila subgroup of immigrans (Drosophila immigrans and Drosophila nasuta) has evidence great divergence in their life history traits such as, body weight, fecundity, hatchability, viability & ovariole numbers due to food preference on ripe citrus fruits. Drosophila immigrans is better adapted to ripe citrus fruits, which are often infected with mould pencillium. On the contrary, oviposition of Drosophila nasuta significantly reduced on ripe citrus fruits. Drosophila immigrans had coevolved with citrus fruits while Drosophila nasuta preferred non citrus decaying organic matter. We confirmed the significant stimulatory effect due to food preference on ripe citrus fruits on life history traits, body weight, fecundity, hatchability, viability & ovariole numbers in i.e. fecundity (47.16) on maize medium food & (66.09) on ripe citrus fruits and opposite results obtain in case of Drosophila nasuta. The significant differences in life history traits of Drosophila immigrans support independent adaptation & divergence of these two related species which belongs to same subgroup.


Proper spatial and temporal accumulation of the TGF-β homolog Gurken (Grk) within the ovary is required for Drosophila oogenesis. Like other spatially restricted ovarian proteins, correct Grk protein distribution is achieved through a combination of mRNA localization and translational repression of unlocalized transcripts. During later stages of oogenesis, when the dorsal-ventral (D-V) axis of the egg is established, Grk protein is tightly localized to the future dorsal-anterior corner of the oocyte. The molecular basis for the translational repression of grk and its subsequent translational activation after localization have not been established. A number of proteins have been indirectly implicated in the translational regulation of grk mRNA. For example, the RNA binding protein, Squid (Sqd), plays a critical role in the translational repression of grk mRNA (as well as its localization). A mechanism for regulating translation of mRNAs is cytoplasmic polyadenylation and deadenylation. Indeed, the CPEB protein Orb is needed for Grk protein accumulation, suggesting that cytoplasmic polyadenylation of grk mRNA may have a role in its translational regulation. It remains unclear, however, whether grk mRNA polyadenylation plays a regulatory role in translational repression. We have analyzed the polyadenylation state of grk mRNA and found that it is
polyadenylated, and that this polyadenylation is markedly reduced in orb ovaries. We have also examined the polyadenylation status of grk transcripts in females carrying mutations in sqd, vasa, encore, and spnF. Current efforts are focused on determining whether the polyadenylation status of grk transcripts correlates with the translational competence of the RNA. Finally, to elucidate whether grk polyadenylation has a role in the translational repression of unlocalized grk mRNA, we are analyzing translation and polyadenylation of grk in females carrying mutations in sqd and enc, vasa or spnF. The results of these analyses should provide insight into mechanisms for post-transcriptional regulation of gene expression.

1010B Characterization of Two Novel EMS Induced Mutants in the Drosophila Trachea. Deanne M Francis. Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA.

The Drosophila tracheal system is derived from 10 pairs of epithelial sacs, each comprised of approximately 80 cells. In response to an FGF (Branchless/Bnl) cue, new branches bud from the tracheal sacs. Each new branch migrates towards the source of the FGF cue, led by one or more “tip cells.” Most, if not all, tracheal cells are capable of becoming tip cells, but tip cell number is restricted by a competition-based “intrinsic” mechanism. We have identified 2 mutants, oak gall and conjoined, which confer a tip cell bias in genetic mosaic experiments. In addition, both oak gall and conjoined mutant cells have a rounded cell morphology, and terminal cells mutant for either gene show specific tubule defects in the area between the terminal cell-stalk cell junction and the terminal cell nucleus. Here we present the initial phenotypic characterization of oakgall and conjoined, and describe the results of our mapping and positional cloning efforts.

1011C The role of the Fox transcription factor fd64a in embryonic salivary gland migration. Caitlin D. Hanlon, Deborah J. Andrew. Cell Biology, The Johns Hopkins Univ, Baltimore, MD.

Cell migration is a diverse and ubiquitous process that can occur with single cells, groups of cells or entire organs. Cell migration is regulated at multiple levels, from the signals providing directional cues, to the substratum through which cells move, to the integration of all of this information within the cell or cell population to facilitate movement. To simplify studying this complicated process, the Drosophila salivary gland provides a unique model system that is both genetically tractable and well-characterized. fd64a, a member of the forkhead box family of transcription factors, is dynamically expressed in the somatic visceral muscles that directly contact the salivary gland during late embryonic stages. The expression pattern of fd64a suggests that it may play an essential role in directing salivary gland migration. To further investigate the function of fd64a, several deficiency lines were obtained. Embryos homozygous for each deficiency, as well as the deficiency combinations, revealed defects in salivary gland development. Staining proposed salivary gland rudiments from the silkworm, revealed a range of defects: the apical membrane appeared rough, uneven, inflated, and/or mis-positioned when compared to wild-type glands. Additionally, the nuclear marker CrebA revealed stunted and mis-positioned glands in both deficiency lines. These data suggest a potential role for Fd64a and its transcriptional targets in directing salivary gland migration during embryogenesis.


The maintenance, migration, and polyadenylation of germ cells and their incorporation into gonads have several conserved aspects in animals. The sophisticated molecular and genetic methods available for Drosophila research make this organism an ideal model for studying germ cell development. By using RNAi based gene silencing technique we have been able both to identify and to analyze genes playing a role in these events. We have developed and successfully applied a high throughput screening method for the identification of novel genes involved in germ cell development. Gene specific dsRNAs were injected into early embryos expressing GFP in their germ cells. Live embryos were observed for 12 hours by video microscopy to follow germ cell development. Injected animals were reared to adult stage and dissected to determine the penetrance of germ cell-less phenotype. In addition to monitor germ cell fate at almost all embryonic stages and in the imago, we were able both to overcome the pleiotropic effects of the genes examined and to study genes with maternal effect. With the help of RNA localization databases we chose for our analysis more than five hundred genes expressed in germ plasm, embryonic germ cell or gonads. After RNAi treatment, 57 of them caused reproducible defects in germ cell/gonad development at embryonic or and adult stages. Silencing effects of 38 out of the 57 examined genes could be confirmed by testing mutant alleles in genetically sensitized mutant background. Based on the detailed analysis of the in vivo video microscopic movies 10 embryonic phenotype categories were established. Candidate genes were grouped using the expression of early embryonic clusters and putative transcriptional targets of these genes were further investigated. This silencing method enables the definition of gene groups involved in the same developmental events and the understanding of the relation between processes separated in space and time.

1013B Control of transit-amplifying divisions and organ size by Smurf in Drosophila male germline development. Pao-Ju Chang1, Chang-Che Hsieh1, Margaret T. Fuller2, Haiwei Pl3. 1) Department of Biomedical Sciences, Chang-Gung University, Tao-Yuan, Taiwan, Taiwan 333; 2) Department of Developmental Biology, Stanford University School of Medicine, Beckman Center B300, 279 Campus Drive, Stanford, CA 94305-5329, USA.

In germline stem cell lineages, the spermatagonia undergo four rounds of transit-amplifying (TA) division before differentiation. We found that smurf, encoding a HECT domain ubiquitin E3 ligase, is required in germ cells to restrict the TA division. 24% of the spermatocyte cysts have 32 cells in smurf mutants. In situ hybridization experiment and analysis of smurf-GFP reporter expression show that smurf expression is initiated in TA cells in early 16-cell stage, and reach highest level in cells entering differentiation program. smurf expression absolutely depends on Bam, the key regulator mediating the TA proliferation to spermatocyte differentiation. Furthermore, the size of testis is increased in smurf mutants and the smurf activity to control testis size is correlated with its ability to inhibit TA proliferation. Thus, our results suggest that Bam ceases TA proliferation in part through activating Smurf expression in spermatogonia-specific transcription factor. Studies in both fly and mammalian cell system have shown that Smurf mediates degradation of several proteins including TGF-β type I receptor, SMAD, Prickle1, MEKK2, Runx2, and RhoA. We are currently investigating what the target(s) of Smurf is in regulation of TA proliferation.

1014C Identification of components involved in recognition and internalization of Leishmania parasite. Miriam MS Costa, Kendi Okuda, Neal Silverman. UMASS Medical School, Department of Medicine, Worcester, MA.

The protozoan Leishmania is the etiological agent of leishmaniasis, a disease affecting several millions of people throughout the world. The pathology of this disease depends of the parasite specie, ranging from self-limiting cutaneous infections to disseminating diffuse cutaneous, mucocutaneous and the lethal visceral leishmaniasis. Macrophages are the primary cell type supporting this protozoan in the mammalian host. The parasites invade macrophages and proliferate intracellularly, in a specialized organelle known as the parasitophorous vacuole. Cell invasion occurs solely by receptor-ligand mediated phagocytosis, driven by host opsonins bound to the parasites and by direct interaction with other unidentified host receptors. Given the high level of conservation between Drosophila and mammalian innate immune responses, we have developed Drosophila, both adult animals and cultured cells, for the study of L. amazonenais interactions with animal
phagocytes. The cell-based studies indicate that *Drosophila* SL2 can be successfully invaded, in an actin-dependent manner, by these parasites, but these cells do not support parasite proliferation. Parasite invasion also involves the GTPases Rho, Rac and CDC42, that are also implicated in the mammalian macrophage infection. A genome wide RNAi screening is ongoing, and will be used to identify more parasite-phagocytosis components. In vivo approaches using flies devoid of functional phagocytes revealed that phagocytosis is a critical mechanism of defense against *Leishmania* parasites injected in the adult hemolymph. Also, hemocyte-specific knock-down of certain scavenger receptors significantly reduced survival following *Leishmania* infection, suggesting that this class of receptor plays an important role in parasite recognition. This study demonstrates that *Drosophila* macrophage-like cells are able to phagocytose and kill *Leishmania* parasites and represent an attractive model to prospect fundamental components involved initial steps of cellular infection by *Leishmania* parasites as well as identify potentially novel anti-parasitic defenses.

1015A

Self-recognition in *Drosophila*: Characterization of the *tu(1)Sz* mutant. Nathan T. Mortimer, Todd A. Schlenke. Department of Biology, Emory University, Atlanta, GA.

Imprecise recognition can be triggered by either recognition of non-self, or by recognition of lack of self. While the identification of mediators of non-self recognition has been the focus of many studies, our understanding of the molecular mechanisms of self and lack-of-self recognition remains incomplete. To address this problem, we have begun to characterize the *Drosophila melanogaster* *tu(1)Sz* mutant, larvae of which mount a cellular immune response directed against their own fat body, presumably because this tissue is missing a self recognition signal. This lack-of-self response is characterized by the precocious differentiation of lamellocytes (a specialized hemocyte subtype) that participate in the encapsulation and melanization of caudal fat body tissue. This mirrors the canonical *Drosophila* encapsulation response triggered by the presence of a foreign body, i.e. a parasitoid wasp egg, in the larval hemocoel. Thus by mapping and molecularly characterizing the *tu(1)Sz* locus, we hope to begin understanding the mechanisms of immune self-recognition, and the basis for lack-of-self mediated immune responses. Previous work has localized the *tu(1)Sz* mutation to the 108B1-108A4 cytogenetic region. Through complementation tests with molecularly defined deletions we have narrowed the *tu(1)Sz* locus to a 24 kb region. Additional rescue experiments using small, defined duplications have further reduced the region to a 14 kb span consisting of 5 candidate genes, which are currently being analyzed. Interestingly, these genetic studies have identified the presence of a second, closely linked mutation that contributes to the *tu(1)Sz* self-encapsulation phenotype. This secondary mutation lies within 40 kb of the 14 kb region described above, and appears to play a role in lamellocyte differentiation.

1016B

Regulation of innate immune response and apoptosis by synthetic microRNAs. Chun-Hong Chen1,2, Wan-Hsun Lin3, Haixia Huang2, Bruce A Hay2. 1) Division of Molecular and Genomic Medicine, National Health Research Institutes, Zhunan Miaoal, Taiwan, Taiwan; 2) Division Of Biology, Mc 156-29, California Institute Of Technology, Pasadena, CA.

Signaling pathways are often regulated by multiple inputs, and produce outputs reflecting the activity of multiple genes. Because of this, inactivation of single genes may provide limited information about function, due to redundancy or compensation. Tools for spatial and temporal silencing of multiple genes would greatly facilitate studies of gene function and pathway analysis. MicroRNAs are processed from transcripts generated by Polymerase II transcription, giving rise to a single, defined 21-23bp RNA fragment that also guides RISC-mediated mRNA cleavage. Here we describe the generation of synthetic microRNAs that silence a number of components of the *Drosophila* apoptotic cell death machinery. We also describe the generation of polycistronic microRNAs that silence multiple, sequence-unrelated genes. We use these to demonstrate that removal of apoptosis activators reaper, head involution (hid) and grim is sufficient to inhibit essentially all developmental apoptosis in the embryo and that these genes have unique and overlapping roles in eye development and in response to DNA damage and innate immunity.

1017C

gut immunity. Luping Liu1,2, Jianquan Ni1,2. 1) Tsinghua Medical School, Tsinghua University, Beijing, Beijing, China; 2) Harvard Medical School, Boston, MA 02115.

Transgenic conditional RNAi in *Drosophila* has become a powerful method to perform genetic screens as it allows knockdown of any gene in any tissue. The approach has the potential to replace more conventional approaches to interrogate gene functions. As is the case with the mammalian gut, *Drosophila* gut epithelia cells act not only as a physical barrier, but also can mount a local immune response by producing antimicrobial peptides and reactive oxygen species when attacked by pathogens and microbes. To quantitatively measure the anti-bacterial response, we developed a luciferase-based transcriptional reporter assay that is specifically regulated by natural infection with pathogens. We tested the efficacy of this assay by knocking down the expression of key components of the innate immunity IMD pathway using in vivo RNAi (Ni et al., 2008, 2009, 2010), and found that this reporter assay has excellent sensitivity and specificity in response to pathogens. We have initiated a pilot of genetic screen and found several new regulators involved in innate pathway.

1018A

Social Regulation of Aggression: A Single Phenome Functions through Two Olfactory Receptor Neurons in a Temporally Differential Manner to Oppositely Regulate the Same Behavior in *Drosophila*. Weiwei Liu1, Xinhua Liang1, Yi Rao1,2, 1) National Institute of Biological Sciences, Beijing, Beijing, China; 2) Beijing Normal University College of Life Sciences, Beijing, China; 3) Institute of Neuroscience, Shanghai, China; 4) Peking University College of Life Sciences, Beijing, China.

When two socially-naive *Drosophila* males meet, they will fight. However, prior social grouping of males reduce their aggression. We have taken a multidisciplinary approach with chemical, electrophysiological and genetic techniques to study how social experience regulates behaviors. We find that olfactory communication is important in modulating *Drosophila* aggression. While acute exposure to the male specific pheromone 11-cis-vaencenyl acetate (cVA) elicited aggression through the Or67d olfactory receptor neurons (ORNs), chronic cVA exposure reduced aggression, not through the habitation of Or67d ORNs. Rather, an interesting role was found for the Or65a ORNs: while Or65a ORNs were not acutely involved in aggression, blockade of synaptic transmission of Or65a ORNs during social grouping or prior chronic cVA exposure eliminated social modulation of aggression. Artificial activation of Or65a ORNs by ectopic expression of dTrpA1 was sufficient to reduce aggression. Social regulation requires interneurons in the antennal lobe. Our results indicate that activation of Or65a ORNs is important for social modulation of male aggression, and demonstrate the acute and chronic effects of a single pheromone are mediated by two distinct types of ORNs, and suggest a chemical method to reduce aggression in animals.

1019B

Genetic basis of sine song evolution in *Drosophila*. Tomoko Sunayama-Morita1,2, Peter Andolfatto1, David L. Stern1,2. 1) Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ; 2) HHMI.

*Drosophila* male courtship song has been shown to be important for species recognition and sexual stimulation in mating. Courtship song rapidly evolves among species in *Drosophila*. In the *melanogaster* subgroup, courtship song consists of two components; a sine song comprised of sinusoidal continuous hums, and a pulse song made up of a series of rapid pulses. *D. simulans*, which is the sister species of *D. melanogaster* sings both pulse and sine song, however, its close sibling, *D. sechellia*, rarely sings sine song. We focus on the sine song evolution between those two species to reveal the genetic basis for the evolution of species-specific behavior. To identify genes that
contribute sine song production in *D. simulans* and *D. sechellia* in an unbiased manner, we carried out QTL mapping by using high-throughput and high-resolution genotyping we recently developed (Andolfatto et al., Genome Res., in press). We have mapped two major epistatic QTL on chromosome 2L and 3, and one minor QTL on chromosome X. We are currently analyzing a series of introgression lines that carry a small region of the *D. simulans* genome in the *D. sechellia* background on those QTLs to identify causal genes.

1020C
Natural variation of post-mating behavior in *Drosophila melanogaster*. Joyce Yushi Kao, Sergey Nuzhdin. Computational Molecular Biology, University of Southern California, Los Angeles, CA.

Studying reproduction barriers is a valuable asset to understanding the dynamics of sympatric populations. We are interested in determining whether there is a geographical pattern of post-mating behavior in *Drosophila melanogaster* females collected from multiple locations along the southeast United States and Caribbean islands. After mating, females exhibit a variety of behavioral changes, but we are most interested in the reduced receptivity to re-mating and increased in egg laying. We will be measuring the effects of reduced receptivity to re-mating by introducing males to mated females 1 day and 6 days after initial mating and recording when females choose to re-mate. Egg laying will be monitored by counting the number of eggs laid over a period of 10 days. In addition to counting the eggs, we also count the number of flies that eclose from those eggs as a measure of fertility. Future directions include whole-genome sequencing to survey the sequence variation in all known genes involved in post-mating behavior.

1021A
Regulation of *Drosophila* Glutamate Receptors by novel genes: *optimus-prime* (*opr*) and *bumblebee* (*bmb*). Subhashree Ganesan, David Featherstone. Univ Illinois at Chicago, Chicago, IL.

Localisation and post-transcriptional regulation of mRNA is increasingly being recognized as an important mechanism in gene expression, and may be important for synaptic plasticity. Using the *Drosophila* NeuroMuscular Junction (NMJ) as a model synapse, we are studying localization and regulation of Glutamate Receptor (GlUR) mRNAs. We observed that GlUR subunit mRNA in the post-synaptic muscle appears in the form of puncta that resemble ‘RNA granules’ or more generally, ‘messenger Ribo-Nucleo-Protein particles’ (mRPNs).

Proteins that are components of mRNPs can possibly regulate translation of the corresponding mRNA. We isolated native GluRIIA mRNPs by RNA affinity purification and identified associated proteins using LC/MS/MS and peptide sequencing. The resultant list of genes was subjected to further short-listing by assaying for changes in GluRIIA protein expression in flies with disrupted gene function. Disruption of CG132149, which we named ‘*optimus-prime* (*opr*)’, causes a partial loss of GluRIIA protein at the NMJ. GluRIIB levels, however, are unaffected. Similarly, disruption of CG17816, which we named ‘*bumblebee* (*bmb*)’ also causes partial loss of GluRIIA protein at the NMJ. Work is ongoing to understand the role of these novel proteins.

1022B

In the developing nervous system growth cones have an essential role in guiding axons to their correct target sites. Directed growth cone movement is a response to extracellular cues produced by the coordinated regulation of F-actin and central microtubule networks. Key regulators of actin dynamics are the so-called nucleation factors, such as the Arp2/3 complex and formins, which use different mechanisms to seed new actin filaments. Formins promote actin assembly by associating with the fast-growing end (barbed end) of actin filaments, and facilitate the formation of unbranched filaments. We have previously examined the function of the *Drosophila* formin *dDAAM* in the embryonic CNS, where this protein shows a strong accumulation in the developing neurites. Genetic analysis suggested that dDAAM plays a major role in the regulation of axonal growth by promoting filopodia formation in the growth cone. Currently, we are investigating the mechanism how dDAAM induced actin assembly might contribute to filopodia formation. To determine proteins that may act together with *dDAAM* in the regulation of axonal growth, we carried out a genetic interaction analysis. We demonstrated that *dDAAM* shows an interaction with *Ena* and *profilin*. Moreover, we identified Rac as the most likely activator of *dDAAM* in the developing nervous system. Additionally, we noticed that *dDAAM* exhibits a strong expression in certain regions of the larval and adult brain as well. Specifically, in the developing mushroom body *dDAAM* is highly enriched in the newly born axons suggesting that *dDAAM* might be a general regulator of *Drosophila* axonal development. Consistently, by loss of function analysis we detected axonal projection defects in the mushroom body. Our poster will provide a detailed analysis of the axonal growth defects exhibited by *dDAAM* mutant adult brains.

1023C

Neuropeptidergic signaling in the lamina, the most peripheral layer of the *Drosophila* visual system, was not detected before. Using antisera to several types of neuropeptides we found a novel type of neuron with wide field tangential arborizations distal to the lamina that expresses myoinhibitory peptide (MIP). These MIP immunoreactive optic lobe (LMO) neurons are located anteriorly in the lateral part of the protocerebrum close to the accessory medulla (AME), branching widely into the optic lobe and to the dorso-lateral and lateral protocerebrum. Similarities in morphology and localization to the main pacemaker neurons expressing pigment dispersing factor (PDF), together with similarities in varicosities projection in the lamina to the serotoninergic large bilateral optic lobe (LBOSHT) neurons inspired us to investigate possible relations between the LMOs and the clock and serotoninergic neurons in the optic lobe. Using Gal4-UAS method combined with several antisera we found no colocalization of LMO neurons with any of clock and serotonergic neurons, however several branches overlap suggesting neuropeptidergic modulation of the two types of signaling in the peripheral visual system.

1024A
A Genetic Modifier Screen of *midline* to Identify Candidate Enhancer and Suppressor Genes that Regulate Interommatidial Bristle Formation in the Adult *Drosophila* Eye. Deepak Kumar, Sandra Leal. Biological Sciences, University Of Southern Mississippi, Hattiesburg, MS.

The *Drosophila* T-box transcription factor *midline* (*mid*) regulates cell-fate specification in multiple tissues across diverse invertebrate and vertebrate species. However, to date, the complex mechanisms by which *mid* regulates cell-fate specification are not yet completely understood. Developmental expression profile studies between *Mid* proteins and transcription factors known to specify motor neuron and interneuron fates within the central nervous system (CNS) reveal little co-expression between these factors (Leal et al., 2009). Thus, to further our understanding of *mid* function as a cell-fate determinant, we are undertaking genetic screens to identify mid-interacting genes. The identification of mid-interacting genes will reveal functional associations that place *mid* within complex signaling pathways regulating cell-fate specification. Specifically, we are using a genetic modifier screen and RNA interference (RNAi) methodology to identify genes that suppress or enhance a dosage-sensitive RNAi interommatidial bristle mutant phenotype observed when *mid* transcripts are reduced in the eye imaginal discs of third-instar larvae heterozygous mutant for third chromosomal deficiencies. This high-throughput screening approach streamlines efforts to identify mid-interacting gene candidates that regulate cell-fate specification within the CNS. A follow-up rescoring assay will determine whether identified gene candidates modify a CNS-specific and dosage-sensitive mid mutant phenotype that affects
even-skipped (eve) expression in a subset of neurons. Presently, we have identified several third chromosomal regions harboring enhancer or suppressor candidate genes that regulate interommatidial bristle formation. The goal of this research is to advance our understanding of mid function as an integral regulator of conserved cell-fate specification pathways within the CNS and other tissues.

1025B

A genetic screen for novel genes in the auditory system. Tongzhe Li1, Andrew Groves1,3,4, Hugo Bellen1,2,3,4. 1) Program in Developmental Neurobiology, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute, BCM, Houston, TX; 3) Department of Molecular and Human Genetics, BCM, Houston, TX; 4) Department of Neuroscience, BCM, Houston, TX.

Hearing is an important peripheral sense related to mechanotransduction and there are over 100 human loci that have been associated with hearing defects. In the fly, normal hearing function plays a role in courtship and mating. Drosophila respond to hearing with a large chordotonal organ - Johnston's Organ (JO) - located in the second antennal segment which is attached to the base of the third segment. Movement of the third antennal segment stimulates the mechanosensory neurons in the JO and produces a sound-evoked potential. To identify novel genes functioning in hearing, we screened a lethal X chromosome collection generated by the lab. The mutants that we screened showed either a loss of synaptic activity using the electroretinogram (ERG) assay or abnormal bristle morphology. We used a sound-evoked potential (SEP) assay to discover functional defects and used immunostaining to examine JO morphology and differentiation. After screening 70-80 available complementation groups from this FLP-FRT screen, we identified several groups showing either reductions of SEP and/or abnormal JO structure. The abnormal JO mutant phenotypes observed included abnormal neuronal morphology and scolopale cell structure. For example, one complementation group, XM18, corresponds to CG4078 which is an evolutionarily conserved gene that is a homolog of regulator of telomere elongation helicase 1 (Rtel1) in mammals. This gene was found to affect genome stability through mediating D-loop disruption in SDSA and can regulate telomere length. Two genome-wide association studies found it to be linked to glioma. The JO of CG4078 mutants exhibit loss of HRP staining in neurons. A defect was also observed in the SEP assay. Another complementation group, XM20, exhibits a loss of Prospero and phaillolidin staining in scolopale cells. We are currently mapping the gene.

1026C

An analysis of interactions between alleles of discs large, strawberry notch and changing a cyclin level. Georgina Portillo-Aguilar, Flora Retano, Josh Duah, Jennifer Zelaya, Catherine Coyle-Thompson. Biology Department, CSU, Northridge, 18111 Nordhoff St. Northridge, CA.

Males with a dlg"nst"nmo" double mutant combination have a severe reduction in the number of bristles on the thorax, head, wings and leg of the fly. These males are also sterile and have a reduced lifespan. This reduced bristle phenotype is suppressed by reducing the level of cyclin A. Individuals heterozygous for dlg"nst"nmo" are pupal lethal and tumors are present. This tumorous phenotype is enhanced when a single copy of nmo" is present in the combination. The phenotypic analysis of both of these interactions will be presented. Funding generously provided by NSF LSAMP Grant # HRD-0802628.

1027A

Characterization of RFX target genes required for cilogenesis in Drosophila. Fabien Soulavie, Anne Laurençon, Joëlle Thomas, Brigitte Chhin, Camille Enjolras, Bénédicte Durand. CGMC, Université Lyon 1, Villeurbanne, France.

Cilia are microtubular structures conserved throughout species from algae to mammals. They play major functions in physiology and development. In human, ciliary dysfunctions are responsible for several diseases called ciliopathies. RFX transcription factors are key regulators of genes involved in cilia assembly. In Drosophila, Rfx is involved in ciliated sensory neuron differentiation. Putative Rfx transcription targets in Drosophila have been identified in our laboratory by an in silico screen (Laurençon et al. 2007). We have selected several candidates for functional studies based on their occurrence in other studies in the literature of cilia associated proteins/genes. We show in transgenic flies expressing promoter reporter constructs that most of the selected genes are expressed in ciliated neurons in Drosophila. We have selected two interesting candidates for further functional analysis. The first gene has an ortholog, in protozoa, which has been shown to be involved in flagellar motility. In Drosophila, we show that this gene is indeed only expressed in ciliated sensory neurons and is downregulated in Rfx deficient flies. As well, we show that the mouse ortholog is regulated by RFX3. Furthermore, we demonstrate that this first candidate is required for sensory perception mediated by ciliated neurons in Drosophila. Our observation opens new perspectives for the understanding of the evolution of an ancestral function of this gene in flagellar motility to cilia mediated sensory perception. The second candidate is expressed in ciliated neurons in Drosophila, and is regulated by the Rfx transcription factor. Several knockdown methods are in progress to characterize the function of this gene in ciliated neurons. This project will provide a global understanding of the function of Rfx in sensory cilia development and function.

1028B

Drosophila katanin 60 regulates microtubule network formation and neuromuscular synapse development. Shan Jin1, Cuan X. Mao1, Zhao H. Xiong1, Young Q. Zhang2, 1) College of Life Sciences, Hubei University, Wuhan, Hubei 430062, China; 2) Key Laboratory of Molecular and Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China.

Katanin is a member of the highly diverse AAA (ATPases associated with different cellular activities) protein superfamily, composed of a heterodimer of 60- and 80-kDa subunits. The 60 kDa subunit uses energy from ATP hydrolysis to disrupt tubulin contacts within the microtubule (MT) lattice, severing the MT fibers but leaving tubulin capable of subsequent repolymerization, whereas the 80 kDa accessory subunit involved in subcellular targeting of katanin. Drosophila katanin 60, encoded by CG10229, disrupts MT arrays when overexpressed in cultured cells (Zhang et al, J Cell Biol, 2007, 177:231-242; Yu et al, Mol Biol Cell, 2008, 19:1485-1498). Up to now, the in vivo functions of katanin have not been well characterized at organismal level. To dissect the functions of katanin, we made katanin 60 null mutants, transgenic UAS and RNAi flies which can be used to manipulate its expressions.

1029C


One of the most important developmental events for the final patterning of the Drosophila eye is the ommatidial rotation process. In this process, developing ommatidia rotate 90° in two 45° steps. It is known that the EGFR and Fz/PCP signaling pathways are involved in this process, however few genes have been proved to specifically affect it. One of these genes is nmo (nmo), a gene encoding a MAP-like
protein kinase. nmo loss of function produces an arrest of the ommatidia at 45°, which suggested an essential role of the gene in the second rotation step. However, it has been shown to regulate the speed of rotation in both steps. Whether there is a connection between nmo and the mentioned signaling pathways in this process is still unclear. To gain further knowledge on the genes affected by nmo loss of function in this process, we analyzed the expression profile of nmo mutant third instar larval eye discs using expression microarrays. We identified a total of 101 downregulated and 104 upregulated genes with respect to wild-type discs. Interestingly many genes fell into functional categories which could be related to the ommatidial rotation process. Expression changes of four of these genes in nmo mutants were validated by QT-PCR. In order to analyze their potential role in the ommatidial rotation process, genetic interaction assays between them and nmo and phenotypic analyses of mutants of such genes have been performed. We found that they interact genetically with nmo, and that mutant alleles of the identified genes show ommatidial rotation defects. These results suggest that these genes are functionally related to nmo and have a role in the process. Since they encode proteins involved in cell adhesion and signaling, their functional characterization will help to decipher the molecular mechanisms underlying ommatidial rotation.

1030A Mechanical tension and the influence of cell proliferation on the maintenance of the Drosophila dorsoventral compartment boundary. Christian Dahnmann1, Jens-Christian Röper1, Maryam Aliee1, Katharina Landsberg1, Constanze Pentzold1, Thomas Widmann1, Frank Jülicher1. 1) Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

Maintaining a straight and sharp compartment boundary during development is important to separate cells with different identities and fates from each other. However, this is a challenging task as cell proliferation causes cells to rearrange and hence to mix within the tissue. Two mechanisms have been proposed to play a crucial role. First, a local increase in mechanical tension could guide cell rearrangements after cell division to maintain the boundary. Second, reduced proliferation of cells next to the compartment boundary could reduce rearrangements. A local increase in mechanical tension has been previously shown for the anteroposterior compartment boundary of the developing Drosophila wing. However, whether a local increase in mechanical tension is common to compartment boundaries remained unknown. Moreover, the contribution of reduced cell proliferation at compartment boundaries has not yet been tested. Here, we confirm that in the developing Drosophila wing the rate of cell proliferation is reduced in a stripe of cells next to the dorsoventral compartment boundary (D/V boundary). Importantly, increasing the rate of cell proliferation did not alter the roughness of the D/V boundary. Moreover, by quantitatively analyzing the movements of the junctional network of cells after laser ablation of individual junctions, we found that mechanical tension is increased at cell junctions along the D/V boundary. Mechanical tension is decreased in the presence of the Rho kinase inhibitor Y-27632, which leads to a reduced actin-myosin contractility. In addition, we confirm that the roughness of the D/V boundary is increased when actin-myosin contractility is reduced in a zipper (encoding Myosin II) mutant. Our results suggest that a local increase in mechanical tension is a common mechanism to maintain compartment boundaries.

1031B Modulation of lifespan and healthspan by the transsulfuration pathway. Hadis Kabil1,2, Omer Kabil1, Robert Wessels2, Lawrence Harshman2, Scott Pletcher1. 1) Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI; 2) Department of Internal Medicine, University of Michigan, Ann Arbor, MI; 3) School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE.

Aging is an inevitable process and a multifactorial trait; both biological and environmental factors play a role in aging. Dietary restriction is a commonly used environmental manipulation which extends lifespan and delays the onset of age-related diseases. However, underlying mechanisms/s of diet-mediated longevity are not clearly understood. Therefore, we used combination of genetic, biochemical and pharmacological tools to gain insight into the molecular basis of dietary restriction modulated lifespan and healthspan. We have identified CG1753 gene as a Drosophila ortholog of the human cystathionine-beta synthase gene and demonstrated that dietary restriction upregulates CG1753 and associated biochemical pathway, the transsulfuration pathway. Moreover, using both RNAi and pharmacologic inhibitor we have showed that diet-mediated lifespan extension is dependent on the activity of the transsulfuration pathway. Further, we also showed that the transsulfuration pathway plays a role in cardiac function, suggesting that fly healthspan is controlled by this pathway.


The TGF-β superfamily of ligands form the largest group of secreted signaling molecules in the animal kingdom, and are involved in regulating a plethora of developmental and homeostatic processes. Recent evidence indicates that TGF-β signaling may also play an important role in regulating metabolism, energy and nutrient homeostasis. However, the mechanism(s) involved in TGF-β dependent regulation of metabolic processes remain unknown. Here we use the Drosophila model system to identify metabolic phenotypes manifested in larvae deficient in TGF-β signaling and determine mechanism(s) by which TGF-β signaling regulates these metabolic phenotypes. Our preliminary data show that null mutations in the Drosophila TGF-β/Activin homologue dawdle and R-Smad homologue smox affect the Insulin signaling pathway. We find that null mutations in daw cause a significant increase in hemolymph sugar concentration indicating an Insulin-Resistance like (InR-like) phenotype. However, circulating sugar level remains normal in smox null mutants. Consistently, we find that smox RNAi clones in the fat body show a cell autonomous increase in Insulin signaling and may explain why smox mutants do not exhibit an InR-like phenotype. We conclude that Drosophila TGF-β/Activin ligand daw positively regulate Insulin signaling at the systemic level. Additionally, we conclude that TGF-β signaling negatively regulates Insulin signaling in the peripheral tissues. This discrepancy may be either caused by differential role of daw and dActivin (the second TGF-β ligand signaling through smox) or by a differential role of TGF-β signaling on Insulin production/secretion and Insulin signaling.

1033A Positive and negative gustatory inputs affect Drosophila lifespan partly in parallel to dFOXO signaling. Joy Alcedo1, Werner Böll2, Ivan Ostojic1. 1) Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland; 2) Institute of Molecular Life Sciences, University of Zurich, Switzerland.

Many biological processes have been conserved between C. elegans and higher organisms. For example, the insulin/IGF-1 pathway regulates the physiology, and consequently the longevity, of worms, flies and mice. Thus, the sensory influence on C. elegans lifespan might also be present in other animals.

We have tested the hypothesis that Drosophila taste mutants will also exhibit lifespan alterations. As in C. elegans, we find that the fly gustatory system has bidirectional effects on lifespan. We show that taste inputs shorten lifespan through inhibition of the fly insulin pathway effector dFOXO, whereas other taste inputs lengthen lifespan in parallel to this pathway. We also find that the gustatory influence on lifespan is independent of food intake levels but depends on the type of food sources, which involve yeast-dependent and yeast-independent effects. Together our data suggest that different gustatory cues can modulate the activities of distinct signaling pathways to promote physiological changes that ultimately affect lifespan.

Since the gustatory (our study) and olfactoryb influences on lifespan are conserved in both worms and flies, it is possible that the sensory influence on lifespan is also found in mammals. For instance, both gustatory and olfactory information are relayed to the hypothalamus, a region in the mammalian brain that controls behavior and physiology. Thus, the processing of this information by the hypothalamus may in
turn modulate lifespan.


1034B

Although aging is universally recognized as a major factor in the aging of multicellular organisms, the changes that occur during aging at the cellular level remain poorly understood. We are particularly interested in the effects of aging on adult germline stem cells (GSCs). These cells exist throughout the lifetime of the organism, and thus might be more susceptible to aging-related damage, but because they will contribute to future generations, these cells need to remain essentially immortal. Using male germline stem cells in Drosophila as a model, we and others have previously shown that while total GSC number decreases only slightly, there is a significant decrease in cell cycle activity in these cells. Because stem cell activity is highly dependent on signals from the surrounding microenvironment, or niche, we are interested to know to what extent aging-related changes in GSCs are caused by cell-extrinsic versus cell-intrinsic factors. In these studies, our lab is taking two approaches. First, using the Gal4/UAS system, we are looking at cell-type specific effects of various genes that have been shown to affect aging, including superoxide dismutase, protein carboxy methyltransferase, and methuselah. Second, we are doing transplantation of both whole testes and individual stem cells into heterochronic backgrounds. Together these studies should elucidate whether stem cell aging is primarily a cell-intrinsic process, or whether it is subject to the age of the surrounding environment.

1035C
Sestrin is a feedback inhibitor of TOR that prevents age-associated pathologies. Jun Hee Lee1, Andrei Budanov1, Eek Joong Park1, Ryan Birse2, Teddy Kim3, Guy Perkins4, Karen Ocorr2, Mark Ellsman4, Rolf Bodmer2, Ethan Bier3, Michael Karin1, 1) Laboratory of Gene Regulation and Signal Transduction, Departments of Pharmacology and Pathology, School of Medicine, University of California San Diego, La Jolla, CA; 2) Development and Aging Program, NASCR Center, Burnham Institute for Medical Research, La Jolla, CA, USA; 3) Section of Cell and Developmental Biology, University of California San Diego, La Jolla, CA, USA; 4) National Center for Microscopy and Imaging Research, and Department of Neurosciences, University of California San Diego, La Jolla, CA, USA.

Sestrin is a family of stress-inducible proteins that are widely conserved across animal species. Sestrins were known to increase activity of adenosine monophosphate-activated protein kinase (AMPK), and inhibit target of rapamycin (TOR). Here, we showed that the Sestrin expression is induced upon chronic TOR activation through JNK and FOXO in a manner dependent on ROS accumulation. In turn, Sestrin suppresses oncogenic cell growth, metabolic dysfunction and age-related tissue degeneration brought about by TOR hyperactivation and/or ROS accumulation. Hence, Sestrin appears to be a negative feedback regulator of TOR that integrates metabolic and stress inputs and prevents pathologies caused by chronic TOR activation that may result from diminished autophagic clearance of damaged mitochondria, protein aggregates, or lipids.

1036A
The Role of CLAMP in Drosophila Dosage Compensation. Jessica Chery, Erica Larschan. MCB, Brown University, Providence, RI.

The initial recognition of target sequences by transcriptional activators is a crucial component of coordinate regulation, but the regulation of this event is not very well understood. One of the best model systems for studying how genes are identified for coordinate regulation is dosage compensation. This highly conserved mechanism allows males (XY) to equalize expression of their single X chromosome with females (XX) and male autosomes. Dosage compensation in Drosophila is regulated by the MSL (Male Specific Lethal) protein complex that associates with TFIID at promoters. We have been investigating the role of a testis-specific TFIIA subunit. TFIIA consists of three protein homologs of more generally expressed subunits are expressed in a tissue-specific manner. Specifically, several homologs of TFIIId subunits are testis-specific, and are necessary for gene expression in the testis and spermatid differentiation. TFIIId is comprised of TBP (TATA-binding protein) and up to fourteen TAFs (TBP-associated factors). In D. melanogaster there are also two homologs of TBP, TRF1 and TRF2, that are widely expressed in the fly, including in the male germ-line. Another General Transcription Factor, TFIIA, physically associates with TFIIId at promoters. We have been investigating the role of a testis-specific TFIIA subunit. TFIIA consists of three protein

1037B
Grainy head phosphorylation is essential for wound-dependent regeneration of an epidermal barrier. Myungjin Kim, William McGinnis. University of California, San Diego, 9500 Gilman Dr. #0349, La Jolla, CA 92093-0349.

Grainy head (GRH) is an important transcription factor that controls the expression of genes that are required for formation and maintenance of an epidermal barrier formation. The role of GRH in epidermis is highly conserved throughout the animal kingdom, from worms to mammals. However, the molecular mechanism of how GRH protein activity is controlled during development and tissue repair still remains elusive. However, we show that GRH is directly controlled by Extracellular signal-Regulated Kinase (ERK) phosphorylation. ERK phosphorylation of GRH is critical for wound-induced expression of GRH target genes in epidermal cells. Our biochemical analyses have shown that the Sestrine 91 in GRH that is principally phosphorylated by ERK. Although the ERK phosphorylation sites in GRH are dispensable for DNA binding affinity of GRH, they are needed for activation of Dopa decarboxylase (Ddc) and misshapen (msn) epidermal wound enhancers after epidermal wounding. These data indicate that ERK phosphorylation is required for epidermal barrier repair. However, GRH with mutated ERK phosphorylation residues can still function to promote barrier gene expression during normal embryonic epidermal development, suggesting that ERK sites are not essential for the developmental function of GRH in establishing epidermal barrier integrity. These results offer mechanistic insight into how tissue regeneration can be initiated by post-translational modification of a key transcription factor that normally mediates the developmental generation of that tissue.

1038C
The flii-s-2 gene is a germ-line-specific homolog of the small subunit of TFIIA. Mark A. Hiller, Alexander Daniel, Margaret Wood, Cynthia Cain. Dept of Biological Science, Goucher College, Baltimore, MD.

The General Transcription Factors are multi-protein complexes and essential for transcription in Eukaryotes. In Drosophila melanogaster, several homologs of more generally expressed subunits are expressed in a tissue-specific manner. Specifically, several homologs of TFIIId subunits are testis-specific, and are necessary for gene expression in the testis and spermatid differentiation. TFIIId is comprised of TBP (TATA-binding protein) and up to fourteen TAFs (TBP-associated factors). In D. melanogaster there are also two homologs of TBP, TRF1 and TRF2, that are widely expressed in the fly, including in the male germ-line. Another General Transcription Factor, TFIIA, physically associates with TFIIId at promoters. We have been investigating the role of a testis-specific TFIIA subunit. TFIIA consists of three protein
subunits. A single gene, *tfiia-l*, encodes a 48 kD polypeptide which is protolytically cleaved to form two proteins, a 30 kD and a 20 kD species. A separate gene, *tfiia-s*, encodes the small subunit, a 14 kD protein. We have shown that the gene *tfiia-s-2* (CG11639) encodes a male germ-line-specific homolog of the 14kD subunit. *In situ* hybridization indicates that *tfiia-s-2* is expressed in gonial cells and primary spermatocytes of the testes. Reverse transcriptase PCR experiments demonstrate that two different messages are encoded by *tfiia-s-2* due to alternative splicing. Both TFIIA-S-2 proteins associate with TFIIA-L in vitro. We propose that three different forms of TFIIA, each containing the TFIIA-L gene product and one of the gamma subunits, are present in *D. melanogaster* testes. These forms of TFIIA may interact with either TFIIID or the testis-specific TFIIID-like complex to regulate gene expression in the testes. We are characterizing the ability of TFIIA-S-2 containing complexes to physically associate with subunits of TFIIID, including TBP and the TBP associated factors (TAFs). In order to probe the function of the testis-specific TFIIA subunit we are creating a null allele by targeted disruption.

1039A

A positive feedback mechanism contributes to X chromosome identification during Drosophila dosage compensation. Marcela Soruccí1, Shouyong Peng2, Lingsheng Dong1, Erica Larschan. 1) MCB, Brown University, Providence, RI; 2) Harvard Medical School, Boston, MA.

The X-chromosome in Drosophila males is an important model for studying domains of coordinate gene regulation because all of the genes on a single chromosome are specifically upregulated. This process of dosage compensation increases transcript levels of the single X chromosome in males by two-fold to equalize expression levels with the two X-chromosomes in females and with the other chromosomes. The key regulator of dosage compensation in Drosophila is the Male Specific Lethal (MSL) ribonucleoprotein complex, which is specifically expressed in males and distinguishes the X-chromosome from the autosomes. The MSL complex is assembled during the transcription of its two non-coding RNA components, RNA X (roX), which are encoded on the X-chromosome and act as “seed” sites for MSL complex recruitment. These “seed” sites also contain MSL Recognition Elements (MREs), which are key 21 bp cis-acting sequences that are two-fold enriched on the X chromosome. The MRE sequences are also required for MSL complex recruitment. However, it is not known how the MREs and non-coding RNAs are functionally integrated to generate X-specificity because the MREs are not X-specific and the MSL components are not sufficient to target the MREs. We recently identified an essential zinc-finger protein called CLAMP that is strongly enriched at the MREs. Here we demonstrate by ChIP and ChIP-seq that the MSL complex and the CLAMP protein associate interdependently at MSL complex high affinity sites distributed across the X-chromosome. Based on these data, we hypothesize that the MSL complex recognizes the X-chromosome via a positive feedback mechanism that amplifies the two-fold enrichment of the MRE sequences to generate X-specificity.

1040B

Long Range Transcriptional Regulation in the Developing Eye. Nicole C. Evans, Christina I Swanson, Scott Barolo. Cell & Dev Biol, University Michigan, Ann Arbor, MI.

Enhancers are cis-regulatory elements that control the pattern and levels of gene expression. Enhancers are often located at great distances from the promoters of the genes that they regulate. Therefore, long-range enhancer promoter interactions are a critical part of transcriptional regulation—but we know almost nothing about how these interactions are managed in the genome. In order to better understand the mechanisms by which enhancers activate transcription from a distance, we are performing an in vivo structure-function analysis of the EGFR/MAPK- and Notch-regulated D-Pax2 cone cell-specific sparking enhancer (spa). In this study GFP expression is driven by the spa enhancer placed either adjacent to, or at a distance from, a heterologous promoter. Using this approach, we have identified a sequence within spa that is required when the enhancer is placed at a distance from the promoter, but is dispensable when the enhancer is proximal to the promoter. To our knowledge, this is the first enhancer region identified that specifically mediates long-range enhancer activity, yet is not required for patterning gene expression, thus we have named it the “remote control” element or RCE. Our current work focuses on determining the capabilities of the RCE, as well as the identification and characterization of proteins that interact with the RCE to allow it to perform its essential activities. This study will provide insight into the mechanisms by which enhancers engage in long-range transcriptional regulation.

1041C

**FTZ-F1 is required for eye development in Drosophila.** Hitoshi Ueda1,2, Abdel-Rahman Sultan2, Kazutaka Akagì3. 1) Dept. of Biol., Fac. of Sci., Okayama Univ, Okayama, Japan; 2) Grad. Sch. of Nat. Sci. and Tech., Okayama University, Okayama, Japan.

*FTZ-F1*, a member of the nuclear hormone receptors, is induced after ecdysone pulse and expressed in temporally restricted manner. It has been reported that its temporally restricted expression is important for embryonic, larval and pupal development of *Drosophila*. However, its expression pattern and function during pupal period have not been analyzed. Western immunoblot analysis during the pupal stage showed that *FTZ-F1* is expressed throughout their development, with especially high levels during late pupal stage. An immunohistochemical study revealed that *FTZ-F1* is strongly expressed in developing eye. To elucidate the role of *FTZ-F1* expression in the eye at late pupal developmental stage, effect of *FTZ-F1* knockdown in developing eye was examined. Results showed that morphological aberrations were observed in the adult flies, indicating that *FTZ-F1* plays an important role for eye development. Furthermore, because it has been shown that *FTZ-F1* is regulated by transcriptional repressor Blimp-1 at prepupal stage, we detected *FTZ-F1* and *Blimp-1* mRNA at pupal stage by RT-PCR. Results revealed increasing of *FTZ-F1* mRNA level after reduction of *Blimp-1 expression level, suggesting that *Blimp-1* may also repress *FTZ-F1* at pupal stage. From these results, we expect that expression timing of the *FTZ-F1* at pupal stage may be regulated by *Blimp-1* and is required for eye development.

1042A

Identifying relevant targets of miR-8 responsible for the pigmentation defect in mutants. Evan J. Waldron, Jennifer A. Kennell. Vassar College, Poughkeepsie, NY.

miRNAs are a class of RNA that has the ability to reduce gene expression by binding to specific messenger RNAs (mRNAs). Adding to the complexity of identifying a microRNA regulatory network, a single microRNA can target multiple mRNAs. We are particularly interested in the role of the microRNA miR-8 in the development of *Drosophila melanogaster*. We have found that flies lacking miR-8 display decreased pigmentation of the abdominal cuticle in addition to the previously reported small body size and neuronal defects. Bioinformatic searches and experiments in cell culture suggest that genes involved in cuticle pigmentation, such as *ebony*, *bab1* and *bab2*, are direct targets of miR-8. We have found that miR-8 expression partially overlaps with *Ebony* and completely overlaps with *Bab2* in the larval brain. *Ebony*, *Bab2*, and miR-8 are also expressed throughout the developing cuticle. However, overexpression of miR-8 in the developing cuticle or wing causes no change in *Ebony* or *Bab2* protein level in *Bab2* mutant. In addition, we found no difference in the mRNA or protein level of *Ebony* or *Bab2* in miR-8 mutants. We conclude that *Ebony* and *bab2* are most likely not targets of *miR-8* in vivo even though they can be targeted using *in vitro* sensor assays. Though *in vitro* sensor assays are useful for verifying the direct regulation of an mRNA by a microRNA, we have found that they are poor predictors for bonafide targets.

1043B

A genetic screen for novel factors regulating motor-driven mRNA localisation in the Drosophila oocyte. Rippei Hayashi1, Sophie Liddell1, Mark Wainwright1, Sheena Pinchin1, Stuart Horswill2, David Ish-Horowicz2. 1) Developmental Genetics Laboratory, London
Research Institute, Cancer Research UK, United Kingdom; 2) Bioinformatics and BioStatistics, London Research Institute, Cancer Research UK, United Kingdom.

Background: In Drosophila, many mRNAs that drive axial patterning are localised to specific subregions of the oocyte cytoplasm by motor-driven transport. Key questions remain as to how these mRNAs are selectively targeted to the specific regions, and how the cytoskeleton networks and the polarity of the cell are established during development to achieve specific localisations. To identify novel regulators of this process, we have developed a genetic screen based on directly observing mRNA localisation in the oocyte. Progress: We're focusing on gone early mRNA, a maternal RNA whose striking localisation in the oocyte determines both body axes of the oocyte and, thereby, of the embryo. We've used a system in which mature oocytes are tagged with binding sites for a fluorescent RNA-binding protein MS2-CP-RFP. The screen is based on random mutagenesis with EMS and making germ line clones using FLP/FRT system, and currently focuses on chromosome 3L. So far we have scored over 4,000 independently mutagenised lines and collected 10 RNA mis-localisation mutants. Complementation tests are underway. One newly-induced mutation, “saturn”, is characterised by early-stage mutant egg chambers in which gone early mRNAs localise to the centre of the oocyte, rather than to the posterior end of the oocyte as in wild-type. This phenotype is suggestive of a defect in microtubule organisation. In later stages saturn oocytes, gone early mRNAs are perinuclear as in wild-type, but the nucleus is mis-positioned at the centre of the animal, rather than to the dorsocentral corner of the oocyte. Experiments aimed at understanding these phenotypes will be presented. We shall also present a method for rapid mapping of EMS-induced mutations using a combination of meiotic recombination and whole-genome deep sequencing.

1044C
Maintenance of undifferentiated state of stem cell precursors in the Drosophila ovary. Shinya Matsuoka12, Miho Asaoka12, Yasushi Hiromi12: 1) National Institute of Genetics, Mishima, Japan; 2) SOKENDAI, Kanagawa, Japan.

Many types of sexually reproducing animal possess germline stem cells (GSCs). GSCs are undifferentiated cells and have an ability to produce not only gametes but GSCs themselves after every cell division. This self-renewal ability allows GSCs to produce gametes indefinitely to maximize success in fertilization. Drosophila is one of such organisms equipped with GSC systems. During larval stage, Drosophila ovary is filled with stem cell precursors, primordial germ cells (PGCs), which are kept in an undifferentiated state. At pupal stage, some of PGCs start to differentiate to eggs, but the rest of PGCs are selected as GSCs and keep being in an undifferentiated state. A previous study has shown that it is a crucial step to prevent PGCs from differentiation to establish adequate number of GSCs. However, how PGC differentiation is suppressed is largely unknown.

Here we show that novel gene gone early is involved in prevention of PGC differentiation. Gone early is a membrane-bound protein with an unknown extracellular domain. When gone early is over-expressed in the oocytes in which it is endogenously expressed, PGC differentiation is excessively blocked. In contrast, in gone early mutant, the increased number of differentiating germ cells is observed and this phenotype can be rescued by expressing gone early, suggesting that the observed phenotype is caused by the mutation in gone early. Although the number of differentiating germ cells in gone early mutant is greater than in wildtype, the number of PGCs does not decline. This is due to dedifferentiation of differentiating germ cells into PGCs in order to ensure certain number of PGCs. Even though PGC number does not decline in gone early mutant, the number of PGCs in favorable background is lower than in wildtype and this defect leads to the decline in the number of GSCs, supporting the notion that preventing PGC differentiation is prerequisite to establish adequate number of GSCs.

1045A
Early oogenesis phenotypes associated with disrupted function of DPR9, a brain expressed Ig domain protein. Nicola Ford, Laura Ponting, Martin Baron. Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom.

Stem cells are important for maintaining tissue homeostasis and are associated with cancer and age related conditions. Therefore, identifying how stem cells are regulated is important for understanding how these diseases progress. The Drosophila ovary is an ideal model system for stem cell regulation as it contains three populations of stem cells which are required to produce a viable egg. We identified a viable P-element insertion mutation which exhibited strongly reduced egg laying and displayed early oogenesis phenotypes including reduced germline stem cell number and a defective supply of follicle cells. Complementation, expression analysis, RNAi and remobilising the P-element in this stock suggested that defective proboscis extension response 9 (dpr9) is required for fertility. RNAi and RT PCR was used to demonstrate that dpr9 functions in the brain. Dpr9 has no known function but carries immunoglobulin domains which share homology with diglons, mammalian neural adhesion proteins, suggesting dpr9 may have a role in adhesion. It is possible that dpr9 also shares a similar function.

1046B

Perhaps the best understood mechanism of hematopoietic stem cell regulation is the inclusion of stem cells within stem cell niche that maintains pluripotency by providing local signals. How signals originating outside the niche might influence the behavior of stem cells is much less understood. Here we show that metabolic stress due to nutritional deprivation dramatically reduces myeloid-like hematopoietic stem cells in Drosophila - Nutritional deprivation signals through Insulin-like Signaling which is a process mediated by Insulin-like signaling (IIS) and dTOR, which regulate expression of wingless, a major signal in stem cell maintenance. Additionally, amino acid levels are critical to stem cell maintenance, based on a result that knocking-down slimfast in stem-like progenitors causes loss-of-stemness in the lymph gland. We also find that developmental changes in Insulin/dTOR activity influence stem cell behavior, indicating that Insulin-like Signaling/dTOR controls normal hematopoietic development in the lymph gland. Taken together, our studies reveal systemic control of hematopoietic stem cells through Insulin-like Signaling/dTOR, in the context of both stress response and normal hematopoiesis.

1047C
Intestinal homeostasis in the absence of epithelial production of JAK/STAT signaling ligands. Guonan Lin, Na Xu, Fu Yang, Rongwen Xi, National Inst Biological Sci, Beijing, China.

The JAK/STAT pathway has critical role in intestinal stem cell (ISC) maintenance, division and multiple lineage differentiation in the Drosophila midgut, but the mechanisms of JAK/STAT signaling activation in ISCs remain to be clarified. We observe that Upd, the major signaling ligand, is exclusively expressed in the surrounding visceral muscle cells in healthy intestine. However, its expression was observed in the epithelium instead by other groups. A weak ligand, Upd3, is also expressed sporadically in some enterocytes, suggesting a model that the dying enterocytes could send a feedback signal for ISC activation. Here we attempt to address the relative contribution of Upd ligands produced from the epithelium versus that produced from the visceral muscle by generating large patches of epithelial cells (>100 cells) depleted for all the JAK signaling ligands, including Upd, Upd2 and Upd3, followed by examining their behaviour. In these epithelial clones, ISCs remain normal division activity, sustain normal lineage differentiation. The JAK/STAT activity also appears unaltered in the mutant ISCs within the clones. These data further support the notion that muscle-derived Upd signals are probably the major ligand source for JAK/STAT signaling activation in ISCs for normal intestinal homeostasis.

1048A
EGFR, Wingless and JAK/STAT signaling cooperatively maintain Drosophila intestinal stem cells. Na Xu, Siqi Wang, Guonan Lin,
The “Ciber-genética” blog (http://ciber-genetica.blogspot.com/) was constructed with the aim of supporting different courses related to

We have used these constructs to confirm the lineage-specific tiling of the projection neurons in the antennal lobe and to trace
2007). Our dBrainbow adaptation can be used to image endogenous fluorescence with three separable colors, but we optimized an
“Ciber-genética” an education Project.

The blog contains links to several journals associated with research and classes in Genetics and Biotechnology, as well in health sciences.

hybridization will be used to identify novel epidermal-specific wound response genes from selected candidates. These novel epidermal
epidermal wounding. Substantial upregulation of various barrier repair, signaling, and innate immunity genes were observed.

Drosophila Brainbow can be used to subdivide complex GAL4 patterns. Julie H. Simpson, Stefanie Hampel, Phuong Chung, Claire McKellar, Donald Hall, Loren Looger. HHMI, Janelia Farm Res Campus, Ashburn, VA.

The Drosophila Brainbow technique combines cell targeting made possible by GAL4 lines with fluorescent label diversity generated by
recombination to subdivide complicated neural expression patterns. This facilitates anatomical analysis of neuronal lineages and individual
neurons. The original mouse Brainbow uses endogenous fluorescence and variable copy numbers to generate color diversity (Livet, et al 2007). Our dBrainbow adaptation can be used to image endogenous fluorescence with three separable colors, but we optimized an
antibody-epitope based version for use in fixed samples. The expression levels are high enough for tracing of fine processes in adult
neural hair cells, and even in flies this allows us to confirm the refined specific tiling of the projection neurons in the antennal lobe and to trace
motor neurons from the subsesophageal ganglion to their neuromuscular junctions in the proboscis. We are using the ability to visualize
different populations within the same animal to dissect other GAL4 lines with complex expression patterns.


The “Ciber- Genética” blog (http://ciber-genetica.blogspot.com/) was constructed with the aim of supporting different courses related to
genetics. The blog provided a useful tool to encourage students to deepen the topics covered in class. The information at the Blog was
updated every day. The students could also have access to the material reviewed in class such as videos and power point presentations.
The blog contains links to several journals associated with research and classes in Genetics and Biotechnology, as well in health sciences.
The blog also contains RSS subscriptions, which allows this blog to be re-purposed in other ways across the Internet, thereby giving the
blog the potential to reach well beyond the immediate links above, and thereby be available to larger audiences. The blog can be reposed on
other websites and subscribed to RSS readers, such as Google Reader (which is the what the one used by us). A great advantage of
blogging as a teaching tool is that students can log on at different times from different places other than with their teacher. This gives a
student a chance to reflect on posts, do research and reply once they have time to do so. This takes teaching and learning to a level beyond
the classroom time schedule. As a communication tool, students will learn to summarize answers, clarify thoughts, and express them accurately. “Ciber- Genética” website (http://cibergenetica.fciencias.unam.mx) was created to improve and expand the Ciber-Genética blog.
The main objective is to integrate in one place resources, relevant and current information that can improve the teaching-learning process.
The website contains a link to “Ciber- Genética” blog, has a section of members, a section on academic events and a glossary. One of the most
interesting qualities of the website is that visitors can register so they can have access to educational materials of great value in the most
interesting sections of our site such as courses, research, dissemination and teaching, in which visitors can access materials such as presentations, videos, books and articles in PDF format.
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