# EDRC2009

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# Abstract Book

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**Genome-wide manipulations of flies.**

**Hugo J. Bellen**[1], Koen Venken[1], Robert Levis[3], Joseph Carlson[2], Ellen Popodi [4], Karen Schulze[1], Thom Kaufman[4], Roger Hoskins[2] and Allan Spradling[3]
[1]HHMI, Baylor College of Medicine, Houston, TX; [2]Lawrence Berkeley National Laboratory Berkeley, CA; [3]Carnegie Institution of Washington and HHMI, Baltimore, MD; [4]Indiana University, Bloomington, IN

The Gene Disruption Project: we have generated and collected P-element , PiggyBac, and Minos insertions in almost 65% of the annotated fly genes. We are currently using a 'NEW MINOS CONSTRUCT' that carries two attP sites in opposite orientation, called MIMIC, to increase the coverage and to improve the versatility of new insertions. About 30% of MIMIC insertions integrate in introns. Using Recombination Mediated Cassette Exchange we have integrated numerous constructs into introns of known genes. Preliminary data show that tagging the genes with Red Fluoresent Protein by this method is efficient and allows in vivo detection of fusion-proteins encoded by most tagged genes. The P[acman] vector and the P[acman] libraries: we have developed a transformation vector (P[acman]) that allows integration of small to large DNA fragments into defined attP docking sites, avoiding mapping and position effects of transgenes. Numerous docking sites have been created throughout the genome and genomic DNA up to 150 kb can be integrated in these specific docking sites. We also created two P[acman] genomic 21 kb and 80 kb libraries that are publicly available. More than 95% of all fly mutations can now be rescued by genomic clones and most genes can be tagged by recombineering allowing protein localization in vivo. To greatly facilitate mapping of genes on the X-chromosome we have selected a tiled pathway of 400 clones (80kB) that cover the entire X-chromosome and have created transgenic flies that cover almost 50% of the X-chromosome.

**Balancing self-renewal and differentiation: regulation of symmetric and asymmetric division in the nervous system**

Egger, B., Gold, K. and **Brand, A.H.**
The Gurdon Institute and Department of Physiology, Development and Neuroscience, University of Cambridge, Tennis Court Road, Cambridge, UK CB2 1QN

Balancing symmetric and asymmetric division is critical for the generation and maintenance of tissues. Unregulated stem cell division, where stem cells or their progeny divide in an uncontrolled fashion, can lead to tumourous overgrowth, while depletion of the stem cell pool by premature differentiation can result in tissue degeneration. In the Drosophila optic lobe, symmetrically dividing neuroepithelial cells generate asymmetrically dividing neuroblasts. To identify the molecular switches mediating this transition, we isolated small groups of neuroepithelial cells or neuroblasts and compared their transcriptional profiles. We find ~200 differentially expressed genes between these two stem cell populations. In particular, genes of the Notch signal transduction pathway are differentially transcribed in a pattern suggesting Notch activity is required to maintain the neuroepithelial state. A small group of cells separating the neuroepithelium from neuroblasts expresses the proneural gene lethal of scute (l'sc). We show that ectopic expression of l'sc, or loss of Notch, induces premature neurogenesis within the neuroepithelium. To identify key molecules regulating the transition from symmetric to asymmetric stem cell division, we are mapping the transcriptional targets of l'sc throughout the genome and are also investigating the roles of other genes that exhibit restricted expression in the optic lobe. These encode adhesion molecules, transcription factors, and RNA binding proteins.

**Visualizing Assembly and Transport of the oskar mRNP in the Drosophila Oocyte**
**Anne Ephrussi**
European Molecular Biology Laboratory, Heidelberg, Germany

Polarized transport of RNA within cells is a highly prevalent and conserved mechanism allowing tight control of protein expression in space and time. oskar encodes the Drosophila posterior determinant, whose accumulation at the posterior of the oocyte is necessary for abdomen and germline formation in the embryo. Spatial restriction of Oskar is critical and is achieved by coordination of mRNA localization and translational control: unlocalized oskar mRNA is repressed and the mRNA translated only upon posterior localization. oskar mRNA localization requires Exon Junction Complex (EJC) proteins and splicing of the first intron. The 3'UTR is also required for posterior targeting of the mRNA, and promotes co-assembly of oskar 3'UTRcontaining transcripts into transport complexes. Translational repression of oskar is mediated by Bruno protein, which binds to the 3'UTR and represses translation via two mechanisms: one, cap-dependent, in which binding of eIF4G and small ribosomal subunit recruitment are inhibited; the other, cap-independent, in which oskar mRNA is oligomerized into large particles, also blocked at initiation. A correctly polarized microtubule network as well as kinesin-1 motor protein are required for active transport of oskar mRNA to the oocyte posterior pole. New insight into the regulation of oskar mRNA transport and the steps in assembly of the oskar mRNA transport complex will be presented.

**The Systemic Immune Defense of Drosophila**
**Hoffmann J**
Institut de Biologie Moléculaire et Cellulaire CNRS Université de Strasbourg

Insects are prone to infections by many microorganisms and have been known for over a century to mount an efficient defense reaction. Phagocytosis is a central arm of this defense, as is the infection-induced rapid induction of hundred of genes which code for peptides and proteins which concur to fight the invaders. Prominent among the induced proteins are the antimicrobial polypeptides. We and others have addressed the molecular mechanisms underlying the antimicrobial host defense against fungi, bacteria and viruses in the model system Drosophila. Over the last twenty years, results from our groups and from other laboratories have provided insights into the mechanisms of recognition of infecting agents and the subsequent signaling events which lead to gene reprogramming. The presentation will cover recognition events of bacterial, fungal and viral infections, and the signaling cascades induced by these events . Parallels will be drawn with innate immune defenses in other groups, particularly in mammals. The compelling similarities between the defense mechanisms of the various phyla strongly suggest that a basic platform of innate immune mechanisms evolved very early in evolution of multicellular organisms and has persisted in all later groups with adaptations/variations around this ancestral platform. Recent reviews relating to this presentation are: The Host Defense of Drosophila, B.Lemaitre and J.A.Hoffmann, Annu. Rev. Immunol. 2007, 25, 697-743 The Drosophila systemic Immune response : sensing and signaling during bacterial and fungal infections, D. Ferrandon, JL Imler, C.Hetru and J.A.Hoffmann, Nat Rev Immunol. 2007, 7, 11, 862-874 Antiviral immunity in drosophila. C. Kemp , JL. Imler. Curr Opin Immunol. 2009, 3-9.

**Growth control by the Fat-Hippo pathway in Drosophila**

**Kenneth D. Irvine**, Yaopan Mao, Venugopala Reddy, Hyangyee Oh, Cordelia Rauskolb, Gongping Sun, Yongqiang Feng, Guohui Pan, Binnaz Kucuk, Aiguo Xu
Howard Hughes Medical Institute, Waksman Institute and Department of Molecular Biology and Biochemistry, Rutgers The State University of New Jersey, Piscataway NJ 08854 USA

The Fat signaling pathway is named for a Drosophila gene, fat, which encodes a cadherin protein that acts as a transmembrane receptor. Fat signaling influences both gene expression and planar cell polarity. The influence of Fat signaling on gene expression is effected through an intersection with the Hippo signaling pathway. Many components of Fat and Hippo signaling acts as tumor suppressors or oncogenes, both in Drosophila and in mammals. We have analyzed multiple steps of Fat signaling, from regulation of the Fat receptor at the membrane, to the intersection with Warts-Hippo signaling, to the regulation of gene expression through the transcriptional co-activator Yorkie. Our recent studies have focused on defining critical regulatory steps in the pathway, on characterizing developmental requirements for the pathway, and on exploring the relationship between vertebrate and invertebrate Fat signaling.

**Controlling the balance between self-renewal and differentiation in Drosophila neural stem cells**

**Juergen A. Knoblich**

Stem cells are characterized by their ability to create two kinds of daughter cells. On one hand, they can selfrenew and create identical copies of themselves. On the other hand, they give rise to more lineage-restricted cells, which ultimately undergo terminal differentiation. We use Drosophila neuroblasts as a model system to understand how the balance between those different types of progeny is controlled. Drosophila neuroblasts divide asymmetrically: One daughter cell retains neuroblast characteristics while the other cell divides only once more to create two terminally differentiating neurons. During each neuroblast division, the proteins Numb, Prospero and Brat segregate into one of the two daughter cells where they cooperate to prevent selfrenewal and induce terminal differentiation. Mutations in these segregating determinants lead to overproliferation of the neuroblast pool and the formation of a stem cell derived tumor. To understand the biological processes and regulatory networks controlled by these factors, we have carried out a genome-wide RNAi screen for defects in neuroblast self-renewal. We use a library of transgenic RNAi lines targeting over 80% of the genes in the Drosophila genome. By expressing each of the over 20,000 RNAi constructs contained within this library in neuroblasts, we have identified over 600 genes involved in lineage specification in the Drosophila brain. We have quantified the resulting phenotypes to generate a phenotypic barcode expressing the degree of abnormality in neuroblast size, proliferative capacity and daughter cell number. Hierarchical clustering of the resulting phenotypes can classify genes by biological function and identifies, for example, 5 new genes involved in cytokinesis, 110 new genes regulating cell growth and selfrenewal and 10 new tumor suppressors. How genome-wide analysis in vivo opens new avenues to understand biological processes will be the key topic of my talk.

**From cell mechanic to tissue morphogenesis**

Romain Levayer, Matteo Rauzi and **Thomas Lecuit**
IBDML, campus de Luminy case 907, 13288 Marseille cedex 9

Tissues exhibit a remarkable dual property of robustness and plasticity. This relies on unique mechanical properties of the cell cortex and on adhesive interactions between cells. Our group seeks to understand the fundamental molecular mechanisms responsible for this property. This is essential to understand morphogenesis of developing embryos and organs, and is severely affected in a number of disease. To that end we develop a range of approaches, from the genetic and pharmacological perturbations of molecular components, the quantitative imaging of proteins using a variety of photonic methods, probing of the physical properties of cells within intact tissues, and predictive computational modelling of morphogenesis at different scales (molecular to tissue scales). I will present our current research characterizing how adhesion and cortical tension regulate the dynamic remodelling of cell contacts in the primary epithelium of Drosophila embryos. I will first focus on how actin controls E-cadherin organization and how E-cadherin-actin interactions control force transmission at cell interfaces. I will also report new findings concerning the spatial distributions of tensions in epithelial cells and how regulation of different tensile networks controls cell shape and cell dynamics.

**A-01 Abstract not available**
**St Johnston D.**

**A-02 Investigating cell fate decisions of the intestinal stem cell.**
Carolina N. Perdigoto, Francois Schweisguth, Allison J., **Bardin A.**
CNRS URA2578, Developmental Biology Department, Pasteur Institute, Paris, France.
Intestinal Stem Cells (ISC) replenish gut tissue throughout the lifetime of the adult fly. The ISC division is asymmetric in its two daughter cell fates: one remains an ISC and the other becomes a progenitor cell, the enteroblast (EB), which can differentiate into an enterocyte (EC) or enteroendocrine (ee) cell. We are interested in the mechanisms by which the fates of the stem cell and its differentiated progeny are achieved. The Notch signaling pathway is activated in the EB and promotes EB, ee, and EC fates (Micchelli, 2006; Ohlstein, 2006; Ohlstein, 2007). However, how Notch signaling is modulated to provide EB fate and two distinct differentiated cell fates is not understood. Given the role of Notch signaling in many stem cell fate decisions, it is important to understand how it can promote alternative fates in a single precursor cell. In a mutagenesis screen we found that Gmd is specifically required for self-renewing decisions of the ISC but not the production of differentiated cells. Gmd (GDP-mannose 4,6-dehydratase) is important for fucosylation and fringe-mediated glycosylation of Notch (Okajima, 2005; Sasamura, 2007). Interestingly, Gmd plays a fringe-independent role to limit symmetric ISC divisions. Notch signaling is activated in Gmd mutant clones, but precise asymmetric fate decisions do not occur leading to an overabundance of ISCs. Our data suggest that Gmd is required for highly robust Notch activation needed to limit symmetric division of the ISC. We propose a model in which different cell fate decisions have distinct requirements for Notch signaling: the asymmetric decision between ISC and EB requires high levels of Notch signaling whereas further differentiation of the EB into EC or ee cells can occur with lower Notch levels.

**A-03 Interdependence of macrophage migration and ventral nerve cord development in Drosophila embryos.**
**Evans, I.**, Hu, N., Skaer, H., Wood, W.
Department of Biology and Biochemistry, University of Bath, Bath, BA2 7AY, UK N Hu and H Skaer - Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3

During embryonic development Drosophila macrophages (hemocytes) undergo a series of stereotypical migrations to disperse throughout the embryo. One major migratory route is along the developing ventral nerve cord (VNC) where hemocytes are required for the correct development of this tissue. Here, for the first time, we show that a reciprocal relationship exists between hemocytes and the VNC and that defects in nerve cord development prevent hemocyte migration along this structure. Using live imaging, we demonstrate that the axonal guidance cue Slit and its receptor Robo are both required for hemocyte migration but not autonomously in hemocytes. We go on to show that the failure of hemocyte migration along the nerve cord in slit mutants is not due to a lack of chemotactic signals within the nerve cord but rather due to a failure in VNC detachment from the overlying epithelium which provides a physical barrier to hemocyte migration. This block of hemocyte migration in turn disrupts the formation of the dorsoventral channels within the VNC further highlighting the importance of hemocyte migration for correct CNS development. This study reveals an intriguing interplay between the developing nervous system and the blood cells within the fly and demonstrates that the development of these two distinct cell types are closely coupled and codependent upon one another.

**A-04 The co-repressor dNAB interacts with Brinker to regulate competition for the Dpp survival signal.**

Ziv, O.[1*]; Suissa, Y.[1*]; Neuman, H.[1]; Dinur, T.[1]; Geuking, P.[2]; Rhiner, C.[3]; Portela, M.[3]; Lolo, F.[3]; Moreno, E.[3]; **Gerlitz, O.**[1]

[1]Developmental Biology and Cancer Research, IMRIC, The Hebrew University-Hadassah Medical School, 91120 Jerusalem, Israel. [2]Institut fur Molekularbiologie, Universitat Zurich, Winterthurerstrass

The proper development of tissues requires morphogen activity that dictates the appropriate growth and differentiation of each cell according to its position within a developing field. Elimination of underperforming cells less efficient in receiving/transducing the morphogenetic signal is thought to provide a general fail-safe mechanism to avoid developmental misspecification. In the developing Drosophila wing, the morphogen Dpp provides cells with growth and survival cues. Recent studies established a 'survival factor-capture' model where cells continuously compete for the limited Dpp survival signal. Thus, cells less efficient at competing for Dpp, upregulate the transcription-repressor Brinker (Brk), thereby activating apoptosis. Here we find that the transcriptional co-repressor dNAB is a Dpp target in the developing wing that interacts with Brk to eliminate cells with reduced Dpp signaling through the JNK pathway. In addition, both dNAB and Brk are required for dMyc-induced cell-competition, a process that depends on reduced Dpp transduction in outcompeted cells. We further show that in contrast to Groucho, a known co-repressor of Brk, dNAB is not required for Dpp-dependent patterning, whereas Groucho does not promote JNK-mediated cell death. These findings imply that the choice of Brk co-repressor determines the specificity of target gene repression, thereby modulating different Dpp outputs. We propose a novel mechanism whereby the morphogen Dpp regulates the responsiveness to its own survival signal by inversely controlling the expression of a repressor, Brk, and its co-repressor, dNAB.

**A-05 Drosophila Musashi family proteins regulate stem cell behaviour through different mechanisms in the ovary and testis.**

**Siddall, NA**; Kalcina, M; Johanson, TM; McLaughlin, EA; Hime, GR Anatomy and Cell Biology, University of Melbourne, Parkville, Australia, ARC Centre of Excellence in Biotechnology and Development, Callaghan, Australia, School of Environmental and Life Science,

Recent studies have shown that the Musashi (Msi) family of RNA-binding translational repressors are expressed in proliferative cells of the mammalian central nervous system, intestine, stomach, and breast tissue. Furthermore, expression of Msi proteins, in particular Musashi1, can be linked to stem cell over-proliferation in some cancers. Using the Drosophila testis as a model system, we recently demonstrated that Drosophila Msi is required intrinsically in germline stem cells (GSC's) for maintenance of the GSC fate. In this study, we further investigated whether loss of Msi function affects stem cell behaviour in the Drosophila ovary. In contrast to the testis, we have found that Msi is not required intrinsically in GSCs for thee maintenance of GSC identity, but rather is required in the somatic niche to regulate GSC and early germ cell behaviour, suggesting that Msi proteins can modify stem cell behaviour through different mechanisms. Additionally, we have identified a novel Drosophila Msi family member, which we have called Real Musashi (Remsi). We show that Remsi is expressed in the Drosophila testis and ovary by in situ hybridisation. Knockdown of Real Musashi by RNAi in somatic stem cells of the testis results in detachment of the GSCs from their niche environment and a failure of inhibition of germ cell proliferation. These studies highlight the functional importance of Msi family proteins in various stem cell populations, and show that Msi can also function in the stem cell niche environment to regulate stem cell behaviour.

**A-06 The assembly of the musculoskeletal tissue in Drosophila – identification of novel tendon-specific essential signals.**

**Talila Volk**, Bess Wayburn, Eliezer Gilsohn
Department of Molecular Genetics, Weizmann Institute of Science, Rehovot 76100, Israel

The correct assembly of muscles and tendons into a single contractile unit is essential for proper muscle function. The transcription factor Stripe regulates tendon cell identity and is both necessary and sufficient for targeting muscles to interact with their corresponding tendon cells. We identified two Stripe down stream genes, slowdown (slow) and leucine rich tendon (lrt), both essential for the correct musculoskeletal assembly. Slow protein contains two EGF and single EMI domains and is secreted out of tendon cells. Functional analysis indicated that Slow is required for proper distribution of the muscle-specific integrin receptors at the MTJ, which is critical for muscle function. In slow mutant larvae we detected rupture of muscles and tendons, which led to sluggish larvae locomotion and partial larvae lethality. Slow forms a protein complex with Thrombospondin (Tsp), the major ligand of the muscle-specific integrin. Further analysis suggested that Slow-Tsp interaction controls integrin receptor distribution at the muscle leading edge upon MTJ formation. Thus Slow regulates the distribution of the integrin receptors through its association with Tsp. LRT is a leucine-rich repeat domain trans-membrane protein expressed by tendon cells. High levels of LRT are detected in muscle-bound tendon cells. Functional analysis demonstrated that LRT is required for arresting extra-filopodia formation of the muscle cell upon its arrival to the corresponding tendon cell. Molecular analysis suggests that LRT functions through its interaction with Robo receptors on the muscle cells. Taken together these results reveal novel molecular signals directing the establishment of proper musculoskeletal assembly during Drosophila embryogenesis.

**A-07 Color vision in flies**

Johnston, R.; Jukam, D.;, Vasiliauskas, D.; Vogt, N.; Nériec, N.; Rister, J. & **Desplan, C.**
Center for Developmental Genetics, Dept. Biology, New York Uiversity 1009 Silver Center, 100 Washington Square East, New York NY 10003, USA email: cd38@nyu.edu

The Drosophila compound eye is made of 800 unit eyes (ommatidia) that each contains eight photoreceptors: Six are involved in motion detection while two (R7 and R8) play a major role in color vision. These ommatidia can be grouped into four categories: p ommatidia contain UV-sensitive Rh3 in photoreceptors R7 and Blue-Rh5 in R8 while y ommatidia express another UV-Rh4 in R7 and green-Rh6 in R8. The p and y subsets are distributed stochastically throughout the retina in a 30:70 ratio. Comparison between the inputs of R7 and R8, and between p and y ommatidia allows flies to discriminate between colors. Dorsal Rim Area (DRA) ommatidia express UV-Rh3 in both R7 and R8. They measure the vector of light polarization for navigation on cloudy days. A fourth subset located in the dorsal third of the eye coexpresses UV-Rh3 and -Rh4 in yR7 and serves to detect solar vs. anti-solar orientations, also for navigation. We analyze the specification of the different subsets of photoreceptors Processing of color information occurs in the medulla that receives input from R7 and R8 in ~800 ‘columns’, the functional units in the medulla. We are addressing how medulla cells process color information coming from R7 (sensitive to UV) and R8 (sensitive to blue or green) and send it to higher processing centers in the lobula complex and central brain to mediate color behavior. Through silencing subsets of medulla neurons using specific Gal4 lines, we test the consequence for color discrimination. We have adapted to color vision the flight simulator originally designed by the Dickinson/Frye labs. In an operant paradigm, the fly is trained to associate color with a reward or punishment before being tested in the absence of the reward.

**A-08 Lumen formation and cell elongation during tracheal terminal branch development.**

**Gervais, L.**; Casanova, J
Institut de Biologia Molecular de Barcelona-CSIC Institut de Recerca Biomedica de Barcelona, Barcelona, Spain

How a fine tube forms inside a cell as it reaches its target tissue in angiogenesis and in the respiratory systems remains an open question. We have addressed this issue by analyzing the development of the terminal branches of the Drosophila trachea, the insect respiratory system. We have realized a detailed description of the cellular shape modification and cytoskeleton reorganization that accompany terminal cell tubulogenesis. Based on this description, we have explored the contribution at the cellular level of the signaling pathways and transcription factors involved in this step. We have identified some of the effectors in that mechanism and establish the reinforcing roles of the Drosophila homologs of FGF and the SRF transcription factor in this process. Given their similarities, we propose that this can be a shared mechanism to couple cell elongation to lumen generation in the formation of fine respiratory tubes.

**A-09 Heterochromatin Protein 1 (HP1) and Without Children (Woc) are required for niche morphogenesis and proper germ line stem cell differentiation.**

**Maimon, I.**; Gilboa, L.
Department of Biological Regulation, Weizmann Institute of Science, Rehovot, Israel

Niches are important for stem cell regulation but little is known about how these niches form or the mechanisms by which they affect stem cell behavior. In the Drosophila ovary, germ line stem cells (GSCs) are contacted by Cap and Escort cells, required for GSC maintenance and differentiation respectively. We show that two proteins, the chromatin modulator, HP1, and the transcription factor Without children (Woc), are required for proper niche formation and for GSC differentiation. Woc was isolated in a screen for genes that affect GSC fate. Reducing woc expression specifically in the somatic cells of an adult ovary results in niche morphogenesis defects; Escort cells are viable but do not send proper extensions to contact germ cells. Consequently, GSCs differentiation is arrested at the cystoblast stage. Woc was shown to associates with telomeres. Heterochromatin protein 1 (HP1), encoded by su (var)205, also resides on telomeres. Mutations in either gene cause telomere fusions. We found that, similar to Woc, reducing HP1 expression in the soma results in escort cells defects, coupled to a failure of GSC differentiation. Other telomere capping proteins do not affect GSC fate. To understand why niche cells do not contact germ cells correctly, we explored niche formation during larval stages. RNAi of either woc or su(var)205 results in a decreased number of somatic intermingled cells (ICs), a group of cells that contact GSC precursors. This is probably a specific failure of IC differentiation or proliferation, since over expression of Woc results in too many ICs. Our data indicate that Woc and HP1 might function together to specify normal niche fate or morphogenesis. In the absence of proper contact with niche cells, GSCs fail to differentiate.

**A-10 Lipids controlling germ and heart cell biology.**

**Renault, A.**; Haack, T
Max Planck Institute for Developmental Biology, Tübingen, Germany

Lysophospholipids are derivatives of phospholipids that include lysophosphatidic acid and sphingosine 1-phosphate. We are interested in understanding how such lipids act extracellularly to regulate cell behaviour including proliferation, survival and migration. Genetic screens have uncovered a role for lysophospholipids in germ cell migration. The wunen genes encode lipid phosphate phosphatases: transmembrane enzymes which can dephosphorylate lysophospholipids. They are located at the cell surface with their catalytic domain facing outside of the cell and thus are able to dephosphorylate extracellular substrates. Wunens act as germ cell repellents: Germ cells avoid wunen expressing somatic tissues. Our model is that germ cells require a lysophospholipid attractant that is destroyed by wunen expressing somatic cells. Recently we have uncovered a role for wunens in heart development. Wunens are strongly expressed in heart cells and in wunen mutants the heart is malformed. The heart is formed by coalescence of rows of heart cells which are initially located bilaterally but use dorsal closure events to move dorsally towards the midline and each other. We have analysed the movement and morphology of heart cells in wild-type and mutant. In the mutant we find that heart cells are specified properly and move towards the midline. In the majority of embryos all of the heart cells meet normally at the midline but in some embryos meeting at the midline is incomplete and small gaps remain. In both cases however the heart cells subsequently loose adhesion between rows generating a malformed heart. Our goal is to discern which and how lysophospholipids influence heart cell behaviour and to understand the extent of the similarities in germ and heart cell biology.

**A-11 Polarisation of upd mRNA is essential for JAK/STAT activation during border cell migration.**

**Van De Bor V.**, Cerezo D., Zimniak G. and Noselli S.
IBDC, University of Nice-Sophia Antipolis, UMR 6543 CNRS , Parc Valrose, 06108 Nice Cedex 2, France.

Cell migration is a complex mechanism which is essential in animal development and also involved in cancer cell spreading. A variety of signalling pathways are known to play a role in cell migration including the conserved JAK/STAT pathway (Janus kinase/ Signal Transduction and Activator of Transcription).We are using Border Cells (BC) migration in Drosophila ovary to study the regulation of JAK/STAT signalling. BC are a group of 6 to 10 follicular which migrate in between the nurse cells to reach the oocyte and form a structure required for fertilization, the micropyle. BC are recruited from the epithelium by a pair of specialised cells, the polar cells (PC), which secrete the ligand Unpaired (Upd), activating the JAK/STAT cascade in neighbouring cells. It is essential that the right number of BC be specified since an excess or a deficit of recruited cells prevent migration. Here, we found that PC have a highly stable and polarised network of microtubules required for efficient activation of the JAK/STAT pathway. We show that upd mRNA transcript is asymmetrically localised along this microtubules network in a Dynein-dependent manner. We demonstrate that in absence of upd mRNA localisation, the ligand is no longer efficiently secreted leading to recruitment and migration defects. Hence, we propose a model whereby upd mRNA apical localisation is necessary to concentrate the ligand prior to BC specification. These findings reveal that JAK/STAT signalling is controlled by a novel post-transcriptional mechanism involving polarized accumulation of ligand mRNA, important for Epithelial-Mesenchymal transition and cell migration.

**A-12 Crumbs (Crb) maintains segmental patterning during Drosophila germ band retraction.**

**Vincent, S**; Perrimon, N; Axelrod, J
Ecole Normale Supérieure de Lyon - Harvard Medical School Stanford School of Medicine

Differential cell adhesion sorts distinct cell types within organs and segments in order to generate compartments. An unknown mechanism prevents cells sharing the same identity but residing in different organs or segments from mixing if they come into close contact. Here we describe an aberrant tissue fusion phenotype during the germ band retraction in crb mutants in the Drosophila embryo: the dorsal regions of different abdominal segments fuse, indicating that Crb suppresses unwanted cellular interactions amongst cells of the same identity present in successive segments. Interestingly, we show that Crb mediates homophilic inter-cellular interactions through its extracellular domain. We further observe that the extracellular domain is required for Crb to stabilize the adherent junctions. In crb mutant, we detect an increase in actin protrusions which actively recruit cells of the same type from neighboring segments, leading to aberrant fusion. We propose a model where Crb homophilic interaction maintains adherens junctions while suppressing actin protrusions so that cell sorting is limited to a given organ. These results provide insights to understand the cellular mechanism at play during compartment formation and tissue regeneration.

**B-01 WingX – Towards Systems Biology of Wing Development**

**E. Hafen** and the WingX Consortium
Institute of Molecular Systems Biology, ETH Zürich, University of Zürich, University of Basel, University of Lausanne and EPFL.

The wing of Drosophila is a model organ well suited for a systems biology approach. This simple two-dimensional structure is undoubtedly one of the best-studied organ systems. Decades of developmental, cell biological, and above all genetic research have provided a detailed understanding of the genetic networks that govern growth and patterning of this structure and thus provide a solid foundation for a systems approach. The detailed biological knowledge of wing development also reveals the limits of the genetic and cell biological approaches, which focus on single genes or regulatory pathways. Although these analyses have provided the basic toolkit for wing development they have failed to provide insight into how a wing of a specific size and shape is formed. The transition to a systems level understanding of wing development requires new approaches involving the expertise and technical developments that come from engineering, physics, and computer science. The goal of this project is to generate quantitative datasets that serve as input for multi-scale modeling approaches of wing development and morphogenesis. These models will make experimentally testable predictions on the biophysical properties and molecular regulatory circuits that control the basic size and shape of the wing. The WingX project is sponsored by SystemsX.ch, the Swiss Initiative in Systems Biology. The following six interdisciplinary Research and Technology Programs have been established: (1) Micro-culture systems for ex vivo disc culture, to facilitate imaging and perturbation of wing imaginal discs. (2) Imaging platforms to obtain 5-dimensional datasets (with spatial, temporal, and genomic resolution). (3) Multiscale Modeling using multiscale modeling and reverse engineering algorithms to develop predictive models of wing growth and morphogenesis. (4) A Proteomics program to obtain quantitative data sets on the temporal changes in the wing proteome, glyco-proteome and phosphoproteome. (5) An Epigenomics platform to determine the pattern of epigenetic changes during wing development. (6) A Data Management platform. (7) A Teaching Program that indroduces physics and engineering concepts to cell and developmental biological problems at the MSc and PhD level. A first year progress report will be presented.

**B-02 The WW domain protein Kibra acts upstream of Hippo.**

**Poernbacher, I.**; Baumgartner, R.; Buser, N.; Hafen, E.; Stocker, H.
ETH Zuerich, Institute of Molecular Systems Biology (IMSB), Hafen group.

This work was supported by a DOCfFORTE grant of the Austrian Academy of Sciences (ÖAW) (to P.I.) and by a grant of the Swiss The conserved Hippo kinase pathway plays a pivotal role in organ size control and tumour suppression by restricting proliferation and promoting apoptosis. Whereas the function of the core kinase cascade, consisting of the serine/threonine kinases Hippo and Warts, in phosphorylating and thereby inactivating the transcriptional coactivator Yorkie is well established, much less is known about the upstream events that regulate Hippo signalling activity. The FERM domain proteins Expanded and Merlin appear to represent two different signalling branches that feed into the Hippo pathway. Signalling initiated by the atypical cadherin Fat has been suggested to act via Expanded but how Merlin is regulated has remained elusive. Here, we provide genetic evidence that the WW domain protein Kibra is a Hippo signalling component. Loss of Kibra results in a subtle overgrowth phenotype, whereas Kibra overexpression induces apoptosis. Genetic epistasis analyses indicate that all these effects are mediated via Yorkie and that Kibra acts upstream of Hippo and Merlin. Kibra acts synergistically with Expanded, and it physically interacts with Merlin. Thus, Kibra predominantly acts in the Merlin branch upstream of the core kinase cascade to regulate Hippo signalling.

**B-03 Kibra is a regulator of the Salvador/Warts/Hippo signalling network.**

**Alice Genevet**[1], Michael C. Wehr[1], Ruth Brain[2], Barry J. Thompson[2] and Nicolas Tapon[1]
(1) Apoptosis and Proliferation Control Laboratory, Cancer Research UK, London Research Institute, 44 Lincoln's Inn Fields, London, WC2A 3PX, United Kingdom (2) Epithelial Biology Laboratory, Cancer

The Salvador (Sav)/ Warts (Wts)/ Hippo (Hpo) (SWH) network is a major conserved regulator of tissue growth that inhibits cell proliferation and promotes apoptosis. The core of the pathway consists of a kinase cascade leading to the phosphorylation and inactivation of the Yorkie (Yki) transcription co-activator. Merlin (Mer) and Expanded (Ex) are thought to be regulators of the pathway though how they promote Hpo/Wts activation remains unclear. In an in vivo RNAi screen for growth regulators, we have identified the WW-domain-containing protein Kibra (Kib), the Drosophila orthologue of mammalian KIBRA, as a new member of the SWH network. Kib physically interacts with Mer and Ex and the Kib/Mer interaction is conserved in human cells. Loss of kib induces a hpo-like phenotype and kib genetically interacts with hpo. Like Mer and Ex, apically localised Kib is transcriptionally regulated by Yki. We propose that Kib is part of an apical scaffold that promotes SWH pathway activity.

**B-04 Actin Capping Protein restricts cell proliferation through the Hippo signalling pathway.**

García Fernández B.; Brás-Pereira C.; Jezowska B.; Gaspar P.; Rebelo S.R.; Zhang T.; Pignoni F. and **Janody F.**
Instituto Gulbenkian de Ciência, Rua da Quinta Grande 6, P-2780-156 Oeiras, Portugal - Department of Ophthalmology, Harvard Medical School and the Massachusetts Eye and Ear Infirmary, 243 Charles Street

The conserved Hippo tumor suppressor pathway is a key signalling cascade that controls growth. Here we report that the actin-Capping Protein ?? heterodimer, which prevents the addition or loss of monomeric actin to the actin filament barbed end, restricts cell proliferation and excessive growth by promoting activation of the Hippo signalling pathway. Genetic interactions suggest that Capping Protein acts at the level of the two cytoskeletal proteins Expanded/Merlin, which both lie upstream of the pathway. Interestingly, Expanded, like Capping Protein, restricts actin filament polymerization near the apical membrane. Since accumulation of actin filaments per se does not induce upregulation of Yorkie target genes, our data support a model whereby Expanded/Merlin and Capping Protein stabilize a specialized population of actin filaments that stimulates the interaction between Hippo and Warts, highlighting the crucial role of a tight regulation of the actin cytoskeleton to prevent loss of proliferation control.

**B-05 The steroid hormone Ecdysone inhibits systemic growth by repressing dMyc activity in Drosophila fat cells.**

**Delanoue, R**; Salidina, M; Leopold, P;
IBDC, University of Nice-Sophia Antipolis, UMR 6543 CNRS , Parc Valrose, 06108 Nice Cedex 2, France.

How steroid hormones shape animal growth remains poorly understood. In Drosophila, the main steroid hormone, ecdysone, restricts systemic growth during juvenile development. Here, we show that ecdysone controls animal growth rate systemically by specifically acting on the fat body, an organ that retains endocrine and storage functions of the vertebrate liver. We demonstrate that fat body-targeted loss of function for the Ecdysone receptor (EcR) increases dMyc expression and its cellular functions such as ribosome biogenesis. Moreover, changing dMyc levels in this tissue is sufficient to affect animal growth rate. Finally, the growth reduction induced by silencing dmyc in the fat body is epistatic over the growth increase induced by fat body-specific inhibition of EcR. In conclusion, we propose a model whereby rising ecdysone levels at the end of juvenile life systemically impede on larval tissue growth though the inhibition of dMyc functions in the fat body.

**B-06 microRNAs and growth control.**

Héctor Herranz, Xin Hong, Lidia Pérez, Ana Ferreira, Daniel Olivieri, Stephen M. Cohen and **Marco Milán**
ICREA and Institute for Research in Biomedicine (IRB Barcelona) Baldiri Reixac, 10-12, 08028 Barcelona, Spain

Regulation of gene expression at the transcriptional level plays a central role in development and physiology, but the importance of post-transcriptional gene regulation is increasingly recognized. miRNAs, endogenous small noncoding RNAs, 22 nucleotides long, that repress target transcripts, confer a novel layer of post-transcriptional regulation. Dcr-1 is a critical element for miRNAs biogeneis. Since a considerable proportion of mutants lacking single miRNAs show no or relatively mild defects, impairing Dcr-1 activity has been frequently used to analyze the net input of the miRNA pathway in a given biological process. Loss of dcr-1 shows defects in Drosophila and vertebrate stem cell maintenance and causes a delay in G1-S transition in these cells. In the developing mouse limb, loss of dcr-1 leads to growth defects. The main effectors mediating the activity of Dcr-1 in these processes are not identified. We have used the wing imaginal disc of Drosophila to analyze at a cellular level the role of miRNAs in a highly proliferative epithelium and identify such effectors.

**B-07 Limitation of neural stem cell symmetric and asymmetric division number by ecdysone in the Drosophila optic lobe.**

**Maurange, C**; Gould, A
Institute of Developmental Biology of Marseille Luminy, CNRS, Marseille, France MRC National Institute for Medical Research, London, United Kingdom

Mammalian neural stem cells (NSCs) can divide symmetrically or asymmetrically. The relative proportions of each division mode are thought to determine the overall patterns of growth in the developing brain. In the Drosophila optic lobe, both asymmetric and symmetric divisions have been described but the timing mechanism regulating the switch between these two modes is unclear. Here we show that inhibiting ecdysone signalling in the developing optic lobe leads to persistent overproliferation in the adult. Ecdysone signalling contributes to shutting down cell proliferation in two distinct ways. First it promotes the conversion of symmetrically-dividing neuroepithelial cells into asymmetrically dividing optic-lobe neuroblasts. And second, the pupal pulse of ecdysone induces timely apoptosis of optic-lobe neuroblasts. We propose a model in which ecdysone specifies the growth pattern and final size of the optic lobe by temporally regulating the balance between symmetric and asymmetric divisions.

**C-01 Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in Drosophila**

Nicolas Buchon, Nichole A. Broderick, Sveta Chakrabarti1 & **Bruno Lemaitre**
Global Health Institute, EPFL, CH-1015 Lausanne, Switzerland E-mail: bruno.lemaitre@epfl.ch Phone:+41 21 693 18 31 Fax: +41 21 693 17 90

Gut homeostasis is controlled by both immune and developmental mechanisms and its disruption can lead to inflammatory disorders or cancerous lesions of the intestine. While the impact of bacteria on the mucosal immune system begins to be precisely understood, little is known about the effects of bacteria on gut epithelium renewal. Here, we addressed how both infectious and indigenous bacteria modulate stem cell activity in Drosophila. We show that the increased epithelium renewal observed upon bacterial infection is largely a consequence of the oxidative burst, a major defense of the Drosophila gut. Additionally, we provide evidence that the JAK-STAT and JNK pathways are both required for bacteria-induced stem cell proliferation. Similarly, we demonstrate that indigenous gut microbiota activate the same, albeit reduced, program at basal levels. Altered control of gut microbiota in immune deficient or aged flies correlates with increased epithelium renewal. Finally, we show that epithelium renewal is an essential component of Drosophila defense against oral bacterial infection. Altogether these results indicate that gut homeostasis is achieved by a complex inter-regulation of the immune response, gut microbiota, and stem cell activity.

**C-02 The role of RacGTPase in Drosophila innate immunity.**

**Boyer, L.**[1]; Dejardin, S.[1]; Magoc, L.[1]; Hinault, C.[2]; Ip, WK 1; Charriere, M[1]; Fracchia, S[1]; Hennessy, E[1]; Erturk-Hasdemir, D[3]; Reichhart, JM[4]; Silverman, N[3]; Stuart, L[1]
[1]Laboratory of Developmental Immunology, Department of Pediatrics, Massachusetts General Hospital, Harvard Medical School, Boston, MA , USA - [2]Joslin Diabetes Center and Department of Medicine, Harvard

We have recently combined a proteomic analysis with a RNAi screen to identify and validate the function of over 600 proteins isolated in association with the Drosophila phagosome. Among them we have identified Rac family members and downstream effectors of Rac signaling. The RacGTPase has been identified as a central element of the host defense against pathogens in mammals. Rac regulates the actin-cytoskeleton organization and other signaling pathways through interactions with specific effectors. Although the role of Rac in Drosophila development has been extensively studied, the in vivo role of Rac and its effectors in Drosophila innate immunity remains poorly defined. Here we show a central role of Rac in bacterial virulence sensing.

**C-03 Intestinal Homeostasis and Serratia Marcescens oral infection: regeneration of the intestinal midgut.**

Nehme, N; Limmer, S; Liégeois, S; Bou Aoun, R; Ayyaz, A; Giammarinaro, P; Cronin, S*; Pospisilik, A*; Schramek, D*; Penninger, J* ; **Ferrandon, D**
UPR 9022 du CNRS, IBMC, 15, Rue René Descartes, 67084 Strasbourg Cedex, FRANCE * IMBA - Dr. Bohr Gasse 3, 1030 Vienna, AUSTRIA

Drosophila melanogaster is a powerful model to study the innate immune system on the level of a whole organism. We accomplished the first genome-wide RNAi screen in vivo to find genes that confer either susceptibility or resistance to oral infection with the highly virulent natural Drosophila pathogen Serratia marcescens. The tissues implicated in the host defense against an oral infection are the gut epithelium, which plays a key role as a border between the internal milieu and the microbe-rich gut lumen, and the macrophage-like hemocytes, that phagocytose bacteria that have crossed the intestinal barrier. Therefore, having used an inducible ubiquitous driver for the primary screen, we used a gut specific and a hemocyte specific inducible driver to express the RNAi-hairpins in secondary screens. By monitoring the survival of the RNAi expressing flies we were able to identify several hundred genes involved in the response to intestinal infections. We found that oral infection with Serratia triggers Jak/Stat activation in the gut epithelium, primarily in intestinal stem cells (ISCs). The Jak/Stat pathway drives ISC proliferation, likely to compensate the extensive cell death caused in the midgut during infection. This whole-genome in vivo RNAi screen intestinal revealed that host defense against intestinal infections is not limited to classical innate immunity pathways but involves processes such as intestinal epithelium homeostasis (Cronin, Nehme et al. Science, 2009). S. marcescens intestinal infection proceeds through several stages, including an extensive early degradation phase that is followed by a rapid regeneration of the midgut epithelium within a few hours. We conclude that host defense against intestinal infections in Drosophila is a highly dynamic process.

**C-04 Uptake of the Necrotic Serpin in Garland and pericardial athrocytes via Lipophorin Receptor-1.**

Sandra Fausia Soukup[1], Joaquim Culi[2] and **David Gubb**[1]
[1]CIC bioGUNE, Technology Park, Building 801-A, 48160 DERIO, Spain - [2]Centro Andaluz de Biología del Desarrollo (CSIC-UPO), Universidad Pablo de Olavide, Ctra. De Utrera Km1, 41013 Sevilla, Spain

In mammals, serpin inhibitors regulate a wide-range of proteolytic cascades that activate rapid physiological responses, such as blood coagulation, inflammation, the complement pathway and angiogenesis. They interact with their target proteinases by a “suicide inhibition” mechanism, which generates an inert, denatured, serpin/proteinase complex. In mammals, humoral serpins are secreted from the liver into the blood plasma. The denatured complex is later endocytosed back into the liver and degraded. In Drosophila, the Necrotic serpin is secreted from the fat-body into the haemolymph, where it controls the humoral immune response. In flies, however, Necrotic is not endocytosed in the fat-body, but in the garland and pericardial athrocytes. The Necrotic-binding receptor for this process is LpR1, a member of the LDLR family. The endocytosed serpin is targeted for lysosomal degradation. We have preliminary evidence that LpR1 binds the denatured Nec/proteinase complex, while the LpR2 receptor may bind the active Nec inhibitor. More importantly, we show that mutations in LpR1 cause a profound effect on the immune response. Thus, our results indicate that the scavenging of serpin/proteinase complexes might be a critical step in the regulation of proteolytic cascades.

**C-05 Wolbachia induces protection to RNA viruses in Drosophila melanogaster.**

Teixeira, L [1,2;] Ferreira, A [2]; Ashburner, M [2]
[1]Instituto Gulbenkian de Ciência, Oeiras, Portugal - [2]Department of Genetics, University of Cambridge, Cambridge, UK

Wolbachia are one of the most widespread intracellular bacteria known. They are maternally transmitted obligatory endosymbionts. In many cases their presence is associated with profound changes on their hosts' reproductive biology. These effects are thought to increase the relative fitness of infected females, hence increasing the vertical transmission of the bacteria. Natural populations of Drosophila melanogaster are frequently infected with Wolbachia. However, there was no known strong phenotype associated with this infection. We have found that maternally transmitted intracellular bacteria confer, to D. melanogaster, resistance to Drosophila C virus (DCV). This resistance is associate with a 10.000 fold reduction in viral titres. We identified Wolbachia as the causative agent of this resistance. DCV is an RNA virus that naturally infects D. melanogaster. We have tested the specificity of Wolbachia induced protection by testing the protection against two other RNA viruses (Nora virus and Flock House virus (FHV)) and a DNA virus (Insect Iridescent virus 6 (IIV6)). Wolbachia infected flies also have lower viral titres upon Nora virus infection. Strangely, although Wolbachia infected flies also survive much better to FHV, viral titres are not significantly affected. On the other hand, we have not detected an interaction between Wolbachia and IIV6. These results indicate that Wolbachia induced resistance is confined to RNA viruses. Together with Hedges et al. 2008, this is the first reported case of a bacterial infection conferring protection against a viral infection. This new type of Wolbachia-host interaction is the first strong phenotype associate with Wolbachia infection in D. melanogaster and may explain its prevalence in natural populations of this host.

**C-06 Genetic control of calcium homeostasis during phagocytosis.**

Liu, J.W.; Xiao, H.; **Franc, N.C.**
The Scripps Research Institute. 10550 North Torrey Pines Road. The Department of Genetics. Mailstop SP-273. La Jolla, CA92037. USA

Phagocytosis is important during development and in the immune response as it leads to the removal of apoptotic cells and pathogens. Yet, its molecular mechanisms are poorly understood. In C. elegans, the CED-2, -5, -10, -12 pathway regulates actin during phagocytosis of apoptotic cells, whereas the role of the CED-1, -6, -7 pathway in phagocytosis is unclear. We report that Undertaker (UTA), a Drosophila Junctophilin-related protein that contains MORN repeats, is required for Draper (CED-1 homologue)-mediated phagocytosis. Junctophilins couple $Ca^{2+}$ channels at the plasma membrane to those of the endoplasmic reticulum (ER), the Ryanodine receptors. We place Draper, its adaptor Dmel\Ced-6, UTA, the Ryanodine receptor Rya-r44F, the ER Ca2+ sensor dSTIM, and the Ca2 +-release-activated $Ca^{2+}$ channel dOrai in the same pathway that promotes $Ca^{2+}$ homeostasis and phagocytosis. Thus, our results implicate a Junctophilin-related molecule in phagocytosis and link Draper-mediated phagocytosis to Ca2+ homeostasis, highlighting a previously uncharacterized role for the CED-1, -6, -7 pathway (Cuttell et al. Cell. 2008, 135 (3): 524-34). Recent progress made in pursuing the dissection of the molecular mechanisms of $Ca^{2+}$ homeostasis during phagocytosis will be presented.

**D-01 Regulation of Dpp signalling range in the Drosophila ovary**

Robin E Harris and Hilary **L Ashe**
Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester, M13 9PT

Dpp signalling is used repeatedly during Drosophila development to pattern cells within the embryo, ovary, etc. Previously we have shown that type IV collagens are important for Dpp gradient formation in the Drosophila embryo, and have extended our studies to the germarium of the Drosophila ovary where Dpp functions as a short range signal to maintain germline stem cells (GSCs). We demonstrate that type IV collagen mutant germaria have extra GSCs, consistent with an increased Dpp signalling range. We show that type IV collagens can bind Dpp, suggesting a model in which collagen IV functions to restrict Dpp diffusion in the germarium. We are also exploring additional mechanisms which negatively regulate Dpp signalling in differentiating cells of the germarium, including post-transcriptional regulation of specific mRNAs. I will present evidence that redundant mechanisms are deployed to restrict Dpp signalling range in the germarium, consistent with the essential requirement to downregulate Dpp signalling, ensuring egg development and propagation of the fly.

**D-02 A role of receptor Notch in ligand cis-inhibition in Drosophila.**

**Becam, I**; Fiuza, M; Martinez Arias, A; and Milan, M
ICREA and Institute for Research in Biomedicine (IRB Barcelona) Parc Científic de Barcelona, C/ Baldiri i Reixac 10 -12, 08028 Barcelona, Spain Department of Genetics, University of Cambridge, Ca

Notch and its ligands mediate short range cell interactions that play a conserved role in inducing cell fate specification. Several regulatory mechanisms have been described to ensure robust polarized signalling from signalsending to signal-receiving cells. High levels of ligand expression activate Notch in nearby cells and exert a cell autonomous dominant negative effect on Notch activity. This regulatory process is called cis-inhibition and helps to restrict Notch activation to signal-receiving cells. By combining genetic mosaics in the Drosophila wing primordium with cell culture assays, here we present evidence in Drosophila that Notch also exerts an inhibitory effect on the activity of the Serrate ligand expressed in the same cell. This regulatory mechanism is independent of Notch mediated transcription and it is executed via the participation of the extracellular domain of Notch in promoting Serrate endocytosis. We show that this process is required to block Serrate mediated activation of Notch in the signal-sending cell population and helps to restrict Notch activation to the signal-receiving cells. Altogether, our results, in concert with the previous results on ligand-mediated Notch cis-inhibition, indicate that mutual inhibition between ligand and receptor in signal-sending cells helps to block Notch activity in these cells and to restrict receptor activation in signal-receiving cells.

**D-03 Novel Conserved Regulatory Elements Control the Tissue-Specificity of the Ras/MAPK Competence Factor pointed.**

**Aamna K**. Kaul, Gavin M. Craig, Helen McNeill and Joseph M. Bateman Wolfson Centre for Age-Related Diseases, King's College London, Guy's Campus, London SE1 1UL , UK

Developmental control in metazoans is orchestrated by a small number of signaling pathways that are used reiteratively. The Ras/MAPK pathway channels signals from several receptor tyrosine kinases to control a variety of developmental processes. pointed (pnt) encodes the primary Ras/MAPK pathway transcriptional activator in Drosophila. We have identified a novel enhancer element for pnt, contained within intronic sequence, which controls its expression in the eye, fat body (FB) cells, oenocytes, glial cells and trachea. This pnt enhancer responds at the transcriptional level to Ras/MAPK signaling. The pnt gene encodes two isoforms, PntP1 and PntP2 and we show that this enhancer controls the expression of pntP2 in all cells types except glia, where it controls the expression of pntP1. We also use comparative genomic analysis to identify several short conserved regulatory elements that control pnt expression in multiple cell types. Regulatory elements controlling pnt expression in FB cells, oenocytes and glia are tightly clustered in overlapping regions, whereas those controlling eye and tracheal expression are more dispersed. Finally we show that the pnt enhancer is regulated by Notch signaling during eye development. These regulatory elements provide a framework for understanding the spatiotemporal regulation of Ras/MAPK signaling in Drosophila.

**D-04 Fine tuning of the BMP/Dpp morphogen activity gradient by a novel feed-back loop.**

**Vuilleumier, R**[1]; Springhorn, A[1]; Patterson, L[2]; Koidl, S[1]; Hammerschmidt, M[2,4]; Affolter, M[3]; Pyrowolakis, G[1]
[1]Institute for Biology I, University of Freiburg, Hauptstr. 1, D-79104 Freiburg, Germany - [2]Max-Planck-Institute of Immunobiology, Stübeweg 51, D-79108 Freiburg, Germany - [3]Biozentrum der Universit

Signaling by Decapentaplegic (Dpp), a member of the BMP family of ligands and the fruit fly orthologue of vertebrate BMP2/4, is crucial in many developmental processes. In the wing imaginal disc, the precursor of the wing of the fly, Dpp forms a long-range gradient and acts as a morphogen to control both growth and patterning of the organ. Our studies on the nuclear interpretation of Dpp morphogen signaling culminated in the identification of small DNA response elements that mediate transcriptional regulation of Dpp target genes. Here we report on the use of such motifs in genome-wide in silico screens for the identification of targets of Dpp morphogen signaling. We describe pent, a previously uncharacterized gene, that is a direct target of transcriptional repression by Dpp. This regulation restricts pent expression to lateral-most regions of the wing disc where Dpp signaling activity is the lowest. Pent mutant wing discs display a severe shrinkage of the Dpp activity gradient resulting in patterning and growth defects of the adult wing. We show that pent encodes for a secreted multi-domain protein that acts over a long distance. Furthermore we find that Pent interacts with members of the heparan sulfate proteoglycan family and regulates their activity to promote long-range distribution of Dpp. Our studies identify pent as an inherent feed-back loop regulator in Dpp morphogen signaling with a crucial role in shaping and fine-tuning the Dpp gradient.

**D-05 Imaging BMP signalling at a stem cell niche synapse.**

Raabe, I; Michel, M; Perez-Palencia; R; von Kannen, S; **Boekel, C**
Center for Regenerative Therapies Dresden (CRTD) Dresden University of Technology Tatzberg 47-49, 01307 Dresden, Germany

Stem cell privileges, especially the ability to self renew, are regulated via the local establishment of permissive signalling microenvironments termed niches that allows potential stem cells to adopt and maintain that fate. The range of the niche signals sets the size of the stem cell pool and therefore needs to be precisely controlled, especially when pathways are involved that are in principle able to signal over large distances. Adhesion between stem cells and niche stromal cells is thought to play a key role for niche function. In the hematopoietic system, the concept of a stem cell niche synapse has been proposed for a presumed signalling centered on the specialized aherens junctions between stem and niche cells. This synapse would precisely target the niche signals to specific stem cells and mediate the spatiotemporal integration of different signalling pathways. We are studying niche-stem cell interactions using the Drosophila germline stem cell (GSC) niche as a genetically tractable model system. In the testis, niche signalling through BMP- type morphogens is tightly confined to those GSCs that directly contact the signal secreting niche cells, although in other tissues the same signals can spread over many cell diameters. We have developed a reporter system with which for the first time BMP receptor activation can be imaged live and at subcellular resolution. We will show that in the GSC niche signalling is indeed limited to synapse like structures at the interface between niche and stem cells, and will present the first results of our experiments aimed at dissecting the mechanistic basis for this spatially confined signalling process.

**D-06 The ISWI-containing NURF complex regulates the output of the canonical Wingless pathway.**

**Song H**; Spichiger-Haeusermann C; Basler K
Institute of Molecular Biology University of Zurich Winterthurerstr. 190 CH-8057 Zurich Switzerland

Wingless (Wg) signalling regulates the expression of its target genes through Pangolin, Armadillo and their interacting cofactors. In a genetic screen for Wg signalling components, we found that ISWI, a chromatin remodelling ATPase, had a positive role in transducing the canonical Wg signal, promoting the expression of the Wg target senseless. ISWI is found in several chromatin-remodelling complexes, including NURF. The effect of interfering with the function of other components of the NURF complex in vivo mimics that of ISWI. The NURF complex is also required for the efficient expression of other Wg target genes. Armadillo interacts directly with the NURF complex in vitro and recruits it to Wg targets in cultured cells. Together, our results suggest that the ISWIcontaining NURF complex functions as a co-activator of Armadillo to promote Wg-mediated transcription.

**D-07 Regulatory inputs and regulatory modules operating in adult muscle precursor cells in Drosophila: a role for EGFR pathway.**

Figeac,N., Jagla,T., Da Ponte, J.-P. and **Jagla, K**.
GReD, INSERM U931, CNRS UMR6247, Clermont University, Faculté de Medecine, 28, place Henri Dunant, 63000 Clermont-Ferrand, France

In spite of their importance, little is known about the behaviour of muscle stem cells and genetic pathways that control their specification. Here we show that in Drosophila muscle stem-like cells, called AMPs send long processes connecting, by the end of embryogenesis, all of them within a parasegment. Interestingly, a 3D view of muscular layer and the network of PNS neurons clearly showed that all AMPs are closely associated with both the differentiated muscle fibers and the PNS axons, sitting either at the top of muscle fibers or on their internal face. In spite of this common behavior and common morphologically features, the AMPs are heterogenous and differ by the expression of muscle identity genes. We also demonstrate that in Drosophila, EGF pathway plays a dual role ensuring specification and maintenance of AMPs. Our finding is supported by the identification of EGF sending “niche??? cells and the EGF-dependent regulatory modules that drive expression in lateral AMPs. Comparison of the AMP regulatory sequences versus those driving expression in a differentiated muscle lineage, i.e. the SBM, showed that the AMP enhancer requires EGF signals for its activity whereas the SBM enhancer acts in an EGFRindependent way. We thus propose that stemness of muscle cells is not an intrinsic property but that it depends on signals emitted from neighboring cells.

**D-08 Activation of apoptosis in response to loss of cell polarity in the embryonic epidermis.**

Golnar Kolahgar*, Pierre-Luc Bardet*, Cyrille Alexandre, and **Jean-Paul Vincent** * Equivalent contribution
MRC National Institute for Medical Research London UK

One homeostatic mechanism of epithelia involves the elimination of defective cells by apoptosis. Although the core machinery of apoptosis is relatively well understood, relatively little is known about how defective cells are recognised. In crumbs mutant embryos, many cells of the epidermis fail to establish apico-basal polarity and undergo apoptosis as a result. Therefore crumbs embryos can be used as a model system to investigate how loss of cell polarity leads to apoptosis. As a first step, we have mapped the spatial and temporal pattern of cell death in the epidermis of crumbs embryos. We found that a strip of 5-10 cells at the dorsal margin survive while, on the ventral and lateral sides, most cells undergo apoptosis, although the initial pattern of caspase activation has a clear segmental aspect. This pattern of apoptosis is anticipated by the segmental expression of reaper. Indeed, genetic evidence shows that, among the three main pro-apoptotic genes, only reaper is required for apoptosis in the trunk epidermis of Drosophila embryos. In order to find out how loss of cell polarity lead to activation of reaper expression, we have obtained genome wide expression data from crumbs mutant and wild type embryos at three key stages, 10, 11, and 12 (apoptosis begins at stage 11 in crumbs mutants). From the results, we can identify the transcriptional signature of one key signalling pathway. We are currently devising functional tests to find out whether this pathway is indeed required for the activation of reaper (and apoptosis) in the ventro-lateral epidermis of crumbs mutants. How loss of cell polarity activates this pathway remains an open question for the future.

**D-09 A component of the wnt signaling pathway in mammals is necessary for ciliated sensory function in Drosophila.**

**Enjolras, C**; Laurencon, A; Thomas, J; Cortier, E; Durand B
CGMC, UMR5534, CNRS Université Lyon1

Cilia and flagella play many biological functions within organisms. In vertebrates, cilia are found on almost every cell and are involved in several key developmental processes. In humans, ciliary dysfunctions are responsible for several diseases called ciliopathies. RFX transcription factors regulate genes involved in cilia assembly from C. elegans to mammals. In Drosophila we have shown that dRfx is required for sensory cilia assembly and we have identified several dRFX target genes by genomic analysis. Surprisingly, Chibby (Cby) was identified in our screen as one of them. Cby is known to interact with beta-catenin and to regulate negatively the wnt/wg pathway both in Drosophila and mammals as demonstrated by RNAi experiments. By functional studies in Drosophila, I observed that Cby is only expressed in ciliated sensory neurons and in sperm cells, and I showed that Cby is indeed down regulated in dRfx-deficient Drosophila. CBY protein is localized close to the basal body at the base of the primary cilia in sensory neurons and close to the centrioles in spermatocytes. Moreover, inactivation of the Cby gene by homologous recombination demonstrated that Cby is required for sensory perception mediated by ciliated neurons of the peripheral nervous system. Cilia and flagella are still present in Cby mutants suggesting that Cby is not required for their assembly per se but may be required for cilia function. Interestingly, Cby inactivated Drosophila are viable and show only light wg associated phenotypes suggesting that Cby modulates the wg signalling pathway in Drosophila, but that it is dispensable for fly development. Thus we demonstrated that Cby has conserved an ancestral function in cilia biology and in the wnt/wg signalling pathway from Drosophila to vertebrates.

**D-10 A novel mode of action for the Notch pathway acts in the selection of the polar cell pair in Drosophila oogenesis.**

Caroline Vachias, Jean-Louis Couderc and **Muriel Grammont**
CNRS 6247, Clermont University, Inserm U931, UMR GReD, UFR Médecine, Clermont-Ferrand F-63001, France.

The three known modes of action by which the Notch pathway promotes cell differentiation are lateral inhibition, lineage decisions and boundary formation. Here we define a novel mode that acts upon a group of equivalent cells to ultimately yield two polar cells, which are key organizers of the Drosophila ovarian follicle. We show that this sequential process begins when one cell becomes refractory to Notch activation and is selected as the initial polar cell. This cell then produces a neuralized-dependent Delta signal that induces a high level of Notch activation in one other cell within the cluster. This Notch activity prevents elimination by apoptosis, allowing its selection as the second polar cell. Because this mode involves an inductive signal that acts upon an equivalence group and results in a particular cell being selected to adopt the same fate as the Dl-sending cell, we choose to term it the inductive selection mode.

**D-11 Vacuolar ATPase activity controls physiologic as well as pathologic Notch signaling activation in Drosophila.**

**Vaccari, T**[1,2] ; Bilder,[1]
[1]Department of Molecular and Cell Biology, University of California, Berkeley, California - [2]Present address: IFOM, Istituto FIRC di Oncologia Molecolare, Milano, Italy.

Recent evidence in Drosophila indicates that endosomal entry enables activation of the Notch receptor. In a effort to identify genes that mediate Notch signaling activation in endosomes, we isolated mutants in vha68-2, vha55 and vhaSFD, 3 genes that encodes the A, B and H subunit of vacuolar ATPase (v-ATPase), respectively. vATPases are proton pumps that energize proton transport across membranes in a variety of tissues. vATPase function is thought to drive progressive acidification of the endocytic compartments enabling cargo trafficking and activation of lysosomal proteases. Consistent with a role of vATPase in proton transport across endomembranes, null v-ATPase mutant cells display impaired acidification of the endosomal compartment in imaginal epithelial tissue. Despite completely impairing v-ATPase activity, v-ATPase mutant cells still display some endocytic uptake activity, and accumulate Notch in the lysosomal compartment. Surprisingly, such accumulation correlates with loss of both physiologic as well as ectopic Notch receptor activation in endosomes. Our data indicate that v-ATPase activity control both activation of Notch signaling in endosomes and degradation of inactive Notch in the lysosome and suggest that acidification is a key regulator of Notch signaling activation in endosomes.

**D-12 The nesthocker mutation reveals requirement of UDP-N-acetylglucosamine for FGF-signaling in Drosophila.**

**Mariyappa, D**; Sauert, K; Turnock, D; Mariño, K; Ferguson, M; Müller H-AJ
Divsion of Cell and Developmental Biology and Division of Biological Chemistry and Drug Discovery, College of Life Sciences, Dow Street, University of Dundee, Dundee, DD1 5EH

Mesoderm migration in the Drosophila gastrula is dependent on FGF signalling. In a genetic screen for maternal effect genes, we identified nesthocker (nst), a mutation with identical mesoderm migration phenotype as that of the FGF-receptor heartless (htl). htl-dependent-MAPK activation at the leading edge of migrating mesodermal cells is absent in nstmat,zyg embryos indicating that Nst is essential for Htl-signalling. Furthermore, genetic interaction between nst and a hypomorphic htl allele is also observed. Genetic epistasis experiments revealed that Nst function is required downstream of the Htl receptor. Additionally, tissue specific rescue experiments also implicate signal receiving-cell specific requirement for nst. We cloned the nst gene and found that it is allelic to CG10627, encoding GlcNAc (N-acetyl phosphoglucosamine) mutase. The nst16923 mutant allele encodes a catalytically inactive enzyme. Furthermore, a genomic construct of CG10627 rescued nstmat,zyg mutant embryos to viability. The level of UDP-N-acetyl hexosamines (UDP-HexNAc), a pool of downstream metabolites of GlcNAc mutase, is reduced to about 10% of wildtype levels in nstmat,zyg embryos. The potential pathways that might be affected by reduced UDP-HexNAc levels are proteoglycan biosynthesis, GPI-anchor formation, N-/O-glycosylation and O-GlcNAcylation. In nst mutants, other heparansulfate proteoglycan-dependant signalling pathways and GPI-anchor formation are not compromised. Global levels of N-/O-glycosylation are also unchanged in nst mutants. Decreased O-GlcNAcylation of several proteins is observed in nst mutants. We are currently investigating how changes in O-GlcNAcylation leads to impaired FGF signaling during Drosophila mesoderm migration.

**E-01 A Fly Approach to Cancer and Diabetes**

Erdem Bangi, Tirtha Das, Justin Graves, Susumu Hirabayashi, Benjamin Levine,Jianbo Na, Sarah Rim, Vivek Rudrapatna, Marcos Vidal, and **Ross Cagan**
Mount Sinai School of Medicine; New York, NY

Drosophila has long been a favorite model for development, and my laboratory continues to work on eye development. More recently, we have begun to bring lessons learned from studies of developing epithelia to studies of diabetes and especially cancer. Utilizing the ability to study the effects of transgenes in situ, we have generated monogenic and multigenic tumor models. Hewing closely to currently human sequencing data, we have generated multigenic models for thyroid, breast, lung, and colorectal tumors. These models have led us to propose a model of cancer metastasis that emphasizes local interactions between epithelial cells within the epithelium. I will discuss how our manipulation of different genetic combinations has shed light on the emergent activities of specific oncogenes and tumor suppressors. In addition to exploring mechanism, we have developed automated systems for screening drugs in whole flies. Our early efforts helped support clinical trials for a compound therapeutic targeting Medullary Thyroid Carcinoma; this compound is currently in Phase III trials. I will discuss our further efforts to identify useful candidate compounds and discuss how these have also informed us about the cancer process.

**E-02 A tumor suppressive activity of Drosophila Polycomb genes mediated by JAK/STAT signaling.**

**Classen AK**[1], Bunker BD[1], Harvey KF[2,3], Vaccari T[4], Bilder D[1]
[1]Department of Molecular and Cellular Biology, University of California, Berkeley, CA 94720, USA [2]Peter MacCallum Cancer Centre, 7 St Andrews Place, East Melbourne, Victoria, Australia, 3002 [3] Dep

A prevailing paradigm posits that Polycomb Group (PcG) proteins maintain stem cell identity by repressing differentiation genes, and abundant evidence points to an oncogenic role for PcG in human cancer. Here we demonstrate using Drosophila that a conventional PcG complex can also have a potent tumor suppressive activity. Mutations in all core PRC1 components cause dramatic hyperproliferation of eye imaginal tissue, accompanied by deregulation of epithelial architecture. The mitogenic JAK/STAT pathway is strongly and specifically activated in mutant tissue; activation is driven by transcriptional upregulation of Unpaired (Upd) family ligands. We show that upd genes are direct targets of PcG-mediated repression in imaginal discs. Ectopic JAK/STAT activity is sufficient to induce overproliferation, while reduction of JAK/STAT activity suppresses the PRC1 mutant tumor phenotype. These findings show that PcG proteins can restrict growth directly by silencing mitogenic signaling pathways, shedding light onto an epigenetic mechanism underlying tumor suppression.

**E-03 The discs overgrown (dco) mutant - a fly model for the role of Casein Kinase Iε in breast cancer.**

**Dolezal, T**; Matulova, J; Kucerova, K; Kubala, E; Bryant, PJ
Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic; Developmental Biology Center, University of California, Irvine, USA

Mutants with altered tissue growth in Drosophila can serve as useful models to study growth regulatory pathways and cancer. One of these mutants, discs overgrown (dco), was characterized in the Bryant laboratory and used along with other Drosophila mutants to identify candidate genes that might be involved in breast cancer. In these studies the human homolog of dco, which encodes the enzyme Casein Kinase I? (CKI?), showed a high frequency of somatic mutations in breast cancer, with some mutations appearing repeatedly and often in association with loss of heterozygosity in the same genetic interval. Since cancerous tissues often contain many mutations it is important to determine which of them have functional consequences related to tissue growth. We have therefore used a reverse-genetics approach to recreate some of the human CKI? mutations in the fly homolog. The results show that the fly equivalent of the commonest reported mutation in breast cancer (L39Q) causes a striking overgrowth phenotype in imaginal discs. The mutant phenotype closely resembles that caused by mutations affecting the Fat/Warts/Hippo signaling pathway, and genetic interaction between dco[L39Q] and the warts mutant further supports the idea that L39Q affects this pathway. Although mutations affecting these genes were characterized more than twenty years ago, it is only recently that the gene products were all linked into a common pathway using both molecular and genetic criteria. Recent work on mouse and human cells suggests that this pathway might be one of the most important targets for mutations leading to breast cancer, so our ability to manipulate it in Drosophila shows how the use of model systems can come full circle in the exploration of the genetic causes of human disease.

**E-04 dMyc oncoprotein levels control lethal giant larvae neoplastic growth in Drosophila epithelia.**

**Francesca Froldi**, Marcello Ziosi, Annalisa Pession and Daniela Grifoni
Dipartimento di Patologia Sperimentale, Alma Mater Studiorum, Bologna, Italy

Neoplastic growth requires the cooperation of several oncogenic mutations leading to major rearrangements in cellular behaviour. Premalignant cells are often removed from normal tissues, but the mechanisms orchestrating such a safeguard process remain in part elusive. In Drosophila, cells mutant for the neoplastic tumour suppressors lethal giant larvae (lgl), scribble (scrib) and discs large (dlg) undergo non-autonomous apoptosis when embedded in a wt background. scrib and dlg mutant clones are eliminated from the imaginal tissues through an Eiger-induced endosomal activation of the JNK pathway, a phenomenon named “intrinsic tumour suppression???. We found that, for lgl mutant clones, this latter does not represent the only mechanism of exclusion. In the wing pouch region of the imaginal wing disc, where cell competition acts in eliminating cells with impaired growth, intrinsic tumour suppression inhibition is not able to rescue lgl growth. lgl mutant cells express very low levels of dMyc protein and when juxtapposed to high dMyc-expressing neighbours, as it is in the wing pouch, they are strongly outcompeted, implicating a role for dMyc-induced cell competition in restraining lgl clonal expansion in this territory. In addition, we show how in different backgrounds and tissues lgl clonal behaviour is always correlated to dMyc protein abundance: an increase is invariantly observed when clones are able to overgrow whereas lower levels are associated with poor viability. Our results provide first evidence that, in addition to intrinsic tumour suppression, cell competition is involved in protecting tissues from tumourous growth and identify a specific function for dMyc oncoprotein in cooperating with lethal giant larvae tumour suppressor to promote malignant overgrowth.

**E-05 Role of Brat/TRIM-NHL genes in brain tumor suppression.**

Jean-Louis Boulay[1], Urs Stiefel[2], Elisabeth Taylor[1], Béatrice Dolder[1], Adrian Merlo[1], and **Frank Hirth**[3]

[1]Department of Biomedicine, University Hospital, CH-4031 Basel, Switzerland; [2]Institute of Zoology, University of Basel, CH-4056 Basel, Switzerland; [3]MRC Centre for Neurodegeneration Research, King‘s College London, London SE5 8AF, United Kingdom

Malignant gliomas are frequent primary brain tumors associated with poor prognosis and very limited response to conventional chemo- and radio-therapies. Besides sharing common growth features with other types of solid tumors, gliomas are highly invasive into adjacent brain tissue, which renders them particularly aggressive and their surgical resection inefficient. Therefore, insights into glioma formation are of fundamental interest of fundamental interest in order to provide novel molecular targets for diagnostic purposes and potential anti-cancer drugs. Human Tripartite motif protein 3 (TRIM3) encodes a structural homolog of Drosophila brain tumor (brat) implicated in progenitor cell proliferation control and cancer stem cell suppression. TRIM3 is located within the loss of allelic heterozygosity (LOH) hotspot of chromosome segment 11p15.5, indicating a potential role in tumor suppression. We analyzed 70 primary human gliomas of all types and grades and carried out somatic deletion mapping as well as single nucleotide polymorphism analysis together with quantitative real-time PCR of chromosome segment 11p15.5. Our analysis identified LOH in 17 cases (24%) of primary human glioma which defines a common 130 kb-wide interval within the TRIM3 locus as a minimal area of loss. We further detected altered genomic dosage of TRIM3 in two glioma cases with LOH at 11p15.5, indicating homozygous deletions of TRIM3. Our results identify TRIM3 as a candidate brain tumor suppressor gene, and suggest that Brat/TRIM-NHL genes are involved in the regulation of progenitor cell proliferation control and cancer stem cell suppression.

**E-06 Phenotypic Recovery of Alzheimer's Disease Model by Quinone-Tryptophan Small Molecules.**

Scherzer-Attali, R[1], Frydman-Marom, A[1], Levy-Sakin, M[1], Peled, S[1], Farfara, D[2], Benramano, T[2], Frenkel, D[2], Gazit, E[1] and Segal, D[1]

[1]Dept. Molecular Microbiology & Biotechnology, Tel-Aviv University, Tel-Aviv 69978, Israel. [2]Dept. of Neurobiology, Tel-Aviv University, Tel-Aviv 69978, Israel

The Amyloid beta (A?) hypothesis proposes that increased levels of the A? protein and its consequent assembly and aggregation from soluble to oligomers and into fibrillar amyloid plaques in the brain are the primary events driving Alzheimer's disease (AD). The early soluble assemblies of A?, may be more pathogenic than the fibrillar structures. We and others have identified a key role of aromatic residues in the molecular recognition and self-assembly leading to the formation of various amyloid assemblies. Aromatic interactions are believed to provide selectivity as well as stability to the interacting molecules. Our strategy is to use small aromatic molecules that would bind the aromatic residues of the A? monomers thereby inhibit the early steps of the molecular recognition and structural transition of the monomers which lead to the formation of the toxic amyloid species. Quinones have been known as inhibitors of various metabolic pathways in the cell, serve as antibacterial, anti-viral, and anti-cancer agents and furthermore were shown to inhibitor several amyloidogenic peptides. We have synthesized a series of N-linked Tryptophanmodified quinones and screened them for anti-A? activity. Two compounds, NQTrp and ClNQTrp, were most effective. They inhibit A? oligomerization and fibrillization in vitro and reduce the cytotoxic effect of A? oligomers towards cultured cells. When fed to Drosophila expressing A?1-42 in their nervous system, these compounds alleviated their Alzheimer's-related symptoms which include defective locomotion and reduced life span, while having no effect on control flies. Injection of Cl-NQTrp to 5xFAD transgenic AD mice, resulted in significant improvement of cognitive behavior and reduction in the specific 56* toxic A? oligomer.

**E-07 A Drosophila model identifies calpains as modulators of the human leukemogenic fusion protein AML1ETO.**

Osman D (a), Gobert V (a), Ponthan F (b), Heidenreich O (b), Haenlin M (a), Waltzer L (a)
a) Université de Toulouse, UPS, CBD (Centre de Biologie du Développement), Bat4R3, 118 route de Narbonne, 31062 Toulouse, France CNRS, CBD UMR5547, 31062 Toulouse, France
b) University of Newcastle

The t(8:21)(q22;q22) translocation is one of the most common chromosomal abnormalities linked to acute myeloid leukemia (AML). AML1-ETO, the product of this translocation, fuses the N-terminal portion of the RUNX transcription factor AML1 (also known as RUNX1), including its DNA binding domain, to the almost entire transcriptional corepressor ETO (also known as MTG8 or RUNX1T1). This fusion protein acts primarily by interfering with endogenous AML1 function during myeloid differentiation, although relatively few genes are known that participate with AML1-ETO during leukemia progression. In order to develop a genetically tractable model for AML1-ETO function, we assessed the consequences of expressing this chimeric protein in Drosophila blood cells. Reminiscent of what is observed in AML, AML1-ETO specifically inhibited the differentiation of the hematopoietic lineage whose development depends on the RUNX factor Lozenge (LZ) and induced increased numbers of LZ+ progenitors. In addition, by performing a large scale RNA-interference screen for suppressors of AML1-ETO in vivo, we found that calpainB is required for AML1-ETO-induced blood cell disorders in Drosophila. Remarkably, calpain inhibition triggered AML1-ETO degradation and impaired the clonogenic potential of the human t(8;21) leukemic blood cell line Kasumi-1, suggesting that calpains play a key role together with AML1-ETO to induce leukemic cell growth. All together, these data indicate that Drosophila provides a powerful model to explore the function of AML1-ETO and to discover new genes that participate in AML development

**F-01 Odour detection by Ionotropic Receptors**

**Benton, R**
Center for Integrative Genomics, University of Lausanne, CH-1015, Lausanne, Switzerland

We recently discovered a novel family of chemosensory receptors in Drosophila melanogaster, named the Ionotropic Receptors (IRs). IRs are structurally related to ionotropic glutamate receptors - a conserved class of ligand-gated ion channel best studied for their roles in synaptic transmission - but have highly divergent ligand binding domains. IR genes are expressed in specific combinatorials in neurons in the antenna that are distinct from those that express Odorant Receptors. IRs localise to the ciliated endings of olfactory sensory dendrites and expression of an IR in an ectopic neuron is sufficient to confer novel odour responses, providing evidence for a direct role in odour recognition. The IRs are therefore likely to define a largely unexplored 'second' olfactory system in Drosophila and other insects. I will present my group's recent progress in understanding the structural, functional and evolutionary properties of the IRs and their neural circuits in mediating odour detection.

**F-02 Male sex peptide is responsible for the loss of siesta sleep in post-mated female Drosophila and an increase in locomotor activity for optimal foraging and egg-laying.**

*__Isaac, R.E.__; *Li, C.; *Leedale,A.E.; #Shirras, A.D.
*Institute of Integrative and Comparative Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK; #Division of Biomedical and Life Sciences, School of Health and Medicine, Lan

Quiescence or a sleep-like state is a common and important feature of the daily lives of animals from both invertebrate and vertebrate taxa, suggesting that sleep appeared early in animal evolution. Recently, Drosophila melanogaster has been shown to be a relevant and powerful model for the genetic analysis of sleep behavior. The sleep architecture of D. melanogaster is sexually dimorphic with females sleeping much less than males during daytime, presumably because reproductive success requires greater foraging activity by the female as well as the search for egg-laying sites. However, this loss of sleep and increase in locomotor activity will heighten the risk for the female from environmental and predator hazards. In this study, we show that virgin females can minimize this risk by behaving like males with an extended afternoon "siesta???. Copulation results in the female losing 70 % of daytime sleep and becoming more active. This behavior lasts for at least eight days after copulation and is abolished if the mating males lack sex peptide (SP), normally present in the seminal fluid. Our results suggest that SP is the molecular switch that promotes wakefulness in the post-mated female, a change of behavior compatible with increased foraging and egg–laying activity. The stress resulting from SP-dependent sleep deprivation might be an important contribution to the toxic side-effects of male accessory gland products that are known to reduce lifespan in post-mated females.

**F-03 Role of a microRNA Pathway in Drosophila Long-Term Memory.**

**Islam, R**; Dan, C; Kunes, SM
Harvard University

Synaptic mRNA localization and protein synthesis are involved in the induction of long-term memory in Drosophila. MicroRNAs (miRNAs) have been suggested to act as local regulators of new protein synthesis involved in synaptic plasticity. We have observed that neural activity controls the biogenesis of miRNAs that have specific targets in neurons, including mRNAs encoding proteins with roles in dendritic structure formation and synaptic function. Our work has focused on let-7-C, a polycistronic miRNA cluster known for its regulatory role during development. These miRNAs are expressed in the Mushroom Body, and are strongly induced by the dopamine signaling pathway that conveys the aversive electric shock stimulus in an odor, electric shock associative conditioning paradigm. We find that the induced miRNAs let-7-C reduce the expression of the protein Abrupt, a developmental regulator of dendrite arbor branching, in a subset of Mushroom Body neurons. Our current effort is focused on determining the role of the regulation of Abrupt by let-7-C in Mushroom Body morphology and in long-term memory formation, maintenance and retrieval. Together, our observations suggest a new pathway by which dopaminergic output modulates miRNA expression in the Mushroom Body of the fly brain to prime synaptic circuitry for change.

**F-04 Input activity modulates size and number of synaptic complexes and active zone number in the adult brain of Drosophila.**

Kremer, M; Leiss, F; Christiansen, F; Paehler, M; Förstner, F; Kloppenburg, P; Sigrist, S and Tavosanis, G
Dendrite Differentiation Group; MPI of Neurobiology; Martinsried; Institute for Biology and Genetics, Sigrist Group, Freie Universität Berlin, Neurowissenschaftliches Forschungszentrum, Sigrist Group

The establishment of long term memories is thought to require activity-induced structural modifications. To approach the mechanisms underlying these phenomena in a genetic system, we have addressed whether alterations of input activity can induce structural changes in the adult brain of Drosophila. We concentrated on a region involved in long term associative olfactory memories, the mushroom body. Previously, we have described in detail the connective organization of the mushroom body input region, the calyx. Here, pre-synaptic boutons of incoming antennal lobe projection neurons and the clawed dendritic endings of mushroom body neurons form synaptic complexes called microglomeruli. In this work, we have developed tools to visualize the post-synaptic compartment and pre-synaptic active zones of microglomeruli and to identify them in a semi-automated fashion. We have addressed the ability of microglomeruli to undergo rearrangements during adult life and in dependence of alterations of projection neuron activity. We found that the number and size of microglomeruli increases during the first seven days of adult life, suggesting that these complex structures can undergo remodelling. To test whether this ability could be influenced by input activity, we genetically silenced a subset of projection neurons and found that action potentials could no longer be elicited in the presence of the leaky potassium channel dORK???C. Microglomeruli formed by silenced projection neurons were both more numerous and larger and contained less pre-synaptic active zones than unaffected microglomeruli. These data provide evidence of structural modifications caused by silencing in the adult fly brain and suggest that compensatory mechanisms can alter the structure of calycal microglomeruli.

**F-05 Coding the way forward in Drosophila larvae.**

**Louis, M**
Center for Genomic Regulation, EMBL-CRG Systems Biology Unit, 08003, Barcelona, Spain

Chemotaxis involves directed navigation toward attractive stimuli (food and mating partners) and away from aversive stimuli (toxins and predators). This process is critical for the survival of any motile organism. While the biochemical pathway governing chemotaxis is reasonably well understood in bacteria, the neural mechanisms allowing animals with complex nervous systems to orient in chemical gradients remains poorly described. We are studying this problem in the simple genetically tractable olfactory system of Drosophila larvae. Using a combination of behavioral analysis, molecular genetics and electrophysiology, we have undertaken a long-term project to identify, and functionally characterize, the circuits underpinning larval chemotaxis. Larvae have a pair of bilateral olfactory organs, each containing 21 olfactory sensory neurons (OSNs). Each OSN expresses one, or occasionally two, specific odorant receptor (OR) genes in addition to the ubiquitously expressed OR gene Or83b. Individual odorant receptors have overlapping but distinct ligand tuning properties. Accordingly, each OSN can be viewed as distinct information channel to the olfactory system. Here, I will examine the contribution of single OSNs to the representation of odor quality and odor intensity. I will present recent findings about the search strategy implemented by larvae to process graded odor stimuli. Our work highlights that larval chemotaxis represents a powerful paradigm to investigate how variable sensory signals are integrated to direct robust decision making.

**F-06 Complex Neural and Metabolic Control of Enteric Physiology in Drosophila.**

Cognigni, P[1]; Bailey, AP[2]; Maddrell, SHP[1]; **Miguel-Aliaga, I**[1]
[1]Department of Zoology, University of Cambridge, Cambridge, UK. [2]Division of Developmental Neurobiology, MRC National Institute for Medical Research, London, UK

Drosophila has greatly contributed to our understanding of how motor neurons innervate and control somatic muscles. By contrast, less is known about the neural control of internal organs such as the digestive tract, and there are currently no behavioural assays to monitor gut physiology. We have developed a new method to visualize and quantify defecation in adult flies under normal feeding conditions. Specific outputs of this behaviour can be used to quantify food intake, water and ion homeostasis and food/water excretion rates, thereby providing an integrated readout for digestive physiology and fluid excretion. Our analysis reveals an unexpected degree of complexity as regards the frequency and nature of excreta. Indeed, defecation behaviour is sexually dimorphic and identifies a new component of the postmating response in female flies. In addition, it reveals distinct metabolic changes associated with fasting, caloric restriction and egg production. We have combined this behavioural output with the selective inactivation of subsets of adult neurons to identify novel roles for Ilp7 and leucokinin neurons in the regulation of food intake and water homeostasis, respectively. Interestingly, neuronal silencing also uncovers a link between reproductive state and digestive physiology, as well as homeostatic mechanisms involving co-regulation of food intake, defecation and water excretion.

**F-07 Distinct functions of larval photoreceptor subtypes in circadian clock entrainment and light avoidance.**

Sprecher S.G.[1]; Mazzoni E.O.[2]; Keene A.C.[2]; Zhen J.[2]; Ng, J.[2]; Yamaguchi S.[2]; Blau J.[2]; Desplan C.[2]

(1) Dept. of Biology, University of Fribourg; ch. du Musée 10, 1700 Fribourg, Switzerland (2) Dept. Biology, New

York University, 1009 Silver Center, 100 Washington Square East, New York, NY 10003-

Sensory input from the visual system acts not only to form an image of the environment but also to control circadian rhythms. However, the neuronal and genetic mechanisms that channel visual information into these two distinct functional outputs remain largely elusive. The larval eye of Drosophila has been implicated in mediating both functions: Rapid light escape responses and entrainment of the circadian clock. Photoreceptor neurons (PRs) of the larval eye connect to the main pacemaker neurons of the larva, the lateral neurons (LNs), providing sensory input to control circadian rhythms. Each eye is only composed of 12 PRs, which can be subdivided into two subtypes. Four PRs express blue-sensitive rhodopsin5 (rh5), while the remaining eight express green-sensitive rhodopsin6 (rh6). We show here that Rh5-PRs and Rh6-PRs mediate both common and distinct functions. For the entrainment of the molecular clock, either PR-subtype is sufficient, while only the Rh5-PR subtype is essential for light avoidance, Rh6PRs being dispensable. Moreover for either function Acetylcholine (ACh) is acting as neurotransmitter. Therefore rapid behavior response and control of circadian rhythms can already be distinguished at the level of PR subtypes.

**G-01 Trafficking the EGFR ligand processing machinery in photoreceptor axons**

Shaul Yogev,Eyal Schejter and **Ben-Zion Shilo**
Department of Molecular Genetics, Weizmann Institute, Rehovot 76100, Israel

The release of signaling molecules from neurons must be regulated, to accommodate their highly polarized structure. In the developing Drosophila visual system, photoreceptor neurons secrete the EGFR ligand Spitz (Spi) from their cell bodies in the eye disc epithelium, as well as from their axonal termini in the lamina, the first optic neuropil. Canonical EGFR activation in all tissues relies on Spi processing by Rhomboid (Rho) proteases in the signal-producing cell, in a late compartment of the secretory pathway. Our previous work has shown that nascent photoreceptors express Rho-1 and Rho-3, the latter being partially localized to the ER, where it attenuates excessive EGFR activation in the eye disc epithelium. This is achieved by premature cleavage of the ligand chaperone Star, preventing it from trafficking Spi out of the ER. Here we examined the role of intracellular compartmentalization of the central components of EGFR ligand processing, in regulating long-distance trafficking of Spi for release from the axonal termini. We find that the ER localization of Spi, Star, and Rho-3 serves as a facilitator of axonal trafficking, as the ER itself is continuous throughout the axon. Indeed, whereas in the eye disc epithelium both Rho-1 and -3 participate in Spi processing, only the ER localized Rho-3 mediates EGFR activation in the lamina. ER localization of Rho-3 thus plays distinct roles at the opposite ends of photoreceptor neurons: signal attenuation in the eye disc, and signal facilitation in the lamina.

**G-02 The Long Range Hedgehog Gradient Is Formed in the Apical Extracellular Space by the Glypican Dally and the Hydrolase Notum.**

**Katie L. Ayers***, Armel Gallet*, Laurence Staccini-Lavenant and Pascal P. Thérond * These authors contributed equally to this manuscript
Institut Biologie du Développement & Cancer – IBDC; Université de Nice Sophia-Antipolis; UMR6543 CNRS; Centre de Biochimie; Parc Valrose; 06108 Nice cedex 2, France.

Cell positioning during developmental patterning is controlled by concentration gradients of morphogens. Dysfunction in morphogens activity or spreading can cause developmental disease or cancer. In the epithelial field, morphogens like the Hedgehog (Hh) peptides diffuse both apically and baso-laterally. How these two parameters are integrated at the cellular level is unclear. Here we show that reducing or increasing the apical Hh gradient causes a dramatic change in long range Hh activity, whereas apical spreading of Hh is not necessary for its short range activity. We provide genetic evidence that the glypican Dally positively regulates apical Hh levels, and physiological release of Dally by the hydrolase Notum in producing cells promotes apical Hh long range activity. Our data suggests that two functional Hh gradients are perceived in epithelial tissues. Thus we propose an additional mechanism in morphogen sensing, whereby cellular response to morphogens integrates the apico-basal values of the extra-cellular gradient.

**G-03 Increased cell bond tension governs cell sorting at the Drosophila anteroposterior compartment boundary.**

[1]Landsberg, K.P.; [2]Farhadifar,R.; [2]Ranft, J.; [1]Umetsu, D.; [1]Widmann, T.J.; [2]Bittig, T.; [1]Said, A.; [2]Jülicher, F.; [1]**Dahmann, C.**
1Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307 Dresden, Germany 2Max Planck Institute for the Physics of Complex Systems, Nöthnitzer Strasse 38, 01187 Dresden, Germany

The subdivision of proliferating tissues into compartments is an evolutionary conserved strategy during animal development. Signals across boundaries between adjacent compartments can result in the local expression of secreted proteins that organize growth and patterning of tissues. Sharp and straight interfaces between compartments are crucial for stabilizing the position of such organizers and therefore for the precise implementation of body plans. Maintaining these boundaries in proliferating tissues requires mechanisms to counteract cell rearrangements caused by cell division; however, the nature of such mechanisms remains unclear. Here we quantitatively analyzed cell morphology and the response to laser ablating cell bonds in the vicinity of the anteroposterior compartment boundary in developing Drosophila wings. We found that mechanical tension is approximately 2.5-fold increased on cell bonds along this compartment boundary as compared to the remaining tissue. Cell bond tension is decreased in the presence of Y-27632, an inhibitor of Rho-kinase whose main effector is myosin II. Simulations using a vertex model demonstrate that a 2.5-fold increase in local cell bond tension suffices to guide the re-arrangement of cells after cell division to maintain compartment boundaries. Our results provide a physical mechanism in which the local increase in myosin II-dependent cell bond tension directs cell sorting at compartment boundaries.

**G-04 miR-9a prevents apoptosis during wing development by repressing Drosophila LIM-only.**

**Fernando Bejarano**, Peter Smibert and Eric C. Lai
Department of Developmental Biology, Sloan-Kettering Institute, New York, NY.

The Drosophila LIM-only (dLMO) gene is a transcriptional regulator that functions in wing and neural development. Classical gain-of-function mutants of dLMO, known as Beadex, exhibit loss of adult wing tissue. This is caused by as little as a two-fold increase in dLMO activity, and closely resembles phenotypes reported for a deletion mutant in mir -9a. We observed that ectopic apoptosis in the wing primordium is associated with both mutant backgrounds, and that mir-9a mutant phenotypes could be rescued by decreasing dLMO dosage or by suppressing apoptosis. We then used in vivo clonal sensor assays to demonstrate direct repression of dLMO by endogenous miR-9a. These data indicate dLMO-induced cell death as the major cause of loss of mir-9a wing tissue, providing evidence for a critical individual miRNA:target relationship. Interestingly, many phenotypes of mir-9a mutant clones were not recapitulated by mutant clones of miRNA biogenes factors encoded by Dicer-1 or pasha, even though all of these situations de- repressed miR-9a and dLMO sensor constructs. These findings indicate that an absence of Dicer conditional phenotypes do not indicate a lack of critical miRNA functions in a given setting, potentially due to the opposing functions of certain miRNAs and/or targets.

**G-05 EGF signaling and the origin of dorsal-ventral polarity in insects.**

**Lynch, J**; Drechsler, A; Roth, S
Institute of Developmental Biology, University of Cologne, Cologne, Germany

The establishment of the anterior-posterior (AP) and dorsal-ventral (DV) axes is a critical step in the development of bilaterally symmetric animals, and often depends on maternal cues. Among the insects, this process is only well understood in Drosophila, where both axes depend on signaling provided by the tgf-alfa like ligand Gurken, whose mRNA is localized around the oocyte nucleus, and whose protein activates the EGF-receptor in the overlying somatic follicle cells. We have sought to test whether this system operates in insects with different modes of oogenesis and embryonic development by cloning and testing the function of tgf-alfa like ligands and EGF receptors from the beetle Tribolium, the wasp Nasonia, and the cricket Gryllus. In all three species, mRNA for the EGF ligand is expressed in the germline, while the EGF receptor mRNA is expressed in the somatic follicle cells, and activation of EGF signaling in the follicle cells is correlated with the position of the oocyte nucleus in late stages of oogenesis. Reduction of the activity of this pathway by RNAi leads to defects in oocyte polarity in all three species. In addition, RNAi against this pathway leads to major embryonic dorsal ventral patterning defects in both the short-germ embryo of Tribolium, and the long germ embryo of Nasonia. These phenotypes include simple ventralization of the embryo, loss of the perpendicular relationship between ventral markers and the AP axis, and in some cases, apparent DV axis duplications. In summary, EGF signaling from germline to soma appears to be a broadly conserved mechanism of providing spatial cues for proper oocyte and egg patterning within the insects, but whose deployment and downstream output is variable due to different modes of oo- and embryogenesis.

**G-06 Facilitated transport of Dpp/BMP draws diversified wing vein patterns in insects.**

**Shinya Matsuda**(1), Naotoshi Yoshiyama(2), Masatsugu Hatakeyama(2) and Osamu Shimmi(1)
(1)Institute of Biotechnology, University of Helsinki, Finland (2)Invertebrate Gene Function Research Unit, Division of Insect Sciences, National Institute of Agrobiological Sciences, Japan.

Variations in insect wing vein patterns have been acquired through evolution. However, the molecular mechanisms for such diversity remain unknown. In the drosophila pupal wing, Dpp/BMP signal is active at all wing veins to initiate crossveins (CVs) formation and maintain longitudinal veins (LVs) fate. Here we show that using a GFP-Dpp, the signaling activity tightly reflects Dpp diffusion by “facilitated transport??? and “active retention??? mechanisms. To initiate PCV formation Dpp diffusion from LVs to posterior crossvein (PCV) requires BMP binding proteins, Sog and Crossveinless. Intriguingly, the transport is restricted to future PCV and majority of the ligands is actively retained in LVs by type I receptor Tkv and through positive feedback mechanism(s), while intervein region can transduce the signal when this retention is experimentally disturbed. These data suggest that the directional transport determines the PCV position. We also found that in more ancient insect, Hymenoptera Athalia rosae (Sawfly), Dpp/BMP signal activity reflects wing vein patterns during prepupal stages. In addition, Dpp knockdown by RNAi inhibits wing venation in Sawfly, indicating the evolutionarily conserved role of Dpp/BMP signal for insect wing venation. Furthermore, fore- and hind- wing vein patterns in Sawfly are initially similar but later become different by inducing CVs at different positions during prepupal stages, suggesting that Dpp transport direction differs between fore and hind wing. These observations suggest that variation in wing vein patterns may be acquired partly by regulating Dpp diffusion at the extracellular level.

**G-07 Cell competition and multicellularity: the Maxwell´s Demon code.**

Christa Rhiner; Jesus Maria Lopez-Gay; Davide Soldini; Sergio Casas-Tinto; **Eduardo Moreno**
CNIO, Madrid, Spain

Cell competition is currently accepted to be a general method by which weaker cells are eliminated from a growing population with the purpose of optimizing tissue fitness. Here we investigate how cells of Drosophila wing imaginal discs distinguish 'winners' from 'losers' during cell competition. Using genomic and functional assays we have identified Maxwell`s Demon (Mwd), a novel gene highly conserved in multicellular animals. What we describe here is probably part of an ancient mechanism used to terminate competitive conflicts among cells, whose evolutionary origin is linked to the emergence of animal multicellularity.

**G-08 Fj is an atypical kinase that regulates Ds/Ft heterodimerization.**

**Repiso,A**; Brittle,A.L; Strutt,D; Lawrence,P.A
MRC Laboratory of Molecular Biology. Department of Zoology, University of Cambridge. MRC Centre for Developmental and Biomedical Genetics Department of Biomedical Science University of Sheffield

Epithelial cells are often coordinately polarized in the plane of the sheet; a feature known as planar cell polarity (PCP). One group of proteins involved in PCP establishment consists of the large cadherin molecules, Dachsous (Ds) and Fat (Ft) and the Golgi resident protein Four-jointed (Fj). Fj is a type II trans-membrane glycoprotein of 583 aminoacids, which can be cleaved both in vivo and in vitro to give a secreted polypeptide of 482 aminoacids. Recently it has been shown that Fj has a rare kinase activity that acts on Ds and Ft. Here we show how this activity regulates Ds/Ft binding.

**G-09 Regulation of Delta signaling by Bearded family members.**

**Soline Chanet**, Nicolas Vodovar, Véronique Mayau and François Schweisguth
Institut Pasteur, CNRS, URA 2578, 75015 Paris, France

Lateral inhibition mediated by Notch receptor signaling regulates the determination of sensory organ precursor cells (SOPs) in Drosophila. The selection of SOPs from proneural cluster cells appears to rely on a negative feedback loop linking activation of the Notch receptor to downregulation of its ligand Delta within each cell. The molecular basis of this regulatory feedback mechanism is not known. We have tested the role of the Bearded (Brd) family genes in this process. The Drosophila genome encodes eight Brd family members that interact with the E3 ubiquitin ligase Neuralized (Neur) and act as inhibitors of Neur-mediated Delta signaling. Genome engineering technologies were used to create speci???c deletions of all eight Brd family genes. We ???nd that the Brd family genes ma, m4, and m6 encoded by the Enhancer of split Complex (E(spl)- C) are dispensable for Drosophila development and that deletion of the ???ve Brd family genes encoded by the Brd Complex only reduces viability. However, deletion of all Brd family genes results in embryonic lethality. Additionally, the ma, m4, and m6 genes act redundantly with the other ???ve Brd family genes to spatially restrict Notch activation in stage 5 embryos. These data reveal that the Brd family genes have an essential but redundant activity. While the activity of all eight Brd genes appears to be dispensable for SOP determination, clone border studies indicate that both the relative activity levels of Neur and Brd family members in?uence competition for the SOP fate during lateral inhibition. We propose that inhibition of Neur–Delta interaction by Brd family members is part of the feedback loop that underlies lateral inhibition in Drosophila.

**G-10 Distinct function of actin and microtubule in nuclear ordering of syncytial embryo.**

Takuma Kanesaki; Jörg Grosshans
Institut für Biochemie und Molekulare Zellbiologie, Georg-August-Universität Göttingen

Drosophila early embryo undergoes thirteen rounds of syncytial mitosis after fertilization and turns out to gain approximately 6,000 nuclei sharing cytoplam. In order to clarify how those numerous nuclei are arragened without compartmentalization by plasma membrane, here we examine nuclear dynamics and positioning in the syncytial embryo with a combination of fluorescent time-lapse imaging and quqntitative image analysis. With a orientational order parameter which describes the regularity of total nulcear array, we show that the nuclear arrangement is changed troughout cell cycles; nuclei first get irregular following chromosome segregation and their order increases during interphase. The nuclear order increases independently of nuclear density. With pharmacological assays, we find that the microtuble network is required for the motility- and ordering of nuclei. Actin microfilaments, which form the cap-like structure at apical side of nuclei, are also required for the ordering. However, in contrast to microtubules, actin suppresses the motility of individual nuclei. We propose that the increase of nulcear regularity is achieved through distinct function of two cytoskeletal networks; overlapping microtubules elongated from neighboring nuclei generate the force to move each nucleus, while actin filament act as molecular matrix which buffers the force of microtubules.

**G-11 The Brakeless and Atrophin transcriptional co-repressors in early embryo patterning.**

**Mattias Mannervik** Wenner-Gren
Institute, Dept. Developmental Biology, Stockholm University, 10691 Stockholm, Sweden

DNA-binding transcription factors depend on co-regulators for their activity. Co-regulators can affect the chromatin structure or by other, less understood, means facilitate or hinder RNA polymerase initiation or elongation. Recently, we identified new co-repressors in the early embryo from genetic screens. Brakeless (also known as Scribbler, or as Master of thickveins) is a Tailless co-repressor, and appears to cooperate with another co-repressor, Atrophin (also known as Grunge). In embryos derived from brakeless germline clones, the Krüppel (Kr) and knirps (kni) gene expression domains expand, and mis-expressed Tailless is unable to repress kni expression. We further found genetic and physical interactions between Brakeless and Tailless, and showed that Brakeless can directly interact with Atrophin. Atrophin is a homolog of Atrophin-1 that causes the human neurodegenerative disease dentatorubral-pallidoluysian atrophy (DRPLA) when a polyglutamine repeat is expanded. By chromatin immunoprecipitation (ChIP), we showed that both Brakeless and Atrophin associate with the Tailless-regulated kni cis-regulatory module (CRM) in early embryos. Presently, we are investigating whether other repressors in the embryo depend on Brakeless for their activity, and the mechanisms by which Brakeless contributes to repression. To understand the global role of Atrophin and Brakeless in gene regulation, we are performing genome-wide ChIP assays with Atrophin and Brakeless antibodies.

**G-12 Modular L/R patterning in D. melanogaster supports an incremental model of asymmetry evolution in Diptera.**

**Suzanne M**, Petzoldt AG, Spéder P, Coutelis JB and Noselli S
Institute of Developmental Biology and Cancer (IBDC), University of Nice Sophia-Antipolis, Parc Valrose, 06108 Nice Cedex 2, France Howard Hughes Medical Institute, The Rockefeller University, 1230

The evolution of morphological traits is an important feature of live organism history, yet we have little insights into the underlying changes in developmental mechanisms. We used genitalia looping as a model system to study the basis of Left-Right (L/R) asymmetry evolution. In Diptera, the degree of genitalia rotation follows discrete steps from 0o to 360o, with species undergoing either no rotation, 180° rotation (inversion) or 360° rotation (circumrotation). Here, we present developmental evidence supporting a model of incremental evolution of genitalia rotation. We used time-lapse imaging in Drosophila melanogaster, a higher Diptera undergoing 360° rotation, to show that circumrotation results from the spinning of two separate ring-shaped domains, each contributing 180° to the rotation of the genitalia. We further show that directionality of each half turn, which is clockwise in wild type, is controlled by the L/R determinant Myosin ID (MyoID). Specific invalidation of myoID in one ring leads to its autonomous inversion, without affecting the rotational behaviour of the other ring. Therefore, genetic and phenotypic analyses demonstrate that both rings are functionally identical but independent. These results suggest that an ancestral 180o L/R ring module first emerged (transition from 0° to 180°) and then was duplicated (transition from 180° to 360°), thus providing a simple additive model for both the origin of circumrotation and the evolution of genitalia rotation.

**G-13 Regulation of Transcriptional Elongation by Spt5 during Drosophila Development.**

**Jennings, BH**[1]; Harvey, R[1]; Ish-Horowicz, D[2]
[1]Transcriptional Regulation Laboratory, UCL Cancer Institute. [2]Developmental Genetics Laboratory, Cancer Research UK, London Research Institute.

Transcription elongation has become recognised as a critical point of control during the expression of many genes during development. For example, genome-wide screens for promoter proximal paused RNAP II in Drosophila have revealed that approximately 20% of genes in S2 culture cells, and 10% in early embryos, have initiated transcription but are transcriptionally paused. In both cases, the sets of paused genes are enriched for genes known to respond to developmental cues and environmental stimuli. There is mounting evidence that the Spt5 protein provides a key junction between developmental regulators of transcription and the core transcriptional elongation complex. Mutations in Spt5 recovered from Drosophila and zebrafish that compromise its ability to cause transcriptional pausing result in discrete developmental defects, indicating that Spt5 mediates gene specific regulation. Spt5 acts as both a negative and a positive regulator of elongation, and the switch between these activities at the promoter proximal checkpoint may provide a critical point of regulation by contextual transcription factors. We have characterized a missense mutation in Drosophila Spt5 (W049) that affects the transcription of a subset of genes during development. Expression of the gene even-skipped (eve) is directly subject to repression mediated by Spt5. Enhancer-reporter constructs reproducing expression of specific stripes of eve expression are affected differentially by Spt5W049 indicating that Spt5 can regulate transcription in an enhancer-specific manner. The aim of our current work is to ascertain the molecular mechanisms by which contextual transcription factors regulate transcriptional pausing during development.

**H-01 Control of lipid droplet structure and body fat content by PERILIPINS**

**Kühnlein, R.P.**
Max Planck Institute for Biophysical Chemistry, Dept. Molecular Developmental Biology, Am Fassberg 11, 37077 Göttingen, Germany, e-mail: rkuehnl@gwdg.de

Intracellular lipid droplets are increasingly acknowledged as organelles executing multiple functions in animal cells. Most importantly, lipid droplets serve as dynamic fat storage reservoir to supply both, building blocks for lipoanabolism and fuel reserve for body energy homeostasis. Body fat of phylogenetically diverse animals is allocated to lipid droplets of specialized tissues such as the mammalian adipose tissue or the fly fat body suggesting evolutionary conservation of their biogenesis and regulation. The surface of lipid droplets is a regulatory compartment boundary covered by a large number of associated proteins, the functions of which attract growing research interest. Most prominent mammalian lipid droplet-associated proteins belong PERILIPIN family. All five mammalian PERILIPINS have been implicated in organismic lipometabolism homeostasis control on the level of lipid droplets. Drosophila encodes two PERILIPINS called PLIN1/LSD-1 and PLIN2/LSD-2. We and others have shown that PLIN2/LSD-2 acts as lipoprotective control factor on lipid droplets of different cell types. In contrary, PLIN1/LSD-1 has been proposed to operate in stimulated lipolysis. Data will be presented on (i) the dual role of PLIN1/LSD-1 as structural determinant and lipolytic gatekeeper of lipid droplets in fat storage tissue and (ii) the phenotype of flies devoid of any PERILIPIN.

**H-02 Role of Drosophila CycD/Cdk4 complex in adult metabolism and gut homeostasis.**

**Mojca Adlesic**, Christian Frei
Institute of Cell Biology Schafmattstr. 18 ETH-Hönggerberg, HPM F 29 CH-8093 Zürich

Survival of all organisms requires that they correctly coordinate the processes of food breakdown, uptake and storage. How this is achieved in the fruit fly is currently poorly understood. We have uncovered a role of the Drosophila Cyclin D/Cdk4 complex in controlling normal midgut cellular composition and we are investigating how these gut defects relate to metabolic changes on the whole organism level. Our findings showed that cdk43 and cycd1 mutant adult male flies had reduced total body fat levels, were starvation sensitive and had reduced glycogen levels. Furthermore, cdk43 mutants had reduced enterocyte number and altered cellular morphology in the anterior midgut, potentially indicating cell specification or differentiation defects. Gene expression studies on adult midguts revealed a decrease in several lipases, suggesting defects in fatty acid and/or lipid breakdown. Consistent with a role of CycD/Cdk4 in regulating whole body nutrient storage through regulation of gut homeostasis, gut specific overexpression of CycD/Cdk4 resulted in flies with increased total body fat levels. Taken together, our data suggest that cdk43 or cycd1 mutant flies have inadequate food breakdown and/or nutrient absorption in the midgut, leading to the observed metabolic phenotypes. We are currently exploring the mechanisms through which CycD/Cdk4 regulates endoreplication, differentiation and cell specification in the adult midgut.

**H-03 Effects of dietary copper on ferritin iron storage.**

**Lucia Bettedi**, Mohamad F. Aslam and Fanis Missirlis
School of Biological and Chemical Sciences, Queen Mary University of London

Iron and copper ions serve as cofactors for enzymes and readily accept or donate electrons in redox reactions. If levels of these metals go unchecked electron transfer to hydrogen peroxide generates hydroxyl radicals, which damage DNA, proteins and membranes. Excess cellular iron is either stored inside the ferritin cavity or exported via a transporter known as ferroportin. Ferroxidases convert reduced ferrous iron (Fe2+) in its non-soluble, oxidized form (Fe3+). The ferritin ferroxidase reaction converts oxygen into hydrogen peroxide and forms insoluble ferrihydrate precipitated inside a protein cavity. In contrast, export of iron via the plasma membrane requires ferrous oxidation via ceruloplasmin (or its homolog hephaestin). Ceruloplasmin activity depends on the binding of six copper ions in its active sites. One of these copper ions receives an electron from ferrous iron facilitating the loading of ferric iron onto transferrin for delivery to peripheral organs. Here we investigate the effect of dietary copper on ferritin iron storage in the insect Drosophila melanogaster, by assuming that the iron region of fly intestine simulates the function of liver iron stores in mammals. We demonstrate how Cu-excess in the diet increases the ceruloplasmin-mediated Fe export from enterocytes of fly midgut. Our study shows that addition of 1mM copper sulfate in an otherwise regular diet drastically reduces the pool of iron stored in ferritin and total iron levels in the fly. Moreover, in homozygote mutant flies for the fly ceruloplasmin-like gene fed on a copper-enriched diet we observe that iron accumulation into ferritin is not altered, indicating that the requirement of ceruloplasmin activity for iron mobilization from tissue iron stores is likely conserved in an insect.

**H-04 Membrane sterols in Drosophila melanogaster: dispensable for cell and organism viability but required for growth and progression through development.**

**Carvalho, Maria**(1); Shwudke, Dominik(1,2); Eaton, Suzanne(1)
1 Max-Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany 2 National Centre for Biological Sciences, Bangalore, India

Although sterol synthesis is energetically very demanding, sterols are abundant components of most eukaryotic cell membranes. Sterols are used to synthesize signaling molecules and they are thought to have essential functions in the membranes. We used Drosophila melanogaster, a sterol auxotroph that incorporates sterols into its membranes under normal conditions, in order to separate different sterol functions and investigate general sterol requirements in eukaryotes. We show that membrane sterols can be reduced six-fold when the larvae are fed with a diet containing low levels of sterols without affecting larval viability. Under sterol-depleted conditions, larvae respond by arresting their growth and by increasing the level of specific lipids - hydroxylated sphingolipids are upregulated up to 20-fold in sterol-depleted membranes. These sphingolipids promote survival when sterol is scarce. Moreover, in contrast to other tissues the central nervous system and apical membranes keep membrane sterol levels high even under sterol-depleted conditions. However, and in contrast to most cell types in the sterol-depleted arrested larvae Drosophila's larval growth requires a specific amount of membrane sterol that is tightly regulated. Different amounts of sterol in the diet lead to changes in adult size but the amount of sterol in the membranes is kept constant. In conclusion, while membrane sterol is essential for specialized functions in some cells, it is not essential for cell viability. A eukaryotic organism such as Drosophila melanogaster presents a lipid metabolism with enough plasticity in order to compensate for the loss of sterols. In this way, many of the functions for which sterols are thought to be essential can actually be performed by other lipids.

**H-05 Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the *Drosophila* midgut.**

Huaqi Jiang1, Parthive H. Patel1, Alexander Kohlmaier2, Marc O. Grenley1, **Bruce A. Edgar2**
1Division of Basic Sciences, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, Seattle, WA 98109, USA 2DKFZ-ZMBH Alliance, Im Neuenheimer Feld 282, D-69120 Heidelberg, Germany

Cells in intestinal epithelia turn over rapidly due to aging, damage, and toxins produced by the enteric microbiota. Gut homeostasis is maintained by intestinal stem cells (ISCs) that divide to replenish the intestinal epithelium, but relatively little is known about how ISC division and differentiation are coordinated with gut epithelial cell loss. We have been using the Drosophila midgut as a model system for genetic analyses of intestinal homeostasis. We find that when intestinal enterocytes (ECs) in the Drosophila midgut are subjected to apoptosis, enteric infection, or JNKmediated stress signaling, they resond by producing cytokines (Upd, Upd2, Upd3). These ligands activate Jak/Stat signaling in intestinal stem- and progenitor-cells, and thereby promote ISC division and gut renewal. Surprisingly, Upd/Jak/Stat activity also promotes Delta/Notch signaling in progenitor cells, and is required, like Notch, for their differentiation into ECs. This requirement extends to healthy midguts, which maintain low levels of cytokine (Upd3) expression and Stat activity. In addition to acting as mitogens directly on progenitor cells, Upd cytokines produced by ECs activate Jak/Stat signaling in the midgut visceral muscle (VM), which responds by expressing an EGFR ligand that is also mitogenic for ISCs. Thus cytokine-mediated feedback between differentiated enterocytes, visceral muscle, and intestinal stem cells plays a central role in both stress-induced gut regeneration and normal gut homeostasis.

**I-01 The unusual dynamic properties of Centrosomin can explain how centrosome size is set**

**Raff, J.**

Centrosomes comprise a pair of centrioles surrounded by an amorphous pericentriolar material (PCM) and they are the major microtubule organising centres in most animal cells, playing important roles in many aspects of cell organisation. Centrosomes can differ in size, and this asymmetry is important during the asymmetric divisions of certain stem cells. Athough there is evidence that the centrioles can somehow influence centrosome size, the mechanisms that determine centrosome size are largely mysterious. Here we show that Drosophila Centrosomin (Cnn) exhibits an unsual dynamic behaviour in that it is incorporated into the PCM only at the surface of the centrioles. Once incorporated, Cnn moves away from the centrioles and spreads throughout the PCM. Importantly, we show that the amount of Cnn associated with the centrioles can effectively determine centrosome size. Thus, Drosophila centrioles can set centrosome size by determining the rate at which Cnn is incorporated into the PCM.

**I-02 dRecQ4 Is Required for DNA Synthesis and Essential for Cell Proliferation in Drosophila.**

Yanjuan Xu[1,2], Zhiyong Lei[1], Hai Huang[1,2], Wen Dui[1,2], Xuehong Liang[1], Jun Ma[1,3] and **Renjie Jiao**[1],*

[1]State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, The Chinese Academy of Sciences, Datun Road 15, Beijing 100101, China [2]Graduate School of the Chinese Academy of Sciences

The family of RecQ DNA helicases plays an important role in the maintenance of genomic integrity. Mutations in three of the five known RecQ family members in humans, BLM, WRN and RecQ4, lead to disorders that are characterized by predisposition to cancer and premature aging. To address the in vivo functions of Drosophila RecQ4 (dRecQ4), we generated mutant alleles of dRecQ4 using the targeted gene knock-out technique. Our data show that dRecQ4 mutants are homozygous lethal with defects in DNA replication, cell cycle progression and cell proliferation. Two sets of experiments suggest that dRecQ4 also plays a role in double strand break repair. First, mutant animals exhibit sensitivity to gamma irradiation. Second, the efficiency of DsRed reconstitution via single strand annealing repair is significantly reduced in the dRecQ4 mutant animals. Rescue experiments further show that both the N-terminal domain and the helicase domain are essential to dRecQ4 function in vivo. The N-terminal domain is sufficient for the DNA repair function of dRecQ4. Together, our results show that dRecQ4 is an essential gene that plays an important role in not only DNA replication but also DNA repair and cell cycle progression in vivo.

**I-03 Monitoring chromosome segregation.**

**Martins, T**; Malmanche, N; Monteiro, A; Barral, Y*; Sunkel, CE#;
Institute for Molecular and Cell Biology, Universidade do Porto, Portugal; #Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal; *Institute for Biochemistry, ETH Zurich

To ensure equal distribution of the genetic material, mitosis has to be successfully completed and chromosome segregation has to occur properly. Little is known about the mechanisms that monitor chromosome segregation during anaphase until cytokinesis occurs. The existence of the NoCut checkpoint was suggested to ensure that no DNA is present in the spindle midzone when cytokinesis takes place. However, whether this is achieved by alterations in spindle length or chromosome condensation remains an open question. To address this, we used Drosophila strains that carry re-arranged extra long chromosomes that could not be segregated by a normal mitotic spindle. These compound chromosomes are composed of two whole second chromosomes fused by Y chromosome heterochromatin and that have a single functional centromere. Using appropriate fluorescent markers for chromatin and tubulin, we studied in vivo and in fixed material the alterations in mitosis that must take place to allow the segregation of these chromosomes and cell viability. We found that depending on cell type, they respond in a different manner to ensure a successfully mitosis. Neural stem cells, took longer to complete mitosis successfully, while embryonic nuclei tends to elongate more their spindles.

**I-04 In vivo analysis of Survivin and Aurora-B function in the developing drosophila.**

**Mathieu, J**; Bardin, A; Moch, C; Sampaio, P; Schweisguth, F; Sunkel, C; Huynh, JR.
Institut Curie, Paris, France, CNRS UMR3215, INSERM U934 Institut Pasteur, Paris, France, CNRS URA2578 Instituto de Biologia Moleculare celular, Porto, Portugal

The Chromosome Passenger Complex is a major regulator of mitosis. Although the functions of its components Aurora-B (AurB) kinase and Survivin (Svv) are extensively studied in tissue cultured cells, nothing is known about their role in the developing Drosophila. We have isolated mutant alleles of ial, encoding Aur-B, and of deterin, encoding Svv. We describe their loss of function phenotypes in the larval neuroblasts and the germline cyst. In the neuroblasts, we find that Svv is required for chromosome congression and segregation, and we directly observed the formation of aneuploid and polyploidy neuroblasts. Moreover, mutant cells are delayed in prometaphase/metaphase, suggesting that the spindle assembly checkpoint is partially active in svv mutant neuroblasts. We further found that Svv is required for proper cytokinesis as no midzone nor midbody are observed in mutant cells. In the germline, we found that loss of either svv or aurB leads to the formation of polyploid cells. In mitosis, these germline cells do condense their DNA although phosphorylation of S10H3 is lost. We are currently analyzing the localization and phosphorylation state of other known AurB substrates in those mitotic cells. Finally, we have started a structure-fonction analysis of Svv by mutating specific residues in a genomic rescue construct. We have begun evaluating the requirement for phosphorylation of Svv by AurB in mitosis by mutagenizing two residues potentially phosphorylated by AurB and testing their ability to rescue the svv mutant phenotype. We found that both variants are able to rescue fly viability and mitosis defects, indicating that none of these residues is individually essential for Svv function. We are now investigating a potential redundancy of these phosphorylation sites.

**I-05 Roles of LIM-domain protein Ajuba in Drosophila development.**

**Sabino, D**; Brown NH; Basto R.
Compartimentation et Dynamique Cellulaires, UMR144 CNRS-Institut Curie, France; Wellcome Trust/Cancer Research UK Gurdon Institute of Developmental Biology and Cancer, University of Cambridge, United Kigdom

Vertebrate Ajuba, a LIM-domain protein, localizes at cell contacts assisting de novo cell-cell adhesion. In addition Ajuba associates with the cytoskeleton and is proposed to be an Aurora-A kinase activator at the centrosome. Despite its apparent importance the knockout of mouse ajuba (JUB) does not cause lethality or fertility defects. This might be due to functional redundancy with other LIM-domain proteins. To overcome the possible redundancy found in mice we searched the role of Drosophila Ajuba (CG11063) the only member of Ajuba-LIMD1 subfamily of proteins in Drosophila. Here we show that Ajuba is extremely dynamic and localizes at cell-cell contacts of epithelial cells, closely to adherens-junctions and actin. Furthermore, Ajuba localizes to the centrosome in specific tissues - larvae brain and imaginal discs. To understand Ajuba's biological function, we generated a Drosophila ajuba mutant. Surprisingly, we found that ajuba mutants are pupae lethal, unlike other adhesion mutants that generally die earlier during embryogenesis. Interestingly, we identified defects in trachea morphogenesis in ajuba mutants, which are currently being characterized. Unexpectedly, we also found defects in the centrosome of neural stem cells in ajuba mutants. In 30% of neuroblasts the centrosomes fail to separate generating cells with one centrosome. Importantly, in these cells the mitotic spindle fails to align along the polarity axis and Miranda, an adapter for cell fate determinant Prospero, fails to be correctly positioned to the cell cortex. Defects in centrosome separation seem to be brain specific since we have not identified defects in other stem cell containing tissues. In conclusion, we show Ajuba has multiple, but tissue-specific, roles during Drosophila development.

**I-06 Control of the endoreplication in Drosophila mechanosensory bristle cell lineage.**

**Salle J**; Gho M; Audibert A
Université Paris 6, UMR-CNRS 7622 Biologie du Développement, 9 quai St Bernard, 75005 Paris

Endoreplication is a variant of the canonical cell cycle characterized by repeated rounds of DNA replication without intervening mitosis leading to polyploidy. In Drosophila, endoreplication occurs in almost all larval tissues and several adult tissues. In order to analyze the mechanism underlying endoreplication, we use mechanosensory organs of Drosophila as a model. Each organ is composed of four cells: two outer cells (the socket and the shaft cells) and two inner cells (the neuron and the sheath cell). The outer cells undergo endoreplication concomitantly with the acquisition of a specific morphology giving rise to the external cuticular structures. Using this system we analyze how replicative state is maintained in cells undergoing terminal differentiation. We found that low level of Cyclin E, that prevents terminal cells division, is sufficient to promote endoreplication. In Addition, we have shown that Cyclin A expression oscillates during endocycle and that loss of Cyclin A leads to a reduction of the ploïdy. These results suggest an involvement of Cyclin A in regulating endoreplication timing and/or replication efficiency. This study is the first report of a role of mitotic cyclin during endocycle regulation in Drosophila.

**J-01 Epithelial polarization**

Andrea Leibfried[1], Robert Fricke[2], Matthew J. Morgan[1], Sven Bogdan[2] and **Yohanns Bellaiche**[1]
[1]Institut CURIE, CNRS, UMR144, Equipe Polarité Cellulaire chez la Drosophile, 26 rue d'Ulm 75248 Paris cedex 05, France. [2]Universität Münster, Institut für Neurobiologie, Badestrasse 9, 4

Integral to the function and morphology of the epithelium is the lattice of cell-cell junctions known as adherens junctions (AJs). AJ stability and plasticity relies on E-Cadherin exocytosis and endocytosis. How E-Cadherin exocytosis and endocytosis is regulated by cell polarity complexes is a central question in the field of epithelial cell polarization. During my lecture, I will present data from Drosophila epithelial cells showing how E-Cadherin exocytosis is regulated by a complex formed of beta-catenin and the exocyst complex. Furthermore I will illustrate how the Par complex composed of Cdc42-Par6-aPKC controls the early E-Cadherin endocytic events. The results shed light on the conserved mechanism regulating epithelial polarization in metazoans.

**J-02 Drosophila Tubulin Binding Cofactor B (TBCB) binds to microtubules, regulates their dynamic and is essential for cell polarity.**

**Benoit B***; Baffet A*; Gourhand V*; Heichette C; Chrétien D; Guichet A*.
*UMR7592, Institut Jacques Monod, Equipe Polarité et Morphogenèse, Paris UMR6026, Interactions cellulaires et Moléculaires, Equipe Tubulin and Interacting Protein, Rennes

Microtubules are polarized polymers crucial for cell architecture, asymmetric transport and division. Microtubule's plus end can be very dynamic and interact with different structures of the cell, like chromosomes or the cortex. In the drosophila oocyte, the microtubule network is essential for axes specification of the adult fly, through asymmetric localisation of polarity determinants. In this study we characterized a new mutant, generated in a screen for polarity genes in the oocyte. We identified the mutation to the uncharacterised CG11242 gene, which is conserved from yeast to human. In mammals, this gene encodes the Tubulin Binding Cofactor B (TBCB), and was shown in vitro to be part of a complex required for alpha and beta Tubulin subunits folding and dimerisation. We showed in vivo and in vitro, that the drosophila TBCB is associated with microtubules and is therefore a MAP. Tbcb mutant ovary cells show a drastic drop of microtubules level, probably triggering the observed polarity defects. Interestingly a set of processes, known as microtubule dependent, is still occurring the mutant ovary, suggesting a selective role of TBCB. Real time imaging of reconstituted microtubule in presence of recombinant TBCB revealed a dramatic increase of microtubule plus end dynamics, with enhanced polymerization speed and catastrophe rate. In fact, TBCB binds the plus end binding protein (EB1) and we found an accumulation of the mutant TBCB protein at microtubule plus ends. In summary, we propose that CG11242 codes for an ortholog of the mammalian chaperone TBCB and identify a new function for this gene: dTBCB regulates MT dynamics in vivo and in vitro through a direct association with MTs, thereby controlling cell polarity during Drosophila development.

**J-03 Polarity orientation memory in neuroblasts.**

**Januschke, J**[1] and Gonzalez, C[1,2].
[1]Cell Division Group, IRB-Barcelona, PCB, c/ Baldiri Reixac 10-12. 08028 Barcelona. Spain. [2]Institucio Catalana de Recerca i Estudis Avançats, Passeig Lluis Companys 23. 08010 Barcelona. Spain

Asymmetrically dividing neuroblasts establish a polarity axis at mitosis in an almost invariant manner over many cell cycles. The mitotic spindle aligns with this polarity axis to ensure segregation of fate determinants to daughter cells. Perturbations in neuroblast polarisation can lead to tumour-like overgrowth. Here we have addressed the question of how neuroblast polarity orientation is kept over consecutive cell cycles, which remains unknown, using a live cell imaging approach. We uncovered a polarity orientation memory effect. Video data revealing the memory effect will be presented and discussed.

**J-04 Drosophila clathrin adaptor AP-1 complex regulates Notch dependent binary cell fate decisions.**

Najate Benhra, Sylvie Lallet, Aurore Dussert, and **Roland Le Borgne**
CNRS UMR 6061-Institut de Génétique et Développement de Rennes Faculté de Médecine 2 av du Pr Léon Bernard CS 34317 35043 Rennes cedex 1 France

During sensory organ precursor (SOP) cell division, Numb segregates asymmetrically into the anterior pIIb daughter cell. Numb establishes a distinct cell fate by promoting the endocytosis of the four-pass transmembrane protein Sanpodo (Spdo) to prevent Notch signalling in the pIIb cell. In an attempt to identify novel membrane trafficking regulators controlling Notch signalling, we isolated the Drosophila AP47 gene encoding the ?1 subunit of the clathrin adaptor AP-1 complex, a protein complex involved in basolateral transport in mammalian polarized epithelial cells. AP47 zygotic mutants die during embryogenesis as reported in C. elegans and mice. Clonal analyses for AP47 or RNAi against any of the four subunits of the AP-1 complex causes a pIIb to pIIa cell fate transformation. Cell fate transformation is not caused by a defect in cell polarity, asymmetric cell division nor in the steady state localization of Notch and its ligands. By contrast, in AP-1 mutant cells Spdo is mislocalized to the apical cortex of pI and both daughter cells where it colocalizes with Notch. AP-1 codistributes predominantly with LqfR, and the T-SNARE Syntaxin16, but also with Rab11+ and HRS+ endosomes suggesting that, like Spdo, AP-1 cycles between the TGN and endosomes. While AP-1 is dispensable for Notch ligands signalling, over expression of Numb suppresses AP-1 loss of function phenotype by promoting Spdo endocytosis and preventing Spdo mislocalization to the apical cortex. Together, our data suggest that Spdo is a cargo for clathrin- and AP-1- coated vesicles and that AP-1 regulates binary cell fate decisions by preventing apical localization of Spdo.

**J-05 Crumbs is required to achieve proper organ size control during Drosophila head development.**

**Emily C.N. Richardson** and Franck Pichaud
MRC Laboratory for Molecular Cell Biology and Cell Biology Unit, University College London, Gower Street, WC1E 6BT, London, United Kingdom

Crumbs (Crb) is a conserved apical polarity determinant, required for adherens junction specification and remodelling during Drosophila development. Interestingly, crb function in maintaining apico-basal polarity appears largely dispensable in the primary epithelium that makes the imaginal discs. Here, we show that crb function is not required for maintaining epithelial integrity during the morphogenesis of the Drosophila head and eye. However, whereas crb mutant heads are properly developed, they are also significantly larger than their wild-type counterparts, as a result of increased cell proliferation. We demonstrate that this excess in cell proliferation can be attributed to an increase in ligand dependent Notch (N) signalling. Moreover, we show that in crb mutant cells, ectopic N activity correlates with an increase in N and Delta endocytosis. These data suggest a role for Crb in modulating endocytosis at the apical epithelial plasma membrane, which we demonstrate is independent of Crb's function in apico-basal polarity. Overall, our work reveals a novel function for Crb in limiting ligand dependent transactivation of the N receptor at the apical epithelial cell membrane.

**J-06 Cell flow reorients the axis of Planar Cell Polarity in the Drosophila wing**

Aigouy, B*; **Sagner, A***; Röper, JC*; Farhadifar, R#; Staple, DB#; Etournay, R*; Jülicher, F#; Eaton, S*;
*Max-Planck-Institute for Molecular Cell Biology and Genetics. #Max-Planck-Institute for the Physics of Complex

Systems Planar Cell Polarity (PCP) proteins form polarized cortical domains that control polarity of external structures like hairs and cilia in vertebrates and invertebrates. The molecular interactions that locally align PCP domains are well studied, but the mechanisms ensuring long-range fidelity of their alignment are still unclear. We investigate the processes that control polarization of PCP domains in the Drosophila wing where PCP signaling regulates the distal orientation of wing hairs and cellular packing. Time-lapse imaging and subsequent quantitative image analysis revealed that planar polarity, as indicated by the localization of PCP proteins, points initially towards the wing margin. Prior to hair outgrowth, this early polarity reorients proximo-distally as dramatic morphogenetic movements reshape the wing. Wing hinge contraction induces characteristic patterns of cell flow, tissue shear and local rotation that elongate and narrow the wing blade. On cellular level these processes cause polarized cell rearrangements that predominantly create new cell interfaces at the anterior and posterior side of each cell and order cell packing. Furthermore, we find that PCP proteins engage in protein clusters that can be tracked for several hours but do not form rapidly on newly formed cell boundaries. Thus, the polarized cell rearrangements lead to an enrichment of PCP clusters at older cell interfaces at the proximal and distal cell side. This novel mechanism utilizes the cellular flows that sculpt the wing to align planar polarity with tissue shape and to ensure long-range fidelity of PCP domain polarization. Therefore, these flows are essential to achieve proper hair orientation in the Drosophila wing and similar mechanisms may generalize to other tissues.

**J-07 Rho signalling during Drosophila cellularization is developmentally controlled by slam.**

**Wenzl, C**; Yan, S; Laupsien, P; Großhans, J
Institut für Biochemie und Molekulare Zellbiologie, Georg-August-Universität Göttingen, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany

Many aspects of cytoskeletal and membrane dynamics are regulated by small GTPases of the Rho family. Essential for proper function of Rho proteins during development is their temporal and spatial control by activating GDP exchange factors (GEFs) and deactivating GTPase-activating-proteins (GAPs), however the regulatory mechanisms controlling these factors are not well understood. Here we analyze how Rho signalling is initiated by localization of RhoGEF2 at the site of membrane invagination during Drosophila cellularization. We show that the PDZ domain is necessary for localization and function of RhoGEF2 and identify Slam as a potential developmental regulator which can physically interact with the PDZ domain of RhoGEF2. Slam regulates RhoGEF2 levels at the furrow canal in a dosage dependent manner and can recruit RhoGEF2 to ectopic sites. Based on these findings we propose that accumulation of Slam at the presumptive invagination site provides a temporal and spatial trigger for RhoGEF2Rho1 signalling.

**K-01 Regulation of Layer-specific targeting in the developing visual system of Drosophila.**

[1,2]Joly, W; [1]Timofeev, K; [1]**Salecker, I**

[1]Division of Molecular Neurobiology, MRC National Institute for Medical Research, London NW7 1AA, United Kingdom - [2]Current address: Génétique du Développement de la Drosophile, Institut de Génétique

A characteristic feature of many areas in the brains of vertebrates and invertebrates is the highly regular organization of underlying neural circuits into modules of layers and columns. Afferent axons have to connect with precise sets of target neurons in each unit to ensure correct information processing in the mature brain. Our understanding, as to how this is achieved during development and what the underlying molecular mechanisms are, is still limited. We use the Drosophila visual system as model to address the question as to how afferent axons are able to target to specific layers during development. The fly visual system comprises eight photoreceptor neuron subtypes (R-cells, R1-R8): R1-R6 axons terminate in the lamina, connecting with sets of lamina neurons within cartridges, while R7 and R8 axons establish connections with target neurons in two of ten neuropil layers (M6 and M3) in the medulla. We will present our findings indicating that the Netrin-Frazzled/DCC/UNC40 guidance system plays a central role in mediating layer-specific targeting of one type of photoreceptor axons in the medulla.

**K-02 Orthodenticle and Kruppel homolog 1 function as part of a post mitotic cellular timer required for photoreceptor morphogenesis in the fly retina.**

**Fichelson, P**; Pichaud, F

Medical Research Council, LMCB, Cell biology unit, Department of Cell & Developmental Biology, University College London.

The temporal regulation of cell differentiation and morphogenesis during development is poorly understood. Drosophila photoreceptors are initially specified in the eye imaginal disc and undergo extensive remodeling during pupal development, including a 90 degree rotation of their apico-basal axis, the rearrangement of their zonula adherens (za), the morphogenesis of their apical membrane and the establishment of neural connections in the optic lobes of the brain. Yet, how the timing of these events is regulated is unknown. We show that the transcription factor Orthodenticle (Otd) controls photoreceptor morphogenesis and neuronal connectivity: otd adult photoreceptors exhibit strong defects in their apical membrane and frequently fail to project to their appropriate target in the brain. This phenotype is associated with a delayed expression of the polarity protein Crumbs that is correlated with a delayed stratification of the apical membrane into a sub-apical domain and the za during pupal development. Using microarray analysis, we found that Otd is involved in the repression of Kruppel homolog 1 (Kr-h1), which encodes a transcription factor. In pupal eyes, loss of function of otd leads to a precocious expression of Kr-h1. Importantly, providing an early pupal pulse of Kr-h1 in an otherwise wild-type background is sufficient to reproduce the developmental delay observed in otd photoreceptors. Conversely, knocking-down the levels of Kr-h1 in otd photoreceptors restores the timing of Crumbs expression and za formation, and partially suppresses the morphological defects observed in adult otd photoreceptors. In conclusion, our work reveals the existence of a novel post mitotic clock regulating the timing of key morphogenetic events during pupal photoreceptor differentiation.

**K-03 The regulation of ciliogenesis: bridging the gap between proneural factors and neuronal differentiation.**

**Jarman, A**; Cachero, S; Simpson, I; zur Lage, P; Ma, L; Newton, F
Centre for Integrative Physiology, University of Edinburgh, Edinburgh, UK

Proneural factors are well known for their role in specification of the precursors of Drosophila sense organs. Much less is known of how their function is linked to the cellular events of subsequent neuronal differentiation. One key process that proneural factors must ultimately initiate is ciliogenesis. This highly conserved process is required to construct the specialised cilia-based dendrite of the sensory neuron. To bridge the gap between proneural factor function and neuronal differentiation, we used cell sorting to isolate chordotonal neural precursors and characterised genome-wide changes in gene expression associated with their development and differentiation. This has revealed the time course of activation of known ciliogenesis genes, including those associated with human disorders such as Bardet-Biedl Syndrome. Surprisingly we find that the expression of a particular subgroup of ciliogenesis genes begins much earlier than anticipated - even before cell cycle exit. Our analysis identifies many new candidate genes for sensory neuron differentiation and ciliary dendrite biogenesis. Mutational analysis of some of these reveals potential ciliary defects. We show that several transcription factors are involved in regulating these differentiation genes, including a novel factor of the forkhead family. In turn, we show that these transcription factors are regulated by the Atonal proneural factor. Thus our data bridge the gap between early neuronal specification by proneural factors and the cellular differentiation program for a particular class of sensory neuron.

**K-04 New functional insights into shot & actin-microtubule dynamics from a novel Drosophila growth cone model.**

**Sanchez-Soriano N.**; Gonçalves-Pimentel C.; Travis M; Beaven R.; Alves-Silva J.; Prokop A.
The University of Manchester, Faculty of Life Sciences

The formation of functional neuronal circuits requires the regulated growth of axons guided by their growth cones (GCs). GC advance requires the coordinated dynamics of F-actin and microtubule (MT) networks. The molecular mechanisms governing these networks, their cross-talk, and their response to extracellular signals are not well understood. Combinatorial genetics available in Drosophila represents an ideal strategy to address such complex interrelations, but suitable fly GC models were strikingly lacking so far. We bridged this gap by establishing a Drosophila GC model using embryonic primary neurons, and we demonstrate its power by presenting novel insights into the regulation of cytoskeletal dynamics. We focus on the actin-MT linking factor Short stop (Shot), a paradigm for its clinically relevant mammalian homologues ACF7 (wound healing) and BPAG1 (skin blistering, nerve degeneration). Using the GC model, 2 independent roles for Shot were identified: one in the organisation of neuronal MTs relevant for axogenesis, and one in F-actin regulation relevant for pathfinding. From ongoing studies we present new insights into domain requirements for MT and EB1 interaction of Shot, and a new functional pathway leading from F-actin regulators through Shot to the organisation of MTs. Notably, insights gained in the Drosophila GC model have a high potential to be translatable into mammalian biology, as demonstrated by numerous readouts resembling those of vertebrates, as well as by our parallel work on ACF7 in mouse neurons. We conclude that Drosophila GCs provide a robust platform on which to study the fundamental mechanisms underlying actin and MT dynamics and their relevance for axonal growth. Support: BBSRC, Wellcome Trust, EU, Royal Commission 1851, FCT.

**K-05 Earmuff maintains restricted potentials of transit amplifying cells in Drosophila.**

Mo Weng[1,3]; Krista L. Golden[3]; Caitlin Gamble[3]; **Cheng-Yu Lee**[1,2,3]
[1]Department of Cell and Developmental Biology, [2]Division of Molecular Medicine and Genetics, Department of Internal Medicine, University of Michigan School of Medicine, Ann Arbor, Michigan 48109, USA

Tissue development and homeostasis depend upon mechanisms that consolidate the identity and functional properties of progenitors. The development and maintenance of many tissues depend upon transit amplifying cells which exhibit limited proliferative and developmental capacity, but how restricted potentials are maintained in these cells is poorly understood. In Drosophila larval brains, highly proliferative type II neuroblasts generate intermediate neuroblasts that display limited proliferation and restricted developmental potentials, resembling vertebrate transit amplifying cells. Here we show that the evolutionarily conserved transcription factor Earmuff restricts proliferation and developmental potentials of intermediate neuroblasts. Although earmuff mutant intermediate neuroblasts exhibit normal apical-basal cortical polarity, they overproliferate and can de-differentiate back into apparently normal type II neuroblasts. Overproliferation of erm mutant intermediate neuroblasts is due to loss of Prospero-dependent cell cycle exit, whereas de-differentiation is due to ectopic Notch signaling. We conclude that Earmuff functions to maintain restricted potentials of intermediate neuroblasts during Drosophila neurogenesis.

**K-06 Biogenesis and function of short regulatory RNAs in Drosophila**

**Eric C. Lai**, Joshua Hagen, Katsutomo Okamura, Hong Duan, Nicolas Robine
Sloan-Kettering Institute 1275 York Ave, Box 2525 New York, NY 10065

miRNAs and siRNAs are the predominant known classes of regulatory RNAs in the 21-24 nt range. Our group has focused attention on the biogenesis and function of miRNAs and siRNAs in the Drosophila system. I will discuss recent advances concerning the diversity of processing pathways that generate miRNAs and siRNAs. In addition, I will describe our phenotypic analysis of selected miRNA gene mutants.

**L-01 The Drosophila ENCODE project and recombineering tags into transcription factors.**

**Kevin P. White**, Nicolas Negre, Rebecca Spokony, Robert Grossman, James Posakony, Steve Miller, Ulrich Wagner, Haruhiko Ishii, Bing Ren, Pouya Kheradpour, Chris Bristow, Manolis Kellis, Ralf Kittler, Hugo Bellen, Steve Russell, Rob White

The Drosophila Encyclopedia of DNA Elements (ENCODE) project aims to create a functional annotation map of the D. melanogaster genome. My team is responsible for mapping regulatory elements, including enhancers, promoters and insulators throughout the genome. We are primarily creating this map by using chromatin immunoprecipitation (ChIP) followed by either hybridization to tiling microarrays (ChIP-chip) or next generation DNA sequencing (Chip-seq). So far we have mapped all known insulator proteins in whole animals, as well as six different chromatin marks, PolII and CBP thoughout 12 stages of the life cycle. We have also mapped approximately 50 site-specific transcription factors at different stages of development. This has led to a first draft map of regulatory elements in the Drosophila genome. We are validating a subset of these elements in reporter gene assays. During the first two years of the project we produced over 300 polyclonal antibodies against a wide range of transcription factors. Unfortunately only about 20% of these antibodies are useful for ChIP. To circumvent this problem, we have recently turned to BAC recombineering to introduce protein tags into transcription factor loci. We are creating a large library of tagged factors which can be used for ChIP, immunoprecipitation followed by mass spectroscopy to identify protein complexes, and live imaging (in the case of tagging with fluorescent proteins). All data are available at www.cistrack.org and at www.modencode.org. Interactive data browsing is available at the Flynet database, https://www.cistrack.org/flynet/.

**L-02 The Twin Spot Generator (TSG) : advances in genetic techniques for clonal analysis in Drosophila.**

**Griffin,Ruth**; Norbert Perrimon
Laboratoire Biochimie et Biophysique des Systemes Integres, France. CNRS, UMR 5092, 17 rue des Martyrs, Grenoble F-38054, France; Genetics Department and Howard Hughes Medical Institute, Harvard Medical School, USA

Real-time lineage tracing in flies has advanced with the development of three new genetic tools to specifically label a progenitor's daughter cells (2009 -Nat. Neurosci. Yu et al.; 12, 947–953; Nat. Methods 6, Griffin, R. et al. 601–603; and Evans, C.J. et al. 605–607). I will briefly present the three techniques, and concentrate on the Twin Spot Generator technique, or TSG, that I developed in the Perrimon laboratory. I will outline new modifications underway to improve signal intensity and new strategic directions in the experimental design. Finally, I will discuss the integration of this technique into the Transgenic RNAi Project (TRiP) underway in the Perrimon lab, whereby we will induce systematic expression of RNAis specifically in one marked daughter cell so as to identify phenotypes by direct comparison with the differentially-marked wildtype sister cell. We hope, thereby, to lay the groundwork for integrating clonal analysis into genome-wide screening in vivo.

**L-03 Visualization of multiple protein interactions in living Drosophila embryos using multicolour fluorescence complementation assay.**

**Hudry, B**; Graba, Y; Merabet, S
IBDML - Institut de Biologie du Développement de Marseille Luminy - UMR 6216 - CNRS France

The bimolecular fluorescence complementation (BiFC) assay enables to directly visualize the formation of protein complexes in a living cell or organism. This approach is based on the recovery of fluorescence when two non fluorescent fragments of a fluorescent protein are brought together by the interaction of two protein partners fused to these fragments. This property of re-association in space proximity is shared by several different fluorescent proteins, allowing the development of the multicolour BiFC that offers the possibility to simultaneously analyse multiple protein interactions. Although widely used in cells and plants, the BiFC still remains an unusual approach for measuring protein interactions in model animal organisms. In particular, BiFC has never been used in fly, chicken or mouse embryos. Here we describe the BiFC parameters during Drosophila embryogenesis, by studying interactions between the Hox protein AbdominalA (AbdA) and its PBC cofactor Extradenticle (Exd) fused to split Venus protein fragments. We extended this analysis to other fluorescent protein variants and to other Hox-Exd, or Hox-Hox interactions. Altogether, this work shows that the multicolour BiFC is a simple and highly sensitive method for analysing protein interactions in living Drosophila embryos.

**L-04 A new approach to induce tissue specific loss-of-function in Drosophila melanogaster.**

**Lorensuhewa, N**; Murray, M; Saint, R
Research School of Biology, Australian National University, Canberra, ACT, 2601, Australia

Here we describe the development of a new loss of function technique in Drosophila, based on the N-end rule pathway. We have termed this technique inducible N-end regulated degradation (iNERD). iNERD is a genetically encoded binary system, which allows the conditional degradation of a protein of interest that has been tagged with a peptide sequence. The conditional degradation of the tagged protein is achieved by the expression of another peptide in a tissue specific manner. This enables the conditional tissue specific degradation of the protein of interest in an otherwise normal organism circumventing a number of limitations that are inherent to currently available techniques. We have used iNERD to dissect the roles of the Rho signalling component Pebble (Pbl) during embryogenesis. iNERD induced clones were able to phenocopy pbl loss of function to generate binucleate cells in an otherwise normal embryo in a tissue specific manner. To our knowledge this is the first demonstration of tissue specific loss of function during early embryogenesis. We are currently using this technique to investigate other novel roles of Pbl during embryogenesis. This technique could potentially be applied generally to the genetic analysis of developmental processes previously out of the reach of traditional loss-of function techniques.

**L-05 Tools for systematic analysis of gene expression patterns.**

**Pavel Tomancak**
Max Planck Institute of Molecular Cell Biology and Genetics

In order to visualize gene expression patterns in live embryos, we constructed two complementary genomic fosmid libraries (FlyFos) for Drosophila melanogaster and Drosophila pseudoobscura that permit seamless modification of large genomic clones by high-throughput recombineering and direct transgenesis. The fosmid transgenes rescue mutant phenotypes, recapitulate endogenous gene expression patterns and in some cases allow imaging of gene products in living animals. The D. pseudoobscura transgenes rescue RNAi phenotypes when introduced into the D. melanogaster genome, providing a convenient control for the specificity of the knockdown. These libraries will, in combination with recombineering technology, enable systematic analysis and manipulation of gene activity across species. We are developing a universal tagging system that enables exchange of tags in vivo without the time-consuming transgenesis. We plan to leverage the astonishing efficiency of our toolkit to establish a genome-wide resource of tagged fosmid transgenes and organize a distributed, communitydriven transgenesis effort (http://transgeneome.mpi-cbg.de/). To image the activity of the transgenes we chose Single Plane Illumination Microscopy (SPIM) that allows in toto imaging of large specimens, such as Drosophila embryos, by acquiring image stacks from multiple angles. We developed an algorithm for registration of multi-angle microscopy acquisitions using fluorescent beads in rigid mounting medium as fiduciary markers. So far we obtained unprecedented 4D SPIM recordings of embryos expressing His-YFP in all cells throughout embryonic development. We show that the approach can be used for imaging and registration of multi-angle acquisition in any imaging modality.

**L-06 Combinatorial transcription factor binding predicts spatio-temporal cis-regulatory activity.**

**Zinzen, R. P.**; Girardot, C.; Gagneur, Julien; Braun, Martina; Furlong, E.E.M.
European Molecular Biology Laboratory, D-69117 Heidelberg, Germany

Development requires the establishment of precise patterns of gene expression, which are primarily controlled via transcription factors (TFs) binding to cis-regulatory modules (CRMs). Although active TF binding sites can now be identified at genomic scales, decoding this extensive regulatory landscape remains a key challenge. To address this we used a novel approach to accurately predict spatio-temporal CRM activity based only on in vivo TF binding and CRM activity data. We generated a high-resolution atlas of CRMs describing their temporal and combinatorial TF occupancy during Drosophila mesoderm development. The binding profiles of CRMs with characterized activity were used to train Support Vector Machines to predict five spatio-temporal expression patterns. Transgenic-reporter assays demonstrate the high accuracy of these predictions and reveal an unanticipated plasticity in TF binding leading to similar expression. This data-driven approach avoids assumptions of TF occupancy based on primary sequence and does not require prior knowledge of TF motifs, function or expression, making it widely applicable.

**L-07 Tools for regulatory genomics in Drosophila**

Stein Aerts, Xiao-Jiang Quan and **Bassem A. Hassan**
Laboratory of Neurogenetics Department of Molecular and Developmental Genetics/VIB11 VIB and KU Leuven School of Medicine

All genetic programs are implemented through the decoding of genomic information by transcription factors, resulting in precise, cell specific patterns of gene expression. Therefore, a comprehensive understanding of any genetic program requires the knowledge of all target genes of all transcription factors involved in a given genetic event. Paradoxically, most target genes of almost all eukaryotic transcription factors remain unknown. In addition, target genes are usually identified sporadically precluding generalizable insight into the the transcriptional output of a specific signal. To address these issues, we have developed novel computational and genetic tools that allow the rapid and highly accurate identification of target genes, target enhancers and even candidate binding sites for any transcription factor of interest. These tools will be available as community-wide resources and will be discussed in this presentation.

**M-01 InSIRT (In Situ Integration for Repeated Targeting), to rapidly dissect the cis-regulatory domains of the bithorax complex in Drosophila.**

Carole Iampietro, Maheshwar Gummalla, Annick Mutero, Robert K. Maeda & **François Karch**
Université de Genève Département de Zoologie et biologie animale 30 quai E Ansermet 1211 Genève Switzerland

In the bithorax-complex (BX-C), early embryonic enhancers (initiators), cell-type specific enhancers, silencers and insulators have all been implicated in the control of the three BX-C homeotic gene expression (Ubx, abd-A and Abd-B). Through years of genetic analysis, a picture has been developing where these elements are arranged in the BX-C in discrete chromatin domains, and that each domain functions through the coordinated efforts of these elements to control the expression of Ubx, abd-A or Abd-B in one parasegment. Although transgenic reporters have been extremely useful in identifying BX-C cis-regulatory elements, it has been difficult to characterize these elements in detail using the reporter assays because of the combinatorial interactions that seem to take place between these elements at their endogenous locus. In order to address the function of BX-C cis-regulatory elements in situ, we have combined homologous recombination and ?C31-mediated integration into a powerful new method, which we call InSIRT (In Situ Integration for Repeated Targeting). Using InSIRT, we have created a series of mutations to dissect the iab-6 cisregulatory domain. Here, we show that a 927bp element, called the iab-6 initiator, is necessary but not sufficient for the activity of the entire iab-6 domain. Furthermore, we show that initiators act as domain control regions to turn on neighboring cis-regulatory elements, but not as an actual enhancer to drive homeotic gene expression. Overall, our results clearly demonstrate how targeted genetic modifications can be used to quickly and systematically dissect a cisregulatory region.

**M-02 Neighbourhood continuity is not necessary for correct gene expression in Drosophila testis.**

**Lisa Meadows** [1,2], Yuk Sang Chan [1], John Roote [1], Steven Russell [1,2]
[1]Department of Genetics and [2]Cambridge Systems Biology Centre, University of Cambridge, Downing Street,

Cambridge, CB2 3EH, UK. It is now widely accepted that gene organisation in eukaryotic genomes is non-random and it is proposed that such organisation may be important for gene expression or genome evolution. In particular, the results of several largescale gene expression analyses in a range of organisms from yeast to human indicate that sets of genes with similar tissue-specific or temporal expression are clustered within the genome in gene expression neighbourhoods. While the existence of neighbourhoods is clearly established, the underlying reason for this facet of genome organisation is currently unclear and there is little experimental evidence that addresses the genomic requisites for neighbourhood organisation. Here we report the targeted disruption of selected gene expression neighbourhoods in the Drosophila genome by the synthesis of precisely mapped chromosomal inversions. Comparing gene expression in individuals carrying inverted chromosomes with their non-inverted but otherwise identical progenitors, we find there are no significant differences in the expression of genes that define the neighbourhoods. Thus models explaining neighbourhood organisation in terms of local sequence interactions, enhancer crosstalk or short-range chromatin effects are unlikely to account for this facet of genome organisation. Our study challenges the notion that neighbourhoods are a feature of eukaryotic genome organisation necessary for correct gene expression.

**M-03 The histone H3.3 chaperone HIRA is involved in heterochromatin formation.**

**Orsi, Guillermo A**; Couble, Pierre; Loppin, Benjamin
Centre de Génétique Moléculaire et Cellulaire, CNRS, Université de Lyon, France

HIRA is a highly conserved histone-interacting protein that has been implicated in replication-independent chromatin assembly. In particular, Hira has been found to be a specific component of the chromatin assembly complex associated with histone variant H3.3, which is implicated in a variety of replication-independent chromatin assembly situations. One of such is sperm chromatin remodeling upon fertilization, when paternal chromatin bearing sperm specific proteins must undergo a genome-wide replication-independent replacement with maternally provided histones, involving H3.3. We have previously shown that HIRA has an essential and specialized role in H3.3 assembly during sperm chromatin remodeling. However, since HIRA is ubiquitously expressed, our next goal was to explore further Hira functions in chromatin dynamics. We found that Hira mutations behave as suppressors of variegation. In order to further evaluate a role of HIRA in heterochromatin formation, we built Hira mosaic tissues. Unexpectedly, we found that mutations of Hira seem to dramatically affect nuclear organization in somatic tissues. We also observed that Hira mutations profoundly modify the distribution of hallmark chromatin markers, including HP1, Fibrillarin and H3K4Me2. Together, our results thus suggest a new role for HIRA in heterochromatin formation and maintenance, perhaps shedding light into a particular replication-independent chromatin assembly mechanism.

**M-04 Histones to protamines: preparing sperm chromatin for fertilisation.**

**Rathke, C**., Barckmann, B., Awe, S.; Renkawitz-Pohl, R.
Philipps-University Marburg, Developmental Biology

A major interspecies feature of postmeiotic spermatid maturation is the reorganisation and compaction of chromatin. During mammalian spermiogenesis somatic histones are replaced first by transition proteins and later by highly basic protamines. Previously, we could show that Drosophila sperm contain protamines as well as Mst77F, representing at least one further chromatin component. Further, we were able to characterise two different transition protein like proteins, which are expressed testis specifically at the time of histone to protamine transition. Both of this mark distinct regions of chromatin. Furthermore, we observed that in Drosophila spermiogenesis histones are degraded at the early canoe stage and that just before this stage, hyperacetylation of histone H4, monoubiquitylation of histone H2A as well as many other histone modifications occur. Using cultured developing spermatid cysts we were able to follow the histone to protamine transition by in-vivo imaging and concluded that it takes about five hours to switch the chromatin configuration from histones to protamines. Additionally, we clearly show that H4 hyperacetylation is essential to allow further development of the haploid male germ cell. Finally, we analysed mutants lacking both protamine genes and demonstrate a 21 % increase in X-ray induced mutation rate of prot? sperm. These data support the long-standing hypothesis, that the switch from a histone- to protamine-based chromatin protects the paternal genome from mutagens. In summary, these analyses show that a lot of processes of chromatin reorganisation are highly conserved between Drosophila and mammalian spermatid maturation.

**M-05 Crosstalks between the piRNA- and the siRNA-dependent repression of transposable elements in the somatic ovarian cells.**

Pelisson, A; Serrano, V; Chambeyron, S; Brun, C & Bucheton, A.
Human Genetics Institute (IGH/CNRS) 141 rue Cardonille 34396 Montpellier Cedex05, France. mailto: pelisson@igh.cnrs.fr

In view of their size distribution, the ovarian small RNAs that are homologous to transposable elements (TEs) mostly correspond to piRNAs (24-28nts), which are processed from the transcripts of 'piRNA cluster loci' in a still rather elusive way. By contrast, the somatic tissues corresponding to the rest of the fly rather contain shorter TE-siRNAs (21-nts) that result from the dicing of duplex RNAs by the Dicer-2 endonuclease. These two categories of small RNAs appear specialized in two quite different TE RNA silencing pathways. However, we will present data suggesting that the siRNA pathway may compensate for the impairment of the piRNA pathway, at least in the somatic ovarian tissues. For instance, repression of the gypsy retroelement in the follicle cells does not require the Dicer-2 function, as long as piRNAs are produced by two doses of the flamenco (flam) piRNA cluster; in heterozygous flam mutant ovaries, however, loss of the Dicer-2 function results in a significant increase of gypsy transcripts and proteins .

**M-06 The endogenous siRNA pathway is involved in heterochromatin formation in Drosophila.**

**Bougé, AL***; Fagegaltier, D*; Berry, B*; Poisot, E**; Sismero, O***; Coppée, JY***; Théodore, L**; Voinnet, O**** and Antoniewski, C*.
*Institut Pasteur, Drosophila Genetics and Epigenetics; CNRS-URA2578, 25 rue du Dr Roux, F75015 Paris, France **CNRS-UMR/UVSQ/EPHE 8159, Université Versailles-St Quentin, 45 avenue des Etats- Unis

A new class of small RNAs (endo-siRNAs) produced from endogenous double-stranded RNA (dsRNA) precursors was recently shown to mediate transposable element (TE) silencing in the Drosophila soma. These endo-siRNAs might play a role in heterochromatin formation, as has been shown in S. pombe for siRNAs derived from repetitive sequences in chromosome pericentromeres. To address this possibility, we used the viral suppressors of RNA silencing B2 and P19. These proteins normally counteract the RNAi host defense by blocking the biogenesis or activity of virus-derived siRNAs. We hypothesized that both proteins would similarly block endo-siRNA processing or function, thereby revealing the contribution of endo-siRNA to heterochromatin formation. Accordingly, P19 as well as a nuclear form of P19 expressed in Drosophila somatic cells were found to sequester TE-derived siRNAs while B2 predominantly bound their longer precursors. Strikingly, B2 or the nuclear form of P19, but not P19, suppressed silencing of heterochromatin gene markers in adult flies, and altered histone H3-K9 methylation as well as chromosomal distribution of histone methyl transferase Su(var)3-9 and Heterochromatin Protein 1 in larvae. Similar effects were observed in dcr2, r2d2 and ago2 mutants. Our findings provide evidence that a nuclear pool of TE derived endo-siRNAs is involved in heterochromatin formation in somatic tissues in Drosophila.

**N-01 A conserved role of folded gastrulation and concertina during amnioproctodeum invagination in insects**

**Siegfried Roth**, Cornelia von Levetzow, Rodrigo Nunes da Fonseca
Institute of developmental biology, University of Cologne, Germany

Mesoderm and posterior midgut invagination represent the most conspicuous morphogenetic movements during gastrulation in Drosophila. The coordinated cell shape changes of both movements require the ligand Folded gastrulation (Fog) and the maternally provided intracellular effector Concertina (Cta). Since Fog is a fast-evolving protein so far found only in Drosophillid genomes, the role of the Fog/Cta pathway in gastrulation was proposed to be restricted to higher Diptera. Using a modified bioinformatic approach we detected genes with low similarity to Drosophila fog in all insect genomes sequenced so far. Functional analysis of fog in the beetle Tribolium castaneum revealed a strict requirement for posterior amniotic fold and hindgut formation as well as a weak requirement for mesoderm invagination. Identical knockdown phenotypes were observed for Tc-cta, indicating that both genes act in the same pathway as in Drosophila. Interestingly, Tc-cta's role in Tribolium is mainly zygotic, since a Tc-cta mutant obtained by insertional mutagenesis shows a zygotic phenotype similar to that cause by RNAi. We speculate that the Fog/Cta pathway is required for amnioproctodeum invagination in a wide range of insect orders and possibly presents one of the "archetypical" characters of insect development.

**N-02 Evolutionarily conserved juvenile hormone signaling controls both holometabolous and hemimetabolous insect metamorphosis.**

**Marek Jindra**[1] and Barbora Konopova[2]
[1]Biology Center ASCR, Branisovska 31, Ceske Budejovice 37005, Czech Republic-
[2]University of South Bohemia, Branisovska 31, Ceske Budejovice 37005, Czech Republic

We study metamorphosis to understand how hormonal cues regulate development and how the signaling has changed during evolution of insects undergoing diverse types of metamorphosis. Differentiation of tissues at metamorphosis is promoted by ecdysteroids and prevented by the morphostatic juvenile hormone (JH). How JH acts at the molecular level to preclude premature metamorphosis is unknown, mainly because JH cannot block initiation of metamorphosis in the highly evolved fly, Drosophila. Therefore, we study insects whose entry to metamorphosis strictly depends on JH absence. Using systemic RNAi, we have found that a homolog of the Drosophila JHresistance gene, Methoprene-tolerant (Met), mediates the morphostatic effect of JH in the red flour beetle (Tribolium castaneum), a species representing basal holometaboly. Here, we show that this function of Met is conserved in an evolutionarily distant hemimetabolan true bug (Pyrrhocoris apterus). Met-deficient bug larvae (or nymphs) undergo precocious metamorphosis, developing adult characters such as articulated wings, genitals and adult-specific pigmentation before reaching their final larval stage. Conversely, loss of Met function prevents exogenous JH from blocking the adult differentiation and inducing an extra larval stage. All these Met RNAi effects correspond with disrupted JH signaling. We further show that Met regulates expression of a JH-responsive gene Krüppel-homolog 1 (Kr-h1), whose loss also induces premature adult morphogenesis in both Tribolium and Pyrrhocoris larvae. Met and Kr-H1 are therefore essential, and currently the only known, transducers of the anti-metamorphic JH signal in insects with both holometabolous and hemimetabolous metamorphosis. Supported by LC07032 from MSMT and A500960906 from GAAV.

**N-03 Comparison of gene expression in Drosophila embryogenesis: evidence for the hourglass model of development.**

**Kalinka, A. T.**, Jastrzebowska, K., Preibisch, S., Gerrard, D., Corcoran, D., Ohler, U., Bergman, C., and Tomancak, P.
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; University of Manchester, Manchester, UK; Duke University, Durham, NC, USA.

The hourglass model of development has been proposed to explain the observed higher variance in early and late metazoan embryogenesis relative to the phylotypic stage; the reduced phenotypic variance in this stage is assumed to result from an increased covariance between genes and developmental processes acting to constrain divergent evolution. Thus far, however, evidence for the existence of a 'waist' in the hourglass is based largely on phenotypic data. To gain insight into the variance of gene expression patterns in Drosophila embryogenesis, we have compared the expression of 3591 developmentally-expressed genes in six Drosophila species in a time-course microarray experiment. Time-points were taken every two hours for the duration of embryogenesis, and three biological replicates were conducted per species (melanogaster, simulans, ananassae, persimilis, pseudoobscura, and virilis). A general linear model was applied to the intensitites to both normalise the data and extract contrasts for gene-byspecies and gene-by-species-by-time interactions. The results show that the majority of interspecies variance in gene expression occurs in early and late development and the 'waist' of the hourglass corresponds to the segment polarity pathway. Gene sets identified as divergent include maternal, metabolic, and transcription factor genes. I discuss the results in relation to the microevolution of insect development.

**N-04 The origin and diversification of Drosophila sex combs.**

**Artyom Kopp**, Olga Barmina, Kohtaro Tanaka, and Chen-Siang Ng
Department of Evolution and Ecology, University of California – Davis, Davis CA 95616, USA

The sex comb of Drosophila is an excellent model for reconstructing the genetic and molecular mechanisms responsible for the origin and diversification of new morphological traits. This male-specific array of modified bristles evolved recently in one Drosophila lineage, and shows dramatic diversity within that lineage. Sex comb evolution is associated with the origin of novel interactions between HOX and sex determination genes. Activity of the sex determination pathway was brought under the control of the HOX code to become segment-specific, while HOX gene expression became sexually dimorphic. At the same time, both HOX and sex determination genes were integrated into the intrasegmental spatial patterning network, and acquired new joint downstream targets. Together, these changes reflect the assembly of a novel sex-specific developmental pathway under sexual selection. Similar sex comb morphologies evolved independently in multiple Drosophila species. Convergent evolution at the phenotypic level reflects convergent changes in the expression of HOX and sex determination genes, involving both independent gains and losses of regulatory interactions. However, the downstream cell differentiation programs have diverged between species, and in some evolutionary lineages similar adult morphologies are produced by different morphogenetic mechanisms. Sex combs often change on microevolutionary timescales, and can differ spectacularly between sibling species and show quantitative variation within species. Our results suggest that quantitative changes in the expression of multiple genes can produce qualitatively novel morphological phenotypes.

**N-05 Nuclear pore protein 160 causes hybrid female sterility and hybrid inviability.**

**Sawamura, K**[1]; Mashino, S[2]; Maehara, K[2]
[1]Graduate School of Life and Environmental Sciences, University of Tsukuba [2]College of Biological Sciences,

University of Tsukuba We previously made an introgression from D. simulans to D. melanogaster and found that homozygous introgression causes female sterility (Sawamura et al., 2000). The recessive gene for female sterility was localized to a small chromosomal region by recombination and deficiency mapping (Sawamura et al., 2004). We presently narrowed the region. Female sterility was complemented by Df(2L)BSC343 but not by Df(2L)BSC242, which means the candidate genes are Gr32a, CG6230, CG14921, Csl4, and Nup160. Females heterozygous for the introgression and PBac{RB}RfC38[e00704] (or P{lacW}l(2)SH2055[SH2055]) were sterile (or semi-sterile), producing only undeveloped eggs when crossed with wild type, although those heterozygous for P{EP}Nup160[EP372] were fertile. As Nuclear pore protein 160 (Nup160) is affected by these insertion mutations, this gene is responsible for the female sterility. It was recently reported that the D. simulans Nup160 is nonfunctional and results in inviability in D. melanogaster/D. simulans hybrids; those carrying hemizygous D. simulans Nup160 are not rescued by the Lethal hybrid rescue (Lhr) mutation of D. simulans (Tang and Presgraves, 2009). We previously demonstrated this hybrid inviability by using the introgression (Sawamura, 2000), and our present results lead us to the same conclusion reported by Tang and Presgraves (2009). We conclude that Nup160 causes not only hybrid inviability but also female sterility in different genotypes. As Hybrid male rescue (Hmr) gene of D. melanogaster is also known to have such dual functions in the D. melanogaster/D. simulans hybrids (Barbash and Ashburner, 2003), particular hybrid incompatibilities can affect more than one hybrid fitness component.

**O-01 dgrasp mRNA localisation in the Drosophila follicle cells during epithelium remodeling.**
**Rabouille, C**
Department of Cell Biology UMC Utrecht The Netherlands

During epithelium remodelling such as the flattening of the Drosophila follicle cells at stage 10B, the alpha integrin subunits are unconventionally secreted through a pathway that is built de novo. This pathway depends on the Golgi protein dGRASP that, at this particular stage of development is ectopically localised at the plasma membrane. We show that the biogenetic process starts with the upregulation and the localisation of a small subset of targeted mRNAs (including dgrasp), near the basal plasma membrane of the remodelling epithelial cells, leading to the local synthesis of the protein. Here, we show that dgrasp mRNA upregulation is triggered prior to the remodeling by the tension of the underlying oocyte and by applied external forces at the basal side of the follicular epithelium. We show that integrins are also involved in dgrasp mRNA upregulation and that tension leads to the recruitment of RhoA to the plasma membrane where it participates to its remodelling but not to the upregulation of dgrasp mRNA. This involves the LIM protein PINCH that can cycle to the nucleus. We propose that integrins are involved in triggering the biogenesis of their own unconventional secretion route that they use to strengthen adhesion and ensure epithelial integrity at the next stages of development, perhaps by acting as mechanosensors of the underlying tension through RhoA and PINCH. Last, we investigated the mechanism underlying the localisation of the dgrasp transcript, both for the cis and trans acting factors. A number of preliminary results will be discussed.

**O-02 Pulling to hold: mechanosensitivity of integrin-mediated muscle attachment.**
**Wellmann, J**; Brown, NH
Gurdon Institute, University of Cambridge, UK

Integrin transmembrane receptors and a complex of associated proteins form adhesive structures connecting the cytoskeleton with the extracellular matrix. A key function of this integrin complex in the Drosophila embryo is to anchor somatic muscles to the epidermis, allowing the transmission of contractile force. To bear this mechanical load, elaboration of muscle attachment sites needs to parallel the increasing strength of muscle contractions during late embryogenesis. To answer whether the development of the adhesion structures at muscle ends is regulated by actomyosin-generated force itself, we used different mutations to reduce muscle contractility. We show by quantitative live imaging that accumulation of all analyzed adhesion components is tension dependent, and that levels of different adhesion proteins are affected differentially by the loss of contractile force. We also show that mechanosensitive mechanisms regulate integrin-mediated adhesion on different levels including transcription and protein recruitment.

**O-03 Analysis of in-vivo dynamics of integrin-mediated adhesion in fly muscles.**

Lin Yuan, Michael J. fairchild, Alexander D. Perkins, **Guy Tanentzapf**
Department of Cellular and Physiological Sciences, Life Science Institute, University of British Columbia, 2350 Health Sciences Mall, Vancouver BC Canada V6T 1Z3

While transient cell adhesion underlies many morphogenetic and cell migration processes during animal development, stable, long-term cell adhesion maintains tissue architecture throughout the life of the organism. It is known that transient cell adhesion requires mechanisms to allow ongoing adhesion complex turnover so that adhesive contacts are constantly being broken and remade. However it is presently not known whether adhesion complex turnover plays a role in the maintenance of stable long-term adhesion. Moreover, it is unclear how the change from transient cell adhesion to long-term cell adhesion at the conclusion of tissue morphogenesis is accomplished. We have studied integrin turnover in the fly myotendinous junctions (MTJs), long-term adhesive contacts that mediate Cell-ECM attachment, using Fluorescence Recovery After Photobleaching (FRAP) in live fly embryos and larva. Our analysis shows that the IAC undergoes turnover in the MTJs and that we can measure the proportion of IAC molecules that undergo turnover using FRAP to calculate the mobile fraction (MF). We will present our analysis of the mechanisms that underlie IAC turnover. Our results show that IAC turnover is mediated by clathrin-dependent endocytosis and that the small GTPase Rab5 regulates the MF of IAC components. Altering the MF of IAC proteins weakened MTJs and gave rise to defects in the MTJ. Moreover, while the MF of IAC proteins is high in the embryo, the MF decreased during larval stages. Based on our data we propose that in the MTJ maintaining tissue integrity requires the presence of a dynamic population, of a set size, of IAC components and that changing the size of this population affects the stability of Cell-ECM adhesion.

**O-04 Interaction between Drosophila bZIP proteins A3-3 and Jun prevents replacement of epithelial cells during metamorphosis.**

Petra Sekyrova[1], Dirk Bohmann[2], Marek Jindra[3] and Mirka Uhlirova[4],
[1]University of South Bohemia, Branisovska 31, Ceske Budejovice 37005, Czech Republic -[2]Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY 14642, USA- [3]Biology Center ASCR, Branisovska 31, Ceske Budejovice 37005, Czech Republic - [4]Institute for Genetics, University of Cologne, Cologne 50674, Germany

Epithelial sheet spreading and fusion underlie important developmental processes. Well-characterized examples of such epithelial morphogenetic events have been provided by studies on Drosophila, and include the embryonic dorsal closure, formation of the adult thorax, and wound healing. All of these processes require the basic regionleucine zipper (bZIP) transcription factors dJun and dFos. Much less is known about morphogenesis of the fly abdomen, which involves replacement of larval epidermal cells (LECs) with adult histoblasts during metamorphosis. Here, we implicate dATF3, the single Drosophila ortholog of the ATF3 and JDP2 bZIP proteins, in abdominal morphogenesis. During the process of epithelial cell replacement, transcription of datf3 is down-regulated. When experimentally sustained, datf3 expression in the LECs leads to accumulation of adherens junctions components such as DE-cadherin, and precludes extrusion of these LECs and their replacement with histoblasts. The adhering LECs consequently prevent the complete closure of the adult abdominal epithelium. This closure defect can be either mimicked and further enhanced by knockdown of the small GTPase RhoA or, conversely, alleviated by stimulating the ecdysone steroid hormone signaling. Both Rho and ecdysone pathways have been previously identified as effectors of the LEC replacement. To elicit its effect, ectopic dATF3 specifically requires its binding partner dJun, as reducing dJun availability suppresses dATF3 gain-of-function phenotypes. Our data thus identify dATF3 as a new functional partner of Drosophila Jun during development.

**O-05 Control of protrusive activity by FGF signaling during mesoderm spreading in gastrulation**

Clark, I.N.B; **Muller, H.A.J**
College of Life Sciences University of Dundee Scotland, U.K.

Tissue interactions and cell rearrangements during gastrulation bring about the establishment and separation of the germ layers. FGF signaling is essential for the dorsolateral spreading of the mesoderm, however it is unclear by which cellular processes this is accomplished and how FGF controls these events on the cellular level. To address these questions we generated fluorescent proteins to visualize cell shape changes during mesoderm spreading in living embryos by two-photon microscopy. We found that FGF signaling is essential for the loss of epithelial junctions during the epithelial-mesenchymal transition (EMT). Unexpectedly the first movement of mesoderm cells during spreading occurs in radial direction towards the ectoderm. The cells form actin-rich dynamic protrusions that intercalate between ectoderm cells, rather than migrating upon their basal surfaces as suggested previously. This radial intercalation movement continues throughout the spreading process, requires the FGF8-like growth factors Pyramus (Pyr) and Thisbe (Ths), and involves recruitment of DE-cadherin to sites of mesoderm-ectoderm intercalations. The dorsal-most cells undergo a repolarization event after which they initiate dorso-lateral migration, which requires Pyr. We find that Pyr is required for normal speed of dorsal edge cells and for the organization of the cell collective during migration. Our data indicate that DE-cadherin mediates adhesion within and between the two germ layers and that FGF signaling drives mesoderm spreading by promoting directional protrusive activity.

**P-01 Studying the signaling mechanisms of the wound response in Drosophila and zebrafish epithelia.**

**Carvalho, L;** Geiger, J; Campos, I; Jacinto, A
Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Portugal

Epithelia act as physical barriers that protect living organisms from the surrounding environment. Therefore, the organization and homeostasis in epithelial tissues requires robust mechanisms that assure their integrity in a variety of biological situations, such as normal cell turnover, inflammation and injury. The cell biology of wound healing has started to be understood but there are still many open questions and the signaling cascades that regulate this process are largely unknown. An intriguing and crucial question is what is the nature of the earliest signals that activate the different events of a wound response. Our main focus is to uncover the signaling pathways required for activation of the wound response. To address this question, our group has performed a screen to identify novel genes involved in the wound healing process in the Drosophila embryonic epithelium. 655 insertional mutants were screened for wound healing phenotypes and 30 genes were identified as novel wound healing genes. We are currently analyzing their function during wound healing and results regarding one of these genes, which until now has remained unstudied in Drosophila, will be presented. In parallel, we are determining whether the signaling mechanisms we find in Drosophila are conserved in vertebrates, by studying the wound healing process in zebrafish embryonic epithelia.

**P-02 The dASPP-Boa complex regulates cell-cell adhesion during Drosophila retinal morphogenesis.**

**Julien Colombani**[1, 2, 3], Paul F. Langton[1, 2, 4], Eunice H. Y. Chan[2], Alexander Wepf[5, 6], Matthias Gstaiger[5, 6] & Nicolas Tapon[2].
1 These authors contributed equally to this work. 2 Apoptosis and Proliferation Control Laboratory, CRUK, LRI, London, UK. 3 Institute of Developmental Biology & Cancer, CNRS, Nice, France.

BACKGROUND: Adherens junctions (AJs) provide structure to epithelial tissues by connecting adjacent cells through homophilic E-Cadherin interactions, and are linked to the actin cytoskeleton via the intermediate binding proteins ???-Catenin and ???-Catenin. Rather than being static structures, AJs are extensively remodelled during development, allowing the cell rearrangements required for morphogenesis. Several ‘non-core’ AJ components have been identified, which modulate AJs to promote this plasticity but are not absolutely required for cell-cell adhesion. RESULTS: We previously identified dASPP as a positive regulator of dCsk (Drosophila C-terminal Src kinase). Here we show that Boa, the Drosophila RASSF8 homolog, binds to dASPP and that this interaction is required for normal dASPP levels. Our genetic and biochemical data suggests that Boa acts in concert with dASPP to promote dCsk activity. Both proteins specifically localize to AJs and are mutually required for each other’s localization. Furthermore, we observe abnormal E-Cadherin localization in mutant pupal retinas, correlating with aberrant cellular arrangements. Loss of dCsk or overexpression of Src elicits similar AJ defects. CONCLUSIONS: Since Src is known to regulate AJs in both Drosophila and mammals, we propose that dASPP and Boa fine-tune cell-cell adhesion during development by directing dCsk and Src activity. We show that the dASPP-Boa interaction is conserved in humans, suggesting that mammalian ASPP1/2 and RASSF8, which are candidate tumor suppressor genes, restrict the activity of the Src proto-oncogene.

**P-03 A RNAi screen in border cells to better understand the molecular mechanisms of cell migration.**

**De Graeve, F.**; Ghiglione, C ; Van de Bor, V ; Cerezo, D ; Noselli, S
IBDC-NICE, CNRS UMR6543, Université de Nice Sophia Antipolis , Parc Valrose, Nice, France.

The formation of complex organs and organisms relies on the concerted development of groups of cells. Aside the well studied migration of individual cells involving Epithelial cell to Mesenchymal cell Transition many other types of cell movements occur during embryo development among which those of groups of interconnected cells which retain their epithelial apical/basal polarization and their adherens junctions to keep the cluster integrity. The border cells (BC) in the Drosophila ovary constitute a genetically tractable example of such type of cell migration in vivo. To identify the factors involved in BC migration, we performed a RNAi screen. Several candidates came out, one example will be illustrated.

**P-04 Vesicular transport, actin reorganisation and ECM adhesion: Git as a hub.**

**Friedlander, J**; Brown, NH
Gurdon Institute (University of Cambridge)

In order to understand the molecular mechanisms of integrin-mediated adhesion, we are studying the function of the G-protein-coupled receptor (GPRC)-kinase-interacting protein, Git. Studies with mammalian cells in culture have shown that Git functions as part of a complex comprising paxillin, p21-activated kinase (PAK) and PAK-interacting exchange factor (PIX). We wished to discover whether Git always works in this complex, or whether it has independent functions. Using the follicular epithelium and the embryonic muscle attachment sites as model systems for integrinmediated adhesion, we are examining the role of each component of the Paxillin-Git-PIX-PAK complex. This has revealed both specific and shared functions for these proteins, supporting the view that they work together, but also demonstrating that they have independent functions. In addition to its role as an adaptor that links PIX to Paxillin, in mammalian cells Git can regulate actin-based protrusions in two ways. It negatively regulates the activity of ARF1 and ARF6 through its ARF-GTPase-activating-protein domain (ArfGAP), thus potentially regulating membrane insertion into extending filopodia. It also positively regulates the activity of the actin regulators Rac1 and Cdc42 through its interaction with PIX, which not only recruits PAK, but functions as a Rac and Cdc42 Guanine-Exchange-Factor (GEF). Consistent with this function we have found that mutant embryos lacking Git have defects in filopodial extensions. To discover the contribution of the different Git activities, we have mutated individual domains of Git. These mutants are clarifying the essential roles of Git in regulating vesicular transport, actin reorganisation and cell-extracellular matrix adhesion.

**P-05 Notch and Prospero Repress Proliferation Following Cyclin E Overexpression in the Drosophila Bristle Lineage.**

Simon F; Fichelson P(1); Gho M and **Audibert A**
Université Pierre et Marie Curie-Paris6, UMR 7622, 9 Quai Saint Bernard, 75005 Paris, France; CNRS, UMR 7622, 9 Quai Saint Bernard, 75005 Paris, France. (1) present address : Medical Research Council

Understanding the mechanisms that coordinate cell proliferation, cell cycle arrest and cell differentiation is essential to address the problem of how “normal??? versus pathological developmental processes take place. In the bristle lineage of the adult fly, we have tested the capacity of post-mitotic cells to re-enter the cell cycle in response to the overexpression of cyclin E. We show that only terminal cells in which the identity is independent of Notch pathway undergo extra divisions after CycE overexpression. Our analysis shows that the responsiveness of cells to forced proliferation depends on both Prospero, a fate determinant, and the level of Notch pathway activity. Our results demonstrate that the terminal quiescent state and differentiation are regulated by two parallel mechanisms acting simultaneously on fate acquisition and cell cycle progression.

**P-06 Setting Centrosome Size in Drosophila.**

**Paul Conduit**; Jordan Raff
Gurdon Institute, Cambridge University, Cambridge, UK

Centrosomes are the main microtubule (MT) organising centres in animal cells. They consist of a pair of barrel shaped centrioles that organise many different proteins, collectively known as the Pericentriolar Matrix (PCM). Centrosomes are dynamic cell organelles that change size throughout the cell cycle. Many centrosome components are now known, but exactly how the centrioles organise the PCM, and how centrosome size is regulated, remains unclear. He we use the Drosophila syncytial embryo to investigate centrosome size control. We show that the levels of Centrosomin (Cnn), a highly conserved PCM component, help determine the levels of other PCM components at the centrosome. Like most centrosomal components, Cnn is in dynamic exchange with a cytoplasmic pool; however, unlike other components, Cnn molecules are added into the centrosome specifically around the centrioles. We reasoned that the balance between the rate of Cnn addition at the centrioles and the rate of Cnn loss from the PCM, may determine centrosome size. To test this hypothesis we attempted to alter the Cnn addition rate by altering the amount of available Cnn in the cytoplasm. Strikingly, we found that increasing the cytoplasmic concentration of Cnn increased the Cnn addition rate, and this subsequently increased centrosome size. Decreasing the cytoplasmic concentration of Cnn decreased centrosome size. Moreover, the Cnn addition rate is cell cycle regulated, and changes in the addition rate correlate with changes in centrosome size. We propose a model of how centrosome size is controlled by the regulation of Cnn addition at the centrioles.

**P-07 Drosophila Cyclin G investigated.**

**Floria Faradji**, Sébastien Bloyer, Neel B. Randsholt and Frédérique Peronnet.
CNRS, UMR 7622; Université Pierre et Marie Curie

Conflicting roles have been assigned to mammalian G-type cyclins (CCNGs). CCNG1 promotes proliferation and is overexpressed in certain cancer cells, suggesting a role in cell division. However, CCNG1 has also been shown to induce G2/M cell arrest and cell death in response to DNA damage. CCNG2 is known as a negative regulator of the cell cycle and induces a G1 arrest. Like CCNG1, CCNG2 is overexpressed in many cancer cells. Although clearly involved in cell cycle regulation, the exact molecular mechanisms by which CCNGs act are thus poorly understood. In this work, Drosophila melanogaster is used as a model to study CCNGs. Fly Cyclin G (CycG) shows homology with both mammalian CCNGs. We find here that overexpression of CycG reduces cell size, decreases cell number and increases the percentage of cells in G1 phase. Based on these data, we hypothesize that CycG coordinates cell growth and cell cycling. We investigate this hypothesis by analyzing the genetic interactions between CycG and major regulators of the cell cycle (Cyclin E, String, Cdc2c… ).

**P-08 Identification of dominant modifiers of bubR1[D1326N] meiotic phenotype.**

**Guimarães, S**.[1]; Sunkel, C.[1,2]; Malmanche, N.[1,3]
1) IBMC, Instituto de Biologia Molecular e Celular, Rua do Campo Alegre 823, 4150 Porto, Portugal 2) ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, 4000 Porto, Portugal

We have previously characterized an EMS-induced separation of function of the BubR1 gene (bubR1[D1326N]) which causes meiotic non-disjunction but has functional Spindle Assembly Checkpoint (SAC) activity during somatic cell division. Using this allele, we demonstrated that meiotic non-disjunction occurs for both exchange and non-exchange homologs and is associated with decreased maintenance of sister chromatid cohesion and of the Synaptonemal Complex (SC) during prophase I progression. In addition, we showed that BubR1 meiotic non-disjunction occurs through Precocious Sister Chromatid Separation (PSCS) and is associated with a deviation of the mendelien ratio toward an increase of Diplo-X versus Nullo-X exceptional progeny. We took advantage of these features to perform a genetic screen design to identify third chromosome deficiencies having a dominant effect on bubR1[D1326N]/bubR1[rev1] meiotic phenotypes. We tested 90 deficiencies covering 84% of the third chromosome euchromatin. Among them, we characterized 33 deficiencies having dominant effect on bubR1[D1326N]/bubR1[rev1] meiotic phenotypes that we classified in 5 groups: (1) enhancer and suppressor of mendelien ratio, (2) enhancer and suppressor of non-disjunction and mendelien ratio, (3) suppressor of non-disjunction, (4) enhancer of non-disjunction and (5) induction of sterility. Furthermore we identified 2 deficiencies inducing a lethal phenotype and affecting bubR1[D1326N] somatic activity.

**P-09 Composition of Condensin complexes in Drosophila melanogaster.**

**Sabine Herzog**, Annika Pfeiffer, Stefan Heidmann
Lehrstuhl für genetik, Universität Bayreuth

Chromosome condensation is a fundamental process required for the faithful transmission of the genetic information during cell division. Central players here are the condensin complexes, which are important for chromosome architecture and segregation. Nevertheless, the exact contribution of these complexes and the underlying molecular mechanisms are not known in detail. We are investigating the structure and function of the condensin I subunit Cap-G in D. melanogaster. In previous studies we could show, that Cap-G contains two independent domains. The N-terminal one mediates mitotic chromatin association whereas the C-terminal one contains an NLS and putative phosphorylation sites. In Drosophila, condensin I is the essentail player during mitosis, whereas the condensin II subunits Cap-H2 and Cap-D3 are dispensable for development to an adult fly and may be involved in meiosis. The third condensin II specific non-SMC-subunit, Cap-G2, has not been described so far in Drosophila. Thus, CapG has been proposed to associate with both condensin complexes. Using a fully functional mRFP tagged Cap-G variant, we analyzed by co-immunoprecipitation and subsequent mass-spectrometry whether Cap-G associates with the two condensin II-specific subunits. While we found with high significance all other condensin I subunits, we failed to detect either Cap-H2 or Cap-D3. In addition, a genetic interaction between Cap-G and Cap-H2 was not evident. Finally, the analysis of proteins associated with EGFP-tagged SMC4, also failed to exhibit association of Cap-D3 and Cap-H2. So our accumulated negative evidence speaks against a participation of Cap-G in a condensin II-like complex in Drosophila and also questions the stable association of Cap-H2 and Cap-D3 with SMC4 in soluble complexes.

**P-10 The role of Imp-alpha2 and Imp-beta interaction in early mitotic divisions of Drosophila embryo.**

Virágh, E. (1,2), Szlanka, T. (1,2), Török, I. (1), Kallakuri, S. (1), **Kiss, I.** (2), Mechler, B.M. (1)
Dept. of Developmental Genetics, German Cancer Research Centre, D- 69120 Heidelberg, Germany - (2) Inst. of Genetics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary

Importin-α proteins are adaptor molecules of nuclear transport, forming complexes with Importin-β and the NLS-bearing proteins which are transferred into the nucleus through the nuclear pore complexes. Recently, a new function of the Imp-α/β complexes has been identified in regulating the dynamics of microtubules during the cell cycle. We studied the role of Imp-α2 in early Drosophila embryogenesis through its interaction with Impβ/Ketel. Eggs from females heterozygous for a null allele of imp-α2 and ketelRE34 were 100% sterile. We had the same result with combinations of ketel null alleles and imp-α2 alleles with altered NLS-binding sites (NLSB- and SNLSB-), while combinations of the null alleles of both genes were always fertile. The sterile interaction was rescueable with wild type transgenic copies of the respective genes. By sequencing ketelRE34, we identified a D725N change in the Imp-α binding domain of the Ketel protein which suppresses the original dominant female sterility by possibly altering the stability of the Imp-α/β heterodimer. By immunostaining we showed that the development of defective embryos was blocked at the early stages because of the formation of abnormal mitotic spindles. Multipolar, barrel-shaped and fused spindles were frequent with missing, extra and/or mislocalised centrosomes. Defects in lamin stucture and chromatin organization were detected as well. These results highlight the importance of the cooperation between Imp-α and Imp-β during early mitotic divisions of Drosophila embryo.

**P-11 Dynamic Behavior of Centromeres during Meiotic Prophase in Drosophila Oocyte.**

**N. Malmanche**, 1 S. Steffensen, 4 R.S. Hawley, 1,2,3 C.E. Sunkel, 4
1) Stowers Institute for Medical Research, Kansas City, Missouri, 64110, USA 2) Department of Physiology, University of Kansas Medical Center, Kansas City, KS 66160, USA 3) American Cancer Society R

In Drosophila female meiosis, the prophase nucleus is organized into two separate domains: the euchromatin and one or two heterochromatic regions known as chromocenters within which the centromeres are localized. However, little is known about the dynamic behavior of centromeres within the chromocenters and the molecular determinants for their organization. Thus, we analyzed both the pairing of homologous centromeres and the aggregation of non-homologous centromere pairs during meiotic prophase. Our results show that the centromeric regions undergo dynamic re-arrangements during prophase I progression while remaining within the chromocenter. The paired centromeres of non-homologous centromeres are often aggregated into one or two foci within the chromocenters in early pachytene oocytes and these aggregations are dissolved as prophase continues. Moreover, by late prophase I (stage 10), homologous centromeres are often visibly separated from each other, an event that may play a critical role in the structural organization and the alignment of bivalents on the anastral meiosis I spindle. We also show that the aggregation of non-homologous centromeres observed in pachytene oocytes requires the function of the Spindle Assembly Checkpoint protein BubR1. Failure to establish centromeric aggregation in bubR1 mutant oocytes is followed by both the fragmentation of the pericentromeric heterochromatin and a failure of proper centromeric orientation at prometaphase-metaphase I. Taken together, our results suggest a multi-step mechanism during prophase I progression to establish the correct organization of centromere and heterochromatin to ensure chromosome segregation during meiosis.

**P-12 Assessing a potential cohesive role for Rad21 and C(2)M during female meiotic divisions in Drosophila.**

**Nagarkar S.**, Lehner CF*, Heidmann S.
Department of Genetics , University of Bayreuth , Bayreuth ,Germany *Institute of Zoology, University of Zurich, Zurich, Switzerland

Accurate chromosome segregation critically depends on the fact that sister chromatids remain physically connected from the time of their synthesis until anaphase. Cohesin, a chromatin-bound protein complex, is required for the physical association of sister chromatids. The mitotic cohesin complex consists of two SMC subunits, Smc1 and Smc3,and two non-SMC subunits Scc1/Rad21 and Scc3. The resolution of sister chromatid cohesion is triggered by the activation of separase which cleaves the Scc1/Rad21 subunit,thereby allowing sister chromatid segregation. During meiosis, Scc1/Rad21 is replaced by the meiosis-specific subunit Rec8 in most eukaryotes. It has been shown that Rec8 is indispensable for meiosis-specific events,such as formation of the synaptonemal complex, reciprocal recombination, and the establishment of pericentromeric cohesion. The Drosophila genome does not contain an obvious Rec8 orthologue. Careful bioinformatic analysis has revealed a candidate, C(2)M, a distant alpha-kleisin family member which plays a role in synaptonemal complex formation and homolog segregation during female meiosis. However,a clear function for C(2)M in cohesion during meiotic divisions could not be shown. We are investigating a potential cohesive role of Rad21 during female meiotic divisions. Ectopic cleavage of a fully functional, TEV-protease cleavable Rad21 variant still allows normal meiotic divisions after in vitro activation, Thus, Rad21 does not appear to be essential for cohesion during meiosis. However, preliminary data reveal a significantly higher proportion of abnormal meiotic figures when Rad21 cleavage is triggered in a C(2)M mutant background. This suggests that Rad21 may function in concert with C(2)M in regulating cohesion during female meiotic divisions.

**P-13 Drosophila Minus is required for cell proliferation and influences Cyclin E turnover.**

**Szuplewski , S**; Sandmann, T; Hietakangas, V; and Cohen, SM.
Temasek Life Sciences Laboratory, 1 Research link, Singapore 117604

Turnover of cyclins plays a major role in oscillatory Cdk activity and control of cell cycle progression. Here we present a novel cell cycle regulator, called minus, which influences Cyclin E turnover in Drosophila. minus mutants produce defects in cell proliferation, some of which are attributable to persistence of Cyclin E. Minus protein can interact physically with Cyclin E and SCF Archipelago/Fbw7/Cdc4 ubiquitin-ligase complex. Minus does not affect dMyc, an other known SCFAgo substrate in Drosophila. We propose that Minus contributes to cell-cycle regulation in part by selectively controlling turnover of Cyclin E.

**P-14 Clathrin adaptors and PI4KII regulate secretory granule biogenesis in the Drosophila salivary gland.**

**Burgess, J** (1,2); Jauregui, M (1); Leventis, P (2); Boulianne, G (1,2); Chang, H (3); Le Borgne, R (4); Kramer, H (5) and Brill, J (1,2)
1. Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada 2. Program in Developmental and Stem Cell Biology, The Hospital for Sick Children, Toronto, Ontario, Canada

Regulated secretion of hormones, digestive enzymes and other biologically active molecules requires formation of secretory granules. To investigate the cellular machinery required for granule formation, we focused on the specialized secretory cells of the larval Drosophila melanogaster salivary gland. These epithelial cells produce mucin-containing 'glue granules' during the last half of the third instar larval stage. We show that the membrane lipid phosphatidylinositol 4-phosphate (PI4P), and the clathrin adaptors AP-1 and EpsinR play a crucial role in glue granule biogenesis. Prior to onset of granule production, PI4KII, AP-1, EpsinR and clathrin co-localize at the TGN. At early stages of granule production, a fluorescently tagged glue protein co-localizes with these markers. Strikingly, nascent granules appear partially coated with AP-1, EpsinR and clathrin. Loss of AP-1 results in a failure to recruit clathrin to the TGN, preventing granule formation. These findings strongly support a model in which sorting for entry into the regulated secretory pathway requires phosphoinositides, coat proteins and clathrin.

**P-15 The SNARE protein Syntaxin 5 is required for copper accumulation in Drosophila.**

**Burke, R**(1); Norgate, M(1); Southon, A(2); Batterham, P(2); Camakaris, J(2)
1) School of Biological Sciences, Monash University, Victoria 3800, Australia. 2) Department of Genetics, The University of Melbourne, Victoria 3010, Australia.

Copper is an essential micronutrient required by animals for the activity of numerous cuproenzymes. While the CTR and ATP7 proteins are well established as highly-conserved copper transporters required for cellular copper uptake and efflux respectively, questions remain as to how trafficking mechanisms distribute copper throughout the cell to the organelles that require it. We determined that Drosophila larvae heterozygous for a loss-of-function allele of the Syntaxin 5 (Syx5) gene have a significantly increased tolerance to a semi-lethal dose of dietary copper, indicating an important role for Syx5 in copper homeostasis. Syx5 encodes a Golgi-localized SNARE protein implicated in both anterograde and retrograde trafficking and required for apical protein localization in the fly. We show that suppression of Syx5 expression in cultured Drosophila S2 cells and mammalian cells results in a significant decrease in cellular copper accumulation. However, we see no change in membrane localization of key copper uptake proteins, nor any impact on ATP7A localization or trafficking in mammalian cells. Using tissue-specific suppression in vivo in Drosophila, we demonstrate that reduction of Syx5 function results in hypopigmented cuticle reminiscent of the copper deficiency phenotype caused by reduction of Ctr1A activity. Genetic interactions indicate that this copper defect is independent of the key copper transport genes Ctr1A and DmATP7. We also present ICP-MS data showing a reduction in the copper content of the copper-tolerant Syx5 - / + flies. This work clearly identifies a central member of the cellular trafficking machinery as being essential for correct copper homeostasis. Further studies should reveal the exact role of Syx5 in copper uptake or efflux.

**P-16 Functional characterization of CG14782, an endosome-associated protein, in Drosophila development.**

**Gailite, I**; Egger-Adam, D; Wodarz, A
Gailite, Wodarz: Department of Stem Cell Biology, University of Goettingen Egger-Adam: Faculty of Biology, University of Konstanz

The PAR/aPKC complex, which consists of Baz (PAR-3), PAR-6 and aPKC, is one of the protein complexes that are crucial for establishment of cell polarity. The PAR/aPKC complex has recently been described to be involved in regulation of endocytosis of apical proteins. In a yeast two-hybrid screen several potential interaction partners of Baz N-terminus were identified, among them a highly conserved protein CG14782. CG14782 shows a polarized localization in epithelial cells and colocalizes with Baz at the apical membrane. CG14782 contains PH and FYVE domains that are implicated in lipid binding. CG14782 specifically binds to phosphoinositides with the highest affinity to PI3P, which is mainly found in early endosomes and multivesicular bodies. Overexpression of GFP-tagged CG14782 leads to formation of enlarged vesicle-like structures in epithelial cells and colocalizes with endocytosed dextran. CG14782-GFP colocalizes with endosome markers and is found in the early endosome fraction in cell fractionation experiments. Therefore both colocalization in epithelial cells and biochemical experiments suggest association of CG14782 with endosomal compartments. CG14782 mutants show genetic interaction with Rab5 and can rescue Rab5 dominant negative phenotype, suggesting a function of CG14782 in regulation of endocytic trafficking.

**P-17 boudin, a Drosophila member of the Ly6 family, codes for a diffusible molecule implicated in septate junction organisation.**

**Hijazi, A** ; Masson, W; Auge, B; Waltzer, L; Haenlin, M; & Roch, F.
Centre de Biologie du Développement, UMR 5547 CNRS/Université Paul sabatier, 118 route de Narbonne, Toulouse -France

We have taken advantage of the model system Drosophila melanogaster to enlarge current knowledge about a poorly characterised family of proteins present in most metazoan genomes, the Ly6 family. This family, including 45 human members, encodes for different glycosylphosphatidylinositol (GPI) anchored proteins and also soluble ligands sharing a structural extracellular domain, called Ly6 domain. Some Ly6 proteins play important roles in cell adhesion, proliferation and migration in different physiological contexts, but only few of them have been characterised in detail. In fact, several human and murine proteins are extensively used as markers for lymphocytes subpopulations and tumoral types, but still have unknown physiological roles. We have identified 36 previously uncharacterised Drosophila proteins as new members of the Ly6 family, setting the basis for their functional analysis in this model organism. We focused in one of these new fly Ly6 proteins, coded by the gene boudin, and characterised its function during development. We found that it is required for tracheal morphogenesis in the fly embryo, contributing to the maintenance of the paracellular barrier and the organisation of septate junctions (SJ) in this tissue. SJ are cell adhesion structures analogous to the vertebrate tight junctions and are instrumental for the properties of epithelia as physiological barriers, regulating the passage of solutes between adjacent cells of the same epithelium. Our work points out for the first time that a Ly6 protein participates in the organisation of these structures during development. Finally, we show that the protein Boudin can diffuse from cell to cell, indicating that this protein could act as a secreted factor during SJ assembly. We are currently investigating the mechanisms of action of this protein, to better understand how SJ are assembled and maintained during development.

**P-18 Planar cell polarity and the cytoskeleton: searching for Rho kinase substrates.**

Dollar, G*; Pfleger+, C; **Jenny, A***
*Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY, USA +Department of Oncological Sciences, Mount Sinai School of Medicine, New York, NY USA

Planar cell polarity (PCP) signaling regulates the establishment of polarity within the plane of an epithelium. Its results are as diverse as the determination of cell fates, the generation of asymmetric, but highly aligned structures (e.g. stereocilia in the human ear or hairs on a fly wing), or the directional migration of cells during convergent extension during vertebrate gastrulation. PCP is governed by the non-canonical Fz/Planar Cell Polarity pathway, in which a Wnt signals through a Frizzled receptor leading to nuclear responses, as well as to cytoskeletal changes mediated by Rho Kinase. In Drosophila, PCP is essential for the orientation of wing hairs and the polarization of the ommatidia in the eye, requiring highly coordinated movement of groups of photoreceptor cells (ommatidial rotation). Thus, key to PCP signaling in flies, and to convergent extension in vertebrates, are cytoskeletal rearrangements and cell migration processes. Central to these is Rho Kinase (Drok in Drosophila), mutations in which or dominant negative forms of which lead to ommatidial rotation and convergent extension/neural tube defects in flies and fish, respectively. We performed a systematic, genome wide screen to identify new Drok substrates using a phosphorylation induced gel-shift assay and are characterizing candidates using in vivo RNAi, mutational analysis and genetic interaction assays. One of the substrates we identified is a formin. Formins regulate actin polymerization dynamics and the Xenopus formin XDAAM was previously shown to be activated by Dishevelled during convergent extension. A functional equivalent of XDAAM has not been identified yet. Indeed, the formin we identified as Drok substrate genetically interacts with Drok and its knock-down causes PCP phenotypes.

**P-19 Interplay between Drosophila importin-α2 and kelch during oogenesis and early embryogenesis.**

**Kallakuri S.**,(1) Szlanka T.,(1,2) Viragh E.,(1,2) Kiss I., (2) and Mechler B.M.(1)
(1) Department of Developmental Genetics, German Cancer Research Centre, D- 69120 Heidelberg, Germany. (2) Institute of Genetics, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

imp-?2 plays a critical role during oogenesis. imp-?2 inactivation leads to ring canal(RG)occlusion and prevents the dumping of the nurse cell cytoplasm into the oocyte by blocking Kelch binding to RGs and the final opening of the RG lumen. Mutations in kelch produce similar RG occlusion. In ovaries Kelch is normally associated with RG whereas Imp-?2 is present in the cytoplasm of germ cells. The absence of physical interaction between Kelch and Imp-?2, as shown by IP, pull-down or Y2H assays, suggests a mechanism by which Imp-?2 binds a factor involved in Kelch deposition on RGs. In imp-?2D14 null females the expression of an imp-?2?IBB transgene defective in Imp-?2 binding to Imp-? allows oogenesis to proceed but affects nuclear divisions in embryos. To test whether kelch may function in embryos we first analysed the distribution of Imp-?2 and Kelch and found that both proteins decorate the mitotic spindle and in particular the interpolar microtubules during anaphase, suggesting a function at the metaphase to anaphase transition point. As a robust genetic interaction was detected between imp-?2 and imp-? we tested whether kelch may interact with imp-?. We found that kel?/imp-?RE34 females, similar to imp-?2D14/imp-?RE34 females, produced normal eggs whose development was arrested during early embryogenesis. The recessive imp-?RE34 allele results from a second site mutation in KetelD, a dominant mutation of imp-?, which changes the Imp-?2/Imp-? binding affinity, and provides a sensitized background to identify interacting genes. Pull-down assays showed a strong physical binding of Kelch with Imp-?. Our data suggests that interplay between Imp-?2, Imp-? and Kelch regulate in addition to RG assembly the dynamics of microtubules during mitosis.

**P-20 Drosophila Cip4 and WASp define a branch of the Cdc42-Par6-aPKC pathway regulating E-Cadherin endocytosis.**

**Leibfried, A** (1); Fricke (2), R; Morgan, MJ(1,3); Bogdan, S (2); Bellaiche, Y (1).
1. Institut CURIE, CNRS, UMR3215/U934, 26 rue d'Ulm, 75248 Paris cedex 05, France. 2. Universität Münster, Institut für Neurobiologie, Badestrasse 9, 48149 Münster, Germany. 3. Present add

Integral to the function and morphology of the epithelium is the lattice of cell-cell junctions known as adherens junctions (AJs). AJ stability and plasticity relies on E-Cadherin exocytosis and endocytosis, but the interplay of possible regulators to orchestrate these events remains to be understood. We show that Cdc42, Par6 or aPKC loss of function is accompanied by the accumulation of apical E-Cad intracellular punctate structures and the disruption of AJs in Drosophila epithelial cells. These punctate structures derive from large and malformed endocytic vesicles that emanate from the AJs; a phenotype that is also observed upon blocking vesicle scission in dynamin mutant cells. We demonstrate that the Drosophila Cdc42 Interacting Protein 4 (Cip4) is a Cdc42 effector that interacts with Dynamin and the Arp2/3 activator WASp in Drosophila. Accordingly, Cip4, WASp or Arp2/3 loss of function also results in defective E-Cadherin endocytosis. Altogether our results show that Cdc42 functions with Par6 and aPKC to regulate E-Cad endocytosis and define Cip4 and WASp as regulators of the early E-Cad endocytic events in epithelial tissue.

**P-21 RNA and protein localisation at the growing tips of Drosophila spermatids.**

**Morris C.**,1 Bhatt A.,2 Barreau C.,3 Benson E.,4 & White-Cooper H.1
1.School of Biosciences, Cardiff University, UK, CF10 3AX 2.BBSRC Polaris House, UK, SN2 1UH - 3. P & M Curie University, Villefrance sur Mer, France, 4. Journal of Biology, 336 Gray's Inn Road, London

A large scale RNA in-situ hybridisation experiment ( >1200 genes) in Drosophila testes, identified a set of 24 genes with an unusual expression pattern. Most testis-specific genes are transcribed in primary spermatocytes; these 24 genes are post meiotically transcribed in elongating spermatids. These transcripts are localised to the distal end of spermatid bundles. The RNA localisation patterns are either 'cups' (shallow staining at the end of the spermatid bundles) or 'comets' (a ball of staining with a speckled tail). This expression is detected just before protamine loading onto chromatin during compaction. To determine comet and cup protein localisation patterns, we used genomic constructs for several comet and cup genes, fusing ORFs to either GFP or RFP. At least 5 independent inserts for each gene have been examined; transgene mRNA localisations recapitulate the endogenous gene expression pattern. Intrigingly, the proteins showed different localisation patterns. All are found in gradients, strongest at the tips of the spermatids, but the extent of the gradient, and the specific protein accumulation patterns, differ. For example, Schuy-GFP is in bright speckles in the cytoplasm, while Wa-cup-GFP is found exclusively in the spermatid mitochondrial derivatives. Further studies will focus on whether the localisation signal is present in the 3' or 5' UTRs or the coding region. The function of these genes and the mechanisms for transcriptional activation and RNA localisation are as yet unknown. However, scotti, a comet gene, is necessary for male fertility. These results suggest that there is significant post-meiotic transcription from several genomic loci in Drosophila spermatids and the transcripts are then localised at the end of the growing spermatid.

**P-22 The Role of Drosophila Endophilin B in Oskar Regulated Endocytosis.**

**Pai, L.M.**; Tsai, Y.M.; Chiang, W.; Li, W.H.; Chang, Y. W.; Tanaka, T.; Nakamura, A.; Li, Y.C.
Institute of Basic Medical Sciences, Chang Gung University, Tao-Yuan, Taiwan. Laboratory for Germline Development, RIKEN Center for Developmental Biology, Kobe, Hyogo 650-0047, Japan

The Endophilin is a highly conserved protein family among many species. All Endophilins are composed of an N-terminal N-BAR domain and a C-terminal SH3 domain. Endophilin A, one member of Endophilin family, can cooperate with dynamin through its SH3 domain and participate in multiple stages in clathrin-coated endocytosis from early membrane invagination to synaptic vesicle uncoating. Endohpilin B, the other member, is reported to involve in the regulation of autophagy. However, the function of Endophilin B in the development of Drosophila is still unknown. Here, we show by immunostaining that the Drosophila Endophilin B localizes at the posterior pole in the oocyte and colocalizes with Oskar. We also find that the localization of D-EndoB is regulated by Oskar, and is dependent on the SH3 domain of Endophilin B. In Drosophila, Oskar determines the polarity of the oocyte and the development of the germ cells. Recent studies reported that the endocytic level and yolk content in the oskar mutant oocyte were reduced. In our study, D-Endophilin B did not affect the pole plasm assembling. However, the endocytic activity at the oocyte posterior significantly decreased in D-endoB null mutant oocyte (endoB54/ endoB54 ). Furthermore, the egg production by endoB54 mutant female is decreased and the percentage of larva hatched from these eggs is less than that from wild type egg. In addition, the yolk content in egg laid by D-endoB mutant females is less than that in wild-type egg. In sum, these data suggest that D-Endophilin B plays a role in regulating fertility through yolk uptake by endocytosis.

**P-23 Zona Pellucida Domain proteins remodel the apical compartment for localized cell shape changes.**

Isabelle Fernandes1,3,*, Hélène Chanut-Delalande1,3,*, Pierre Ferrer1,3, Yvan Latapie1,3, Lucas Waltzer1,3, Markus Affolter2, François Payre1,3,# and Serge Plaza1,3,#
1 Université de Toulouse, UPS; Centre de Biologie du Développement; F-31062, Toulouse, France - -2 Biozentrum der Universität Basel, 4056 Basel, Switzerland. - 3 CNRS, UMR5547, Centre de Biologie du Développement, F-31062, Toulouse, France

The Zona Pellucida Domain (ZPD) defines a conserved family of membrane-anchored matrix proteins that are as yet poorly characterized with respect to their functions during development. Using genetic approaches in flies, we show here that a set of 8 ZPD proteins is required for the localized reorganization of embryonic epidermal cells during morphogenesis. Despite varying degrees of sequence conservation, these ZPD proteins exert specific and non-redundant functions in the remodeling of epidermal cell shape. Each one accumulates in a restricted sub-region of the apical compartment, where it organizes local interactions between the membrane and the extracellular matrix. In addition, ZPD proteins are required to sculpture the actin-rich cell extensions and maintain appropriate organization of the apical compartment. These results on ZPD proteins therefore reveal a functional sub-compartmentalization of the apical membrane and its role in the polarized control of epithelial cell shape during development.

**P-24 Dynamics and homeostasis of the Hedgehog receptor Patched : regulation by NEDD4 Ubiquitin ligases.**

Brigui A., Sanial M. and **Plessis A**.
Institut Jacques Monod, Université Denis Diderot Paris 7 UMR 7592 CNRS, 15 rue Hélène Brion, 75205 Paris Cedex 13 France

The Hedgehog (HH) signalisation pathway plays key roles during development of many animals and its mis-regulation is associated with numerous pathologies. HH action on its target cells involves the 12 transmembrane domains receptor Patched (PTC) and the transducine related protein Smoothened (SMO). In absence of HH, PTC down-regulates the pathway by inhibiting SMO which is endocytosed and targeted to the lysosome. After reception of HH, PTC inhibition is relieved, PTC bounded to HH is endocytosed and degraded in the lysosome while SMO is stabilised at the plasma membrane and activates an intracellular signalling complex which includes the transcription factor Cubitus interruptus (Hooper and Scott, 2005). Numerous links between HH signalling and vesicular traffic have recently emerged and we currently study the role of the C-terminal part of PTC in the regulation of its traffic and activity. We found that PTC interacts with the E3 ubiquitine ligase NEDD4 in a two hybrid screen. We showed that NEDD4 regulates the subcellular localisation of PTC, its accumulation and its activity. Moreover, we identified a second NEDD4-E3 ubiquitin ligase which is also involved in the control of PTC traffic. In parallel, we analysed the role of a conserved PPXY motif that is known to mediate the interaction between NEDD4-E3 ubiquitin ligases and their targets. We found that the PPXY motif is necessary for the normal traffic and accumulation of PTC as well as for its regulation by NEDD4. Since, this domain and the PTC/NEDD4 interaction are conserved in human; their analysis should bring insightful information on the normal and pathological roles of PTC.

**P-25 Characterisation of the role of ubiquitin-like molecules in regulating sub-cellular trafficking of core planar polarity proteins in the Drosophila wing.**

**Searle, E**; Strutt, H; Strutt, D
University of Sheffield Western Bank S10 2TN

Planar polarity describes asymmetry within the plane of an epithelium. It has been studied in detail in the Drosophila wing, where the unidirectional orientation of trichomes depends on correct localisation of a core cassette of planar polarity proteins at the apical junctions (AJ). Frizzled, Dishevelled and Diego localise to the distal edge of the cell, with Strabismus and Prickle at the proximal edge of adjacent cells. Flamingo forms homodimers across the intermembrane space. The mechanism of localisation of the cassette involves vesicular transport of the transmembrane proteins (Flamingo, Frizzled and possibly Strabismus). How is this regulated? Literature on localisation of transmembrane proteins implicates ubiquitination as a trigger for endocytosis and vesicular trafficking. I performed an in vivo screen using RNAi lines to knock down levels of components of the ubiquitination pathway and assayed their effects on polarity in the Drosophila wing. If ubiquitination triggers endocytosis, reducing ubiquitination would be expected to increase levels of core polarity proteins at the AJ. I found a group of genes whose knock down causes a marked accumulation of polarity proteins at the AJ. Surprisingly these corresponded to the Nedd8 conjugating pathway. Nedd8 is a ubiquitin-like molecule which is best characterised in regulation of Cullin based RING E3 ubiquitin ligases. Neddylation of Cullins activates these E3 ligases, increasing ubiquitination of targets. RNAi to Cullin-3 also caused accumulation of polarity proteins at the AJ. I am currently characterising the role of the Nedd8 pathway and Cullin 3 in regulating polarity protein levels and localisation.

**P-26 Endocytic turnover of planar polarity protein complexes controls the establishment of planar polarity in Drosophila.**

**Strutt, H**; Warrington, S; Strutt, D
MRC Centre for Developmental and Biomedical Genetics, and Department of Biomedical Science, University of Sheffield, Western Bank, Sheffield, S10 2TN, UK.

The planar polarity proteins are essential for the correct patterning of many adult tissues in Drosophila. The most well characterised is the adult wing, in which each cell produces a single actin-rich trichome that emerges from the distal cell edge. Prior to hair formation, the planar polarity proteins adopt characteristic asymmetric subcellular localisations at either proximal or distal edges of the apicolateral cell junctions. The planar polarity protein Flamingo localises both proximally and distally, and we have previously shown that distally localised Frizzled and proximally localised Strabismus cooperate to allow stable accumulation of Flamingo at apicolateral junctions. Both Flamingo and Frizzled have also been observed in intracellular vesicles, leading to the suggestion that the levels of polarity proteins at junctions are regulated by endocytic trafficking. In order to understand the contribution of this endocytic trafficking to the asymmetric localisation of polarity proteins, we have directly measured the endocytic turnover of polarity proteins using antibody internalisation assays and FRAP. By comparing rates of turnover in wild type and mutant backgrounds, we find that rates of endocytic trafficking of polarity proteins are increased when they are not in asymmetric complexes. These data support our previously proposed model in which the stability of Flamingo and Frizzled at junctions is dependent on whether they are able to participate in asymmetric junctional complexes. We propose that removal from junctions of Flamingo that is not in stable complexes, combined with directional trafficking of Frizzled and Flamingo to the distal cell edge, drives the establishment of cellular asymmetry.

**P-27 An in vivo RNAi Screen to Look for Genes Involved in Planar Cell Polarity.**

Strutt, H; **Thomas, C**; Thomas-McArthur, V & Strutt, D.
MRC Centre for Developmental and Biomedical Genetics, Department of Biomedical Science, University of Sheffield, Firth Court, Western Bank, Sheffield S10 2TN, UK.

We have performed an in vivo RNAi screen in Drosophila melanogaster to identify new genes involved in planar cell polarity (PCP). Just over 10,000 RNAi lines selected from the VDRC and NIG collections were screened. Lines screened were chosen according to a number of criteria including gene expression patterns, gene ontology and potentially interesting protein domains. As the Drosophila wing is the most widely used and simple model system for studying PCP, we carried out a primary screen in the wing using the MS1096-Gal4 driver at 29°C. Lines giving a wing phenotype in the primary screen were secondary screened at lower temperatures and with a second wing driver. We identified almost 200 lines giving either altered trichome polarity or multiple wing hair phenotypes with one or more driver conditions. In addition, over 1600 lines cause lethality or disrupt wings completely, preventing scoring of potential PCP phenotypes. These lines may prove extremely useful for analysis of multiple aspects of wing development. Investigation of potentially novel genes involved in PCP, and further secondary screens in other tissues are ongoing and will be discussed.

**P-28 In search of germ cell survival factors.**

**Tripathy R.**, Baumbach J., Renault A.
Max Planck Institute for Developmental Biology

The process of cell migration is essential for the development and functioning of most multicellular organisms. Studying germ cell migration in Drosophila embryos gives us a convenient system to understand the dynamics of this process. Usually migration of cells is mediated or controlled by a number of attractive and/or repulsive cues present in their environment. In Drosophila, a number of such cues have been identified as being important for the correct migration of the germ cells from the posterior of the embryo at early development to their final destination in the embryonic gonads. Wunen and Wunen2 provide repulsive cues that cause the germ cells to avoid areas in the soma where they are expressed. Both genes encode lipid phosphate phosphatases and act redundantly. In addition to their role in somatic cells, wun and wun2 are maternally provided and required in germ cells for their survival: In embryos with no maternal wun and wun2, germ cells form normally but all of them die during migration through the midgut. Although in vitro these enzymes can dephosphorylate a number of lipid phosphates such as lysophosphatidic acid and sphingosine-1phosphate, their in vivo substrate is not yet known. To help find the in vivo substrate and also other interactors of wunens, we are performing an enhancer/suppressor screen. We are using the sensitized background of maternal wun2 null embryos, where only half of the number of germ cells survive, and are screening for deficiencies that can suppress or enhance this phenotype. Through this we hope to find genes involved in not only the synthesis of the in vivo substrate but also its modification and transport.

**P-29 The role of the small GTPase Cdc42 in the Drosophila heart during morphogenesis and function.**

**Georg Vogler***, Li Qian**, Jiandong Liu**, and Rolf Bodmer*
*Burnham Institute for Medical Research, La Jolla **Gladstone Institute, University of California, San Francisco

The formation of the heart during Drosophila embryogenesis requires the coordinated alignment of the myocardial and pericardial cells. One important aspect of heart morphogenesis is therefore the establishment and maintenance of cell polarity. A number of factors, including the secreted protein Slit and its receptor Robo localize in a polarized manner during and after heart alignment and further morphogenesis of the heart. It is currently not known which factors create or maintain this polarized distribution and localization of factors. From a screen for genetic interactors with the cardiac determinant tinman we identified the small GTPase Cdc42 as being required for correct adult heart function. In addition, we find that it governs cardioblast cell polarity during embryonic heart morphogenesis. Perturbing cell polarity by either loss of Cdc42 or expression of constitutively active Cdc42 disrupts heart morphogenesis in the embryo. As a consequence, multiple heart lumina are formed. We have screened for factors that influence cardiac cell polarity in a Cdc42dependent manner, in order to understand the mechanisms of cardiac morphogenesis.

**P-30 DaPKC-dependent apical exclusion of Bazooka is required to establish the sub-apical membrane and zonula adherens in a polarized cell.**

**Rhian F. Walther**(1,2) & Eurico de Sá(3) Daniel St Johnston(3) Franck Pichaud(1,2)
(1) MRC Laboratory for Molecular Cell Biology and Cell Biology Unit, (2) University College London, Research Department of Cell & Developmental Biology, Gower Street, WC1E 6BT, London, United Kingdom.

A key step toward establishing epithelial cell apico-basal polarity is the stratification of the nascent apical membrane to generate a sub-apical domain and the zonula adherens (za). In Drosophila, this process depends on the intertwined function of the conserved PDZ-domain factor Bazooka (Baz) and that of the transmembrane protein Crumbs (crb). However, the molecular basis for the interplay between the “Crb and Par-complexes??? is not fully understood. Here, we used the Drosophila photoreceptor as a model epithelial cell and demonstrate that Baz recruits DaPKC-Par6 at the apical membrane via the formation of a transient Baz-DaPKC-Par6 complex. This leads to DaPKC phosphorylation of Baz at the conserved Serine 980, an event enabling the dissociation of DaPKC-Par6 from Baz. We show that Crb and Sdt are driving the exclusion of P-S980-Baz from the nascent sub-apical domain by selectively recruiting the DaPKC-Par6-Cdc42 module. Such molecular sorting is necessary for promoting za assembly and sub-apical membrane morphogenesis. We conclude that in a polarized cell, stratification of the developing apical membrane into a sub-apical domain and a za, is based on the Crb, Sdt-dependent molecular sorting of DaPKC-Par-6 from za-associated P-S980-Baz.

**P-31 Two new components involved in oskar mRNA localization.**

**Waldemar Wojciech**, Claudia Wunderlich, Antoine Guichet and Siegfried Roth
Institute for Developmental Biology, University of Cologne, Germany

Abdomen and germ cell specification in Drosophila requires oskar mRNA localisation to the posterior pole of the oocyte. oskar mRNA is assembled into ribonucleoparticles containing the exon-junction complex (EJC) /nonsense mediated mRNA decay (NMD) proteins Y14, Mago nashi, Barentsz and eIF4AIII which co-localise with oskar to the posterior pole. Here, we show that Thoc5, a component of the THO complex involved in nuclear export of RNPs in yeast and metazoans, and Cdc2-related kinase (Cdc2rk) are involved in oskar mRNA localisation. Interestingly, Thoc5 is not required for nuclear export or nurse cell-to-oocyte transport of oskar mRNA, but rather for transport of oskar mRNA to the posterior pole of the oocyte. Thoc5 interacts with Mago nashi and is necessary for the accumulation of Y14 and Btz at the nurse cell nuclear membrane. However, Thoc5 remains in the nucleus and does not co-localise with Mago nashi and osk at the posterior pole of the oocyte. Thus, Thoc5 presumable takes part in the nuclear assembly of osk RNPs that are competent to interact with the posterior transport machinery. Also the second component, Cdc2rk, is localized to the germline nuclei suggesting an indirect role in osk mRNA transport.

**P-32 Function of slam mRNA for Slam localisation in early Drosophila embryos.**

**Shuling Yan**, Philip Laupsien, Christian Wenzl, Jörg Großhans
Institut für Biochemie und Molekulare Zellbiologie, Universitätsmedizin Göttingen, Germany

Slam is required for germ cell migration and for membrane formation and invagination during cellularisation. Although the biochemical activities of Slam are unknown, its localisation to the furrow canal preceding membrane invagination correlates with its function. Investigating the control of Rho signaling in cellularisation we have found, that Slam recruits RhoGEF2 and Patj to the membrane (see abstract of Wenzl). To further investigate how Slam is localised, unexpectedly we observed costaining of slam mRNA and protein at the furrow canal as well as in basal particles. Moreover they appear to be within the same complex, since slam mRNA coprecipates by Slam immunoprecipation, suggesting that slam mRNA and protein define a novel type of RNP complex. We are able to reconstitute slam RNA particles and localisation by RNA injection into early embryos. By this assay the mRNA localisation signal at the furrow canal was mapped to two regions of each about 400 nt within the coding sequence. Unlike in most other cases of localising RNAs, neither 5'UTR nor 3'UTR is required. To get an understanding the transport mechanisms we also investigate the dynamics of slam particles, which can be visualized and tracked by fluoresence microscopy employing fluoresently labelled RNA or transgenic slam-MS2 constructs.

**P-33 The right place and the right moment: addressing the role of G Protein-Coupled Receptors (GPCRs) in hemocyte migratory behavior.**

**Anna Zaidman-Rémy** and Jennifer Regan, Antonio Jacinto
Instituto de Medicina Molecular, Lisboa (Portugal)

Drosophila hemocytes are multi-task circulating cells that play an active role in innate immunity and tissue homeostasis. The majority of them are plasmatocytes that function as professional phagocytes, removing both pathogenic intruders and apoptotic corpses. One key feature that allows them to achieve these functions is their ability to navigate among tissues and migrate to specific sites in response to various signals. For instance, they are rapidly recruited to wounded epithelia. Plasmatocytes also need to adapt to different environments and requirements throughout the Drosophila life cycle. Indeed, flies undergo drastic changes during development and metamorphosis, creating new constraints for plasmatocyte trafficking and modifying the nature of their tasks. In mammals, GPCRs are implicated in the migratory behavior of immune cells, allowing their navigation through the body, their homing to peculiar tissues and their response to inflammatory signals. GPCRs, their co-player PhosphoInositide 3-kinase (PI3K), and the antagonistic Phosphatase and TENsin homolog (PTEN), are also required for Dictyostelium chemotaxis, suggesting some conservation of their role in cell migration throughout evolution. Interestingly, several Drosophila GPCRs are seen expressed in hemocytes, but their functions have not been documented to date. This prompted us to address systematically the role of GPCRs in Drosophila hemocyte behaviors. Using RNAi in vivo, we screened for GPCR implication both in the hemocyte normal behavioral pattern during development and in their response to induced challenges such as wounds.

**P-34 Horizontal transmission incidents of Wolbachia sp. between Drosophila melanogaster and commensal mites Tyrophagus noxius.**

**Bilousov O**1, Kolodochka L2, Zabludovska S2, Kozeretska I1.
1National Taras Shevchenko University of Kyiv, Department of General and Molecular Genetics, 64 Volodymyrska Street, Kyiv, 01033, Ukraine 2I.I. Schmalhausen Institute of Zoology of National Academy o

Maternally inherited intracellular bacteria from genera Wolbachia, Cardinium and Spiroplasma excite great interest of researchers because of their special features. Recently a possibility of lateral gene transfer from Wolbachia genome into host genome has been shown. Commensal acarid mites Tyrophagus noxius Zachvatkin, 1935 (Sarcoptiformes, Acaroidea, Tyroglyphidae) which can inhabit the same places that the fruit flies Drosophila melanogaster, were investigated as potential participant in process of the horizontal transmission incidents of bacterial infection to the flies. PCR was used to examine the presence of endosymbiotic bacteria in DNA samples. General primers wsp81f and wsp691r that amplify wsp-gene fragments 590 – 636 bp length were taken. This gave us the opportunity to identify Wolbachia strain. For possible lateral transmission establishment, uninfected fruit flies (after antibiotic treatment) had been maintained with infected mites during twelve drosophila generations on standard medium at 25°?. After second generation studied drosophila individuals were not infected. After the 9th generation the presence of Wolbachia sp. wsp-gene was detected in flies of this culture. After the 12th generation flies were still infected by Wolbachia sp. These wsp-gene sequences showed high identity level both in mites and flies. Lateral transmission of Wolbachia could happen through the nutritious medium, because this bacterium can remain viable outside a host cell for up to 1 week. In addition T. noxius is commensal, but not parasite of D.melanogaster. So mite oral apparatus is not adapted for pricking victim’s exterior covers.

**P-35 The Drosophila DPP signal is produced by cleavage of its proprotein at evolutionarily diversified furin recognition sites.**

**Künnapuu, J**; Björkgren, I; Shimmi, O
Institute of Biotechnology, University of Helsinki

Maturation of bone morphogenetic proteins (BMPs) requires cleavage of their precursor proteins by furin-type proprotein convertases. Here, we find that cleavage sites of the BMP2/4/Decapentaplegic (DPP) subfamily have been evolutionarily diversified and can be categorized into four different types. Cnidaria BMP2/4/DPP is considered to be a prototype containing only one furin site. Bilateria BMP2/4/DPP acquired an additional cleavage site with either the combination of minimal-optimal or optimal-optimal furin sites. DPPs belonging to Diptera, such as Drosophila and mosquito, and Lepidoptera of silkworm contain a third cleavage site between the two optimal furin sites. We studied how the three furin sites (FSI – III) of Drosophila DPP coordinate maturation of ligands and contribute to signals in vivo. Combining mutational analysis of furin recognition sites and RNAi experiments, we found that the Drosophila DPP precursor is initially cleaved at an upstream furin recognition site (FSII), with consequent cleavages at two furin sites (FSI and FSIII). Both Dfurin1 and Dfurin2 are involved in the processing of DPP proproteins. Biochemical and genetic analyses using cleavage mutants of DPP suggest the first cleavage at FSII to be critical and enough for long-range DPP signaling. Our data suggest that the Drosophila DPP precursor is cleaved in a different manner from vertebrate BMP4 even though they are functional orthologs. This indicates that the furin cleavage sites in BMP2/4/DPP precursors are tolerant to mutations acquired through evolution and have adjusted to different systems in diversified species. (PNAS 2009 May 26;106(21):8501-6)

**P-36 Evolutionary change of Adh distal factor-1 binding sites in Drosophilidae.**

**Lang, M.**; Juan, E.
Departament de Genètica, Facultat de Biologia, Universitat de Barcelona

There is a growing interest in the evolution of transcription factor binding sites and corresponding functional change of transcriptional regulation. Our intention was to compare orthologous binding sites of the general transcription factor ADF-1 in the promoter regions of the Adh gene in Drosophilidae. ADF-1 binds among others to the distal and proximal promoter regions of the Adh gene in D. melanogaster and only was proven to activate transcription from the distal promoter. We compared ADF-1 binding at orthologous sites of different Drosophilidae species by in vitro and in vivo DNA binding assays. In vitro transcription experiments were carried out in order to approach the functional consequences of cis-regulatory change of ADF-1 binding regions. In D. funebris we detected an expanded footprinted region that contains various binding sites with different binding affinities. The observed ADF-1 binding preferences differ from the ADF-1 binding consensus sequence previously described (England, Heberlein, and Tjian 1990). A new consensus sequence is proposed for high affinity ADF-1 binding sites. Interestingly the expanded D. funebris ADF-1 binding region at the Adh promoter results in an enhanced Adh transcription compared to its orthologous binding region of D. virilis. Evolutionary change of ADF-1 binding regions therefore involves both, rearrangements of multiple adjacent binding sites as well as nucleotide changes within single binding sites that lead to different binding affinities.

**P-37 Host specialization of Drosophila pachea due to mutations in a cholesterol oxygenase gene.**

Sophie Murat[1], Luciano M. Matzkin[2], Takuji Yoshiyama[3], Émilie Guittard[1], Chantal Dauphin-Villemant[1], René Lafont[1], Ryusuke Niwa[3], Hiroshi Kataoka[3] and **Virginie Orgogozo**[1]
1CNRS UMR7622, Université Pierre et Marie Curie, case 24, 9 quai Saint Bernard, 75005 Paris, France. 2University of California, San Diego, USA. 3Department of Integrated Biosciences, Graduat

Numerous species, and perhaps most phytophagous insects, are specialized in a single food resource. However, following long-term association with a particular host, specialist species may eventually loose the ability to feed on alternate hosts. If the loss is irreversible and hosts happen to disappear, then the species is likely to go extinct. Drosophila pachea is specialized on a single cactus host plant named Lophocereus schottii. D. pachea flies require 7-dehydrogenated sterols produced by this cactus to survive and they cannot live on other nearby food sources because they lost the capacity to 7,8-dehydrogenate cholesterol (first enzymatic step of the ecdysone biosynthesis pathway). In insects this enzymatic reaction is catalyzed by the evolutionary conserved Rieske-domain oxygenase Neverland. We investigated here whether D. pachea host specialization is caused by mutations in the neverland gene. We found that several amino acid changes in the Neverland protein are responsible for the loss of 7,8-dehydrogenation in D. pachea. Surprisingly, the neverland gene is still expressed in steroidogenic tissues in D. pachea. In addition, our population genetics study indicates that this locus might have undergone a selective sweep. We provide evidence that the D. pachea Neverland enzyme is able to 4,5-dehydrogenate lathosterol, a sterol present in the host cactus. Together, our data suggest that Neverland may have switched to another function during D. pachea evolution. Since only a few amino acid changes are required to restore Neverland ancestral function, the loss of 7,8-dehydrogenase activity in D. pachea is potentially reversible. D. pachea may thus not be at an evolutionary dead end.

**P-38 oskar and the origin of pole cells.**

**Özüak, O**; Lynch, J; Roth, S
Institute for Developmental Biology, University of Cologne

The specification of the germline is a critical process of animal embryogenesis. In Drosophila, germline fate is conferred upon nuclei that enter a distinct region of the egg cytoplasm at the posterior pole called pole- or germ-plasm. These nuclei are the first to become cellularized (and thereafter are termed pole-cells) and eventually migrate to the embryonic gonad, and give rise to the germline cells of the adult fly. oskar is a critical component of the germ plasm, and in the absence of sufficient levels of this factor neither germ granules nor pole cells are formed, leading to sterile adult flies. oskar orthologs had not been identified in the sequenced genomes of Bombyx, Tribolium, or Apis, leading to the hypothesis that this gene arose within the Dipteran lineage. Interestingly, these species all lack identifiable germ plasm in the early embryo, and do not form pole cells. Here we report the identification of an oskar ortholog in the wasp Nasonia, which appears to have a conserved role in assembling the wasp equivalent of germ granules (oosome), as well as in the production of pole cells. We are currently testing whether Nasonia oskar can function in the ovary and embryo of Drosophila, using multiple localization and ovary expression strategies. The implications of finding an oskar ortholog in Nasonia for the origin of pole cells and maternally assembled germ plasm will be discussed.

**P-39 Comparative mechanics of dorsal closur.**

**Panfilio, K.A.**; Roth, S.
Institute for Developmental Biology, University of Cologne, Gyrhofstraße 17, 50931 Cologne, Germany

Dorsal closure is a well-studied morphogenetic event that occurs during late embryogenesis in Drosophila, and is the process by which the embryonic epidermis extends toward the dorsal midline and replaces the amnioserosa, a vestigial extraembryonic epithelium over the yolk. It is now clear that both the amnioserosa and the dorsal ectoderm participate in Drosophila dorsal closure. Nonetheless, initially the emphasis had been on the embryonic tissue as an active force, as it is this tissue that advances, undergoing marked cell shape changes, and that effects final closure at the midline. In contrast, the amnioserosa reduces in surface area but does not undergo any remarkable structural change. Recent investigation of dorsal closure in other species suggests that the relative roles of the embryonic and extraembryonic tissues are not conserved. In the hemimetabolous milkweed bug, Oncopeltus fasciatus, the amnion exhibits a number of heterogeneous, novel features consistent with a primary role of this tissue in dorsal closure. In contrast, leading edge cells of the embryonic flank do not significantly alter their organization or morphology, but more medial tissue does become arranged into structural supports. Oncopeltus dorsal closure is also relatively slow compared to Drosophila, and features unique embryonic activity during early stages. To assess the degree to which these novelties are common properties of the ancestral amniotic membrane or merely reflect mechanical accommodations in a large egg, dorsal closure is now being investigated in the beetle Tribolium castaneum, whose egg is of intermediate size and retains the ancestral extraembryonic tissue complement.

**P-40 Are transposable elements involved in hybrid dysgenesis in Drosophila?**

**Perepelkina M.P.**; Zakharenko L.P.
Institute of Cytology and Genetics, Siberian Division, Russian Academy of Sciences, pr. Lavrent'eva 10, Novosibirsk, 630090 Russia

The phenomenon of hybrid dysgenesis (HD) was discovered in crosses of flies from genetically or geographically distant Drosophila melanogaster populations. HD manifests itself as various genetic aberrations. Activation of transposable elements (TEs), whose contents in the parental genomes are different, is regarded as the cause of HD. According to our studies, the presence of a full-size hobo is necessary and as usually sufficient for activation of the hobo-element, believed to be the cause of H–E HD. Therefore, we cast doubt on the existence of the H–E HD system. We have shown that the activity of hobo transposition does not decrease with time even in the isogenic strain with completely sequenced genome. The frequency of transposition of the P-element in P–M HD marker strain hybrids in our experiments did not depend on cross direction. In our opinion, another indication that the role of TEs in HD manifestation is exaggerated is that hobo and P are inserted into random loci. It is doubtful that such type of TE transposition, even at the rate 10[ 2] per generation, in a genome containing no more than 100 copies of each TE type, would lead to identical phenotypic consequences in each cross of marker stocks. Another objection against TEs as the key factor of HD is that mutations in genes involved in production of small RNAs, which control TE silencing, do not cause HD. In our opinion, HD is more likely to be caused by unsynchronized functioning of parental genes responsible for gonad formation in the case of P–M HD or emergence of eggs unable to develop in the I–R case. To test this hypothesis, analysis of expression patterns at the developmental stages crucial for HD is needed. Supported by: RFBR, 09 -04-00213a; the Biodiversity Program, 23.29 and 23.30.

**P-41 The evolution of Toll/NF-kB signalling and its role in dorsoventral pattern formation in insects.**

**Dominik Stappert**, Rodrigo Nunes da Fonseca and Siegfried Roth
Institute for Developmental Biology, University of Cologne, Germany

The Toll/NF-kB signalling cascade is crucial for innate immunity in vertebrates and insects. In the long germ insect Drosophila melanogaster Toll/NF-kB signalling has also an essential role in dorsoventral patterning. In the more ancestral short germ insect Tribolium castaneum the Toll/NFkB signalling plays a less extensive role for dorsoventral pattering than in Drosophila. In Drosophila a large number of target genes are regulated by Dm-NF-kB/Dorsal, most of theses genes are also regulated by Dm-Snail and Dm-Twist. The same transcription factors play a crucial role in dorsoventral axis formation in Tribolium, but Tc-twist and Tc-snail knockdown phenotypes already suggest major changes in the way these transcription factors regulate their target genes. These data indicate a high evolutionary plasticity of the dorsoventral gene regulatory network in insects. Therefore we have initiated a detailed comparison of the dorsoventral network of Tribolium to that of Drosophila. The ChIP-seq technique offers an unbiased approach towards this end, which allows the identification of new target genes and the elucidation of the relevant cis-regulatory modules.

**P-42 Cis-regulatory evolution and functional diversification upon gene duplication.**

Alexis Hazbun1,2, Assia Hijazi3, Fernando Roch3, **Élio Sucena**1,2
1- Centro de Biologia do Desenvolvimento, Instituto Gulbenkian de Ciência, Apartado 14, 2781-901 Oeiras, Portugal. 2- Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1700 Lisboa, Portugal. 3- Université de Toulouse UPS, Centre de Biologie du Développement, CNRS UMR 5547,Bâtiment 4R3, 118 route de Narbonne, F-31062 Toulouse,

The profusion of paralogue gene that we observed in all metazoan genomes is the signature of the many gene duplication events that took place in the course of evolution. One way by which such novelty may arise is by the acquisition of new regulatory elements in a gene duplicate capable of driving expression in a new developmental context absent from the ancestral function. We identified in Drosophila the three Finger Domain Protein (TFDP) gene family which codes for membrane receptors and ligands containing a single recognisable extracellular domain involved in protein-protein interactions. Its members have duplicated several times in the Drosophila lineage. While Drosophila genomes encode for 35 TFDP family members, which are often grouped in genomic clusters, in others insects this family has considerably fewer representatives: around 16 in Anopheles and 14 in Tribolium. Documenting the expression patterns of all members of clusters III and V of D. melanogaster, we found that some paralogues share the same expression whereas others have evolved different expression profiles. This preliminary characterization of the pattern of evolution of recently generated paralogues, constitutes a descriptive stepping-stone upon which we may generate hypotheses regarding functional evolution and the emergence of novelty.

**P-43 Adaptative variation in Drosophila melanogaster body size.**

Viginier B; Gibert P.; Yvert G.; David J.R.; Veuille M.; **Terzian C**
INRA/ENVL/Université Lyon 1/Ecole Pratique des Hautes Etudes/CNRS/MNHN/ENS Lyon

Clinal variation in size has been reported for various species of Drosophila and on different continents: body size increases with latitude. It has been known for a long time that part of this variation is genetic: when Drosophila sampled from different populations along a latitudinal gradient are kept under the same conditions, the difference in body size persists. Moreover, the repeatability of such clines implies that they are caused by natural selection : clinal variation in body size is adaptive. In this study, we analyse the latitudinal trends in wing area, cell size and cell number for two isofemale strains known to correspond to the ends of a latitudinal cline: an isofemale line from Draveil, a European temperate population and an isofemale line from Sao Tomé, an Afrotropical population. Draveil and Sao Tomé individuals differ in cell size and cell number: the increase in wing size with latitude is associated with an increase of cell number and cell size. Then, we evaluate whole-genome transcriptional profiles that reflect modifications in gene expression between these two isofemale strains. A genomic scan have been applied to detect selection surrounding the RpL27 gene: ten non-coding sequences (each between 400 and 700 bp length) have been sequenced from 6 Draveil and 6 Sao Tomé independant isofemales strains. The data indicate that a selective sweep occurred at the LpR2.2 sequence (intron 2 of lipophorin receptor 2; HKA test, $P<0.05$) in the Draveil population. Moreover, a bulk segregation analysis using SNPs into the Lpr2.2 sequence indicates that these markers are segregating with the “wing length??? phenotype in F2, suggesting that Lpr2 could be a major QTL.

**P-44 Transcriptional networks during Drosophila mesoderm development**

**Junaid Akhtar**, Martina Braun, Charles Girardot and Eileen E. Furlong
EMBL, Myerhofstrasse 1, Heidelberg D69117, Germany

Cell-fate decisions and tissue specification are controlled through the integration of signalling and transcriptional regulatory networks. Several transcription factors typically impinge on a particular enhancer element or cis regulatory module (CRM) to regulate genee expression in space and time. These regulatory networks are still poorly understood. The Furlong-lab has conducted a series of ChIP-on-chip experiments with interconnected transcription factors (TFs) at multiple stages of development. These studies identified a large number of CRMs that drive expression in specific spatial and temporal domains. To identify the complete repertoire of TFs that are regulating these modules we have developed a high throughput luciferase assay screen to determine the activity of the majority of predicted sequence specific transcription factors (TFs). The assay is performed in 384 well plates, using robotic liquid handling. A total of 220 CRMs will be assayed against 352 TFs, providing an extensive activity matrix that contains information about the direction of regulatory change (activation or repression). Regulatory connections will be further confirmed by EMSA, transgenic CRM-reporter assays and genetic phenotypic analysis. The resulting regulatory network will provide a comprehensive understanding of TF contribution to CRM activity and identify new regulatory components.

**P-45 The transcriptional signature of wing disc regeneration reveals integration of JNK and Notch pathways.**

**Enrique Blanco**, Marina Ruiz-Romero, Florenci Serras and Montserrat Corominas
Departament de Genètica, and Institut de Biomedicina de la Universitat de Barcelona (IBUB), Diagonal 645, 08028 Barcelona, Catalonia, Spain.

Regeneration, the ability of an organism to rebuild a body part that has been damaged, can be studied at the molecular level using model organisms. Drosophila imaginal discs, the larval primordia of adult cuticular structures, are capable of undergoing regenerative growth after transplantation and in vivo culture in the adult abdomen. Using transcriptomics at different time points after injury, we studied the regenerative behavior of fragmented wing imaginal discs implanted into adult females. First, through comparison of cut versus intact discs, we identified effects of implantation on the regeneration process. We next compared the transcriptomes of discs at 0h, 24h and 72h after fragmentation elaborating a catalogue of genes involved in wing disc regeneration: genes with differential expression within the first 24h, genes with differential expression between 24 and 72h, genes with a characteristic different pattern between both time points, and genes with a sustained expression pattern during regeneration. Among these genes, we identified members of the JNK and Notch signaling pathways. Using bioinformatics to inspect potential binding sites for downstream transcription factors, we identified regulatory targets conserved in multiple Drosophilas, which allowed the establishment of a putative relationship between members of different classes. Experimental data from genetic mutants provide evidence for the requirement of selected genes in the regeneration process. Our findings indicate that combinatorial signaling by JNK and Notch regulatory cascades through a common circuitry of genes governs the regeneration of wing imaginal discs.

**P-46 Understanding and modeling the mechanisms coordinating dorsal closure of Drosophila melanogaster.**

**Dubé, N***, Solon, J*#, Brunner, D*
*European Molecular Biology Laboratory, Cell Biology and Biophysics Unit, Meyerhofstrasse 1, 69117 Heidelberg, Germany. #Centro de Regulatio Genomica (CRG), Cell and Developmental Biology Programme, C

Dorsal closure (DC) is a prominent morphogenetic tissue movement in Drosophila melanogaster embryogenesis. To close the opening left in the epidermis after germ band retraction, the epidermal cells move dorsally. They exhibit dynamic cellular protrusions at the leading edge, namely lamellipodia and filopodia. When the filopodia from both sides are close enough to contact, they recognize their matching partner at the dorsal midline and adhere to close the embryo, a process called zippering. This is followed by the formation of permanent cell-adhesion structures. Many force generating processes contribute to this morphogenetic movement: a) pulsed amnioserosa cell contractions (1); b) the contractile activity of an actin cable surrounding the opening (1); c) filopodia zippering (2); and d) amnioserosa apoptosis (3). To understand how these forces are coordinated, we use wild-type and mutant embryos combined with live imaging of actin, microtubules, and cell borders to study the dynamics of DC. The mutants used show defects in filopodia activity or actin cable tension for instance. Our implemented analysis tool allows us to quantify the velocity of closure and we also measure the protrusion length, lifetime, directionality, and density. These values are integrated into an in silico model that includes several forces involved in DC. The parameters are then modified to assess the role(s) of the forces at each step of DC. References: 1. Solon J, Kaya-Copur A, Colombelli J, Brunner D. (2009). Cell 137:1331-42. 2. Jankovics F, Brunner D. (2006). Dev Cell 11:375-85. 3. Toyama Y, Peralta XG, Wells AR, Kiehart DP, Edwards GS. (2008). Science 321:1683-6.

**P-47 Expanded recombineering toolkit for cross species genome manipulation.**

**Ejsmont RK**, Sarov M, Tomancak P
Max Planck Institute for Molecular Cell Biology and Genetics

We developed a toolkit for high-throughput gene engineering in flies, that provides means for creating faithful in vivo reporters of gene expression during Drosophila melanogaster development. The cornerstone of the toolkit is a fosmid genomic library enabling highthroughput recombineering and ?C31 mediated site-specific transgenesis. The dominant, 3xP3 dsRed fly selectable marker on the fosmid backbone allows, in principle, transgenesis of the fosmid clones into any nonmelanogaster species. In order to extend the capabilities of the gene engineering toolkit to include 'evo-devo' studies, we generated genomic fosmid library for D. pseudoobscura. The libraries for both species were constructed in the pFlyFos vector allowing for recombineering modification and ?C31 transgenesis of non-melanogaster genomic loci into D. melanogaster. We are developing a PCR pooling strategy to identify clones for a specific gene from the libraries without extensive clone sequencing and mapping. The clones from these libraries will be primarily used for crossspecies gene expression studies.

**P-48 Epithelial organization revealed by a network of cellular contacts.**

**Luis M. Escudero**, Luciano da F. Costa, Anna Kicheva, James Briscoe Matthew Freeman and M. Madan Babu

Organs are largely sculpted from cells organised in epithelia. The emergence of differences in epithelial organisation is the first step towards establishment of the architecture of an organ. Understanding these changes and how they are initiated is a fundamental question in developmental biology but is hampered by a lack of methods to characterise epithelial organisation. We have developed an objective method to characterise epithelia by integrating information on geometric features of individual cells and their spatial organisation. We represent each epithelium as a network with cells as nodes and cell contacts as links. We demonstrate that the underlying pattern of cell contacts (i.e., network topology) is largely invariant between individuals in a population and that a network representation is important to distinguish objectively the epithelial primordia separated temporally, spatially or coming from different species. Finally, we perturb epithelial organization by reducing the levels of myosin II, a key structural protein, and show that it disrupts both cell morphology and robustness of the cell-contact network, making it different from wild-type epithelia. This suggests that altering levels of myosin II within normal limits could provide the signature pattern to different epithelial types. This framework allows quantification of subtle effects upon gene mutation and can be adapted to characterise other biological systems.

**P-49 Prediction and Global Analysis of BMP Regulated Genes.**

**Fuchs, A**[1]; Bardet, AF[2]; Vuilleumier, R[1]; Charbonnier, E[1]; Stark, A[2] and Pyrowolakis, G[1]
1) Department of Developmental Biology, Institute of Biology I, University of Freiburg, Freiburg, Germany 2) Research Institute of Molecular Pathology, IMP, Vienna, Austria

The TGF-beta/BMP family member Decapentaplegic (Dpp) is a key regulator of developmental patterning and growth in Drosophila. Binding of Dpp to its receptors triggers the activation of the intracellular Smad pathway and subsequent activation or repression of target genes. The repressive branch of Dpp signaling utilizes short motifs called silencer elements (SEs) which were first found in a regulatory region of the brinker gene. A specific arrangement of Mad and Medea binding sites permits signal-induced recruitment of the co-factor Schnurri leading to the repression of the target gene. Brinker is a default repressor of the Dpp signaling pathway and the removal of Brinker allows for transcriptional activation of most of the Dpp target genes. A more recent analysis of the regulatory region of dad gene, another intrinsic component of the pathway, revealed an analogous mechanism to activate target genes. A short motif called activating element (AE) integrates both repressive input by Brinker as well as Dpp dependent activating input by Mad and Medea. Both motifs can serve as a tool to predict targets of Dpp signaling. However, only few examples for such regulation were shown so far. In an attempt to gain a more general view on the Dpp pathway we performed a whole genome in silico screen for SEs and AEs combined with analysis of evolutionary conservation in 12 Drosophila species. Potential targets harboring conserved motifs show enrichment for developmental processes regulated by Dpp signaling supporting the predictive power of the motifs. We are currently proceeding with a large scale transgenic analysis of targets involved in oogenesis, embryogenesis and wing development. This approach should provide a deeper understanding of the events downstream to Dpp signaling.

**P-50 Trans-flyer, a new startup company for the production of Drosophila transgenics.**

**Martina Infanti**, Samuele Giberti and Maria Capovilla
Trans-flyer, Dept. of Biology and Evolution, University of Ferrara, Italy

“Trans-flyer" is a startup company based in Ferrara (Italy) that produces transgenic fruitflies for research laboratories in Italy and in the rest of Europe. The project, supported by European Spinner funds, is carried out by a group of three Italian researchers coming from diverse scientific disciplines. The need to produce transgenic flies for our laboratory, as for many other Italian and European laboratories, drove us to ask the question “Why not set up a company in Italy for the production and sale of Drosophila transgenics????. Hoping that the Drosophila research community would welcome this idea of company, we decided to undertake this project taking advantage of the long-standing experience of its scientific advisor Maria Capovilla, who has been working with flies since the end of the 1980s. We are currently producing transgenic flies by Pelement-mediated transformation, but we are also setting up the site-specific PhiC31 integrasemediated trangenesis technique. In a second phase, we plan to offer additional services, such as cloning of the constructs to be injected and staining of embryos transgenic for reporter genes by immunohistochemistry or in situ hybridization. Overall this project is aimed at providing transgenic flies initially to the Italian Drosophila community, simplifying the bureaucratic procedures of importation of live animals from foreign countries, but is also aimed at gaining a slice of the European market providing competitive prices and short delivery times, in addition to minimizing importation problems. At our poster, you will have the opportunity to meet us and to discuss with us your specific needs and requests.

**P-51 Getting to the heart of cardioblast specification.**

**Junion, G**; Spivakov, M; Braun, M; Furlong, EE
European Molecular Biology Laboratory, Heidelberg, Germany European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, United Kingdom

Studies during the past decade, in species as diverse as Mouse, Chicken, Zebrafish and Drosophila, revealed that the genetic cascades regulating heart development are highly conserved during evolution. For example, the transcription factors Tinman (Nkx family), Dorsocross (Tbox family) and Pannier (GATA family) act as key regulators of heart development in all organisms studied to date. Despite their essential role very few of their direct target genes are known and the architecture of their cis-regulatory modules (CRMs) remains a mystery. In Drosophila embryos, cardiac specification occurs in the dorsal mesoderm at the intersection of Wg and Dpp pathway. We have performed chromatin immunoprecipitation followed by microarray experiments (ChIP-onchip) with Tinman, Dorsocross and Pannier as well as with Wg and Dpp effectors: dTCF and pMad respectively, to identify combinatorially regulated CRMs driving cardioblast gene expression. The corresponding direct target genes will provide a molecular understanding of how these transcription factors drive cardioblast specification. The results obtained will represent an initial global view of the regulatory network governing cardioblast specification in flies, and will help us to identify regulatory modules that are likely to be conserved in all species with a heart.

**P-52 Comparison of RNA in-situ vs. microarray analysis of gene expression in Drosophila imaginal discs.**

**Mejstrik, P**; Ahlfeld, P; Dahmann, C; Tomancak, P;
Max Planck Instituteof Molecular Cell Biology and Genetic, Dresden, Germany

Because cell fate specification and tissue differentiation during development are largely driven by the regulation of gene expression, the comprehensive analysis of the spatial and temporal expression pattern of all genes within an organism is an important step towards understanding the regulatory networks underlying its development. Gene expression patterns in developing animals have been determined at large scale by two methods: DNA microarrays and RNA in situ hybridization (ISH). DNA microarrays can provide quantitative data on the relative changes in gene expression over time in larval imaginal discs (ID). However, the spatial regulation of gene expression within ID can be revealed only by ISH. By identifying genes that are co-expressed in space and time, we can also predict the function of novel genes based on the function of known coexpressed genes. We have just completed a genome-wide RNA in situ hybridization screen and we are now determining and documenting the temporal and spatial expression profile of more than half of Drosophila melanogaster genes. Moreover we performed a comparative DNA microarray survey to measure expression level of all genes in wing, leg, haltere and eye ID. Analysis of this microarray dataset indicates that we can readily identify genes restricted to only certain types of discs and reveal some new interesting genes for which we are now preparing a specific ISH probe. For genes expressed in all discs the correlation between microarray measurements and patterned, not ubiquitous, expression is unreliable but it help us to find genes for which our in situ experiments didn’t work in proper manner.

**P-53 FlyBase : a database for the Drosophila research community.**

**Stefancsik, R**; Brown, N; The FlyBase Consortium <http://flybase.org>;
FlyBase-Cambridge, Department of Genetics, University of Cambridge, Cambridge, United Kingdom

FlyBase is an ever growing database that collects and organises information on the genes and genomes of Drosophilidae with a main focus on D. melanogaster. Various query tools allow interrogation of FlyBase through DNA or protein sequence, by gene or mutant name, or through terms from controlled vocabularies (ontologies) used to capture functional, phenotypic, and anatomical data. One of the most important sources of information for FlyBase is the Drosophila research literature. More than 2000 Drosophila papers are published each year, and FlyBase curators can not capture all data from all papers. As an active researcher, there are a couple of ways that you can help FlyBase continue to provide the most useful information for the Drosophila community in a timely manner. First, you can accelerate the incorporation of your paper into FlyBase by using a new data submission tool that you can find under the 'Tools->Data Submission>Add a paper' menu on our website <http://flybase.org/submission/publication/>. Second, you can tell us how we can improve our current curation criteria, and how curated data are displayed and searched on the FlyBase website. For example, which data types are the most important for us to capture during curation and display on the website? Please contact us with your comments at <http://flybase.org/cgi-bin/mailto-fbhelp.html> or speak to one of the FlyBase staff at this meeting.

**P-54 An advanced LexA-based binary transcriptional system with a refined selection of LexA transactivators for Drosophila.**

**Yagi, R**; Mayer, F; Basler, K
Institute of Molecular Biology, University of Zurich, 8057 Zurich, Switzerland

Drosophila is an important model organism for studying biology and disease, and continuously many tools are being developed for manipulating gene functions in this system. The Gal4-Upstream Activating Sequence (UAS) system is one such tool and it is the most frequently used tool to manipulate dosage and function of a gene in single population of cells. More sophisticated experiments aimed at deciphering the interactions between two genes expressed in distinct populations of cells/tissues have not been possible, as this necessitates manipulating the two genes in the two cell populations with two independent binary expression systems. A suitable second binary expression system has been lacking until now. Several groups have reported the development of LexA based systems; but these published systems had potential problems, including toxicity and weak activity. We have developed and optimized a LexA-based binary expression system that eliminates these problems. Our system comprises a tissue-specific artificial transactivator with a LexA DNA-binding domain which activates transgene expression by binding to a specific sequence called lexA operator. By using less toxic LexA trans-activators, we have reduced artificial effects that arise from manipulating gene function by this manner. In addition, we have developed a selection of LexA transactivators with different activity (e.g., one comparable to Gal4 and the other less active than Gal4). This allows the user to choose an appropriate activity balance with the Gal4-UAS system in order to examine the interaction between two genes whose expression is driven by the different binary expression systems. The optimized system facilitates precise analysis of signaling networks and complicated biological phenomena in Drosophila.

**P-55 Proteome profiling of the male and female reproductive organs of Drosophila melanogaster using nanoIC linear ion trap TOF mass spectrometer.**

Takemori, T; **Yamamoto, M-T**;
Drosophila Genetic Resource Center, Kyoto Institute of Technology

We have conducted proteomic analysis of the reproductive organs using a combination of SDSPAGE and nanoLC-MS system for protein expression profiling of the reproductive systems. After dissection of the wild type Canton-S male and female reproductive organs, each sample was homogenized in SDS lysis solution. The extracted proteins were separated by SDS-PAGE and digested with trypsin. The tryptic peptides were analyzed by nanoLC linear ion trap TOF mass spectrometer. NanoLC-MS system successfully displayed over 2,000 proteins and achieved approximately 10-fold higher sensitivity than was obtained with 2-D gel-based analysis (http://www. dgrc.kit.ac.jp/~jdd/proteome_atlas/ ). The LC-MS based approach is also useful for quantitative proteomic analysis of the reproductive processes in copulation and ovulation. We are currently underway to establish the protein expression maps of the Drosophila reproductive organs based on their LC-MS profiles.

**P-56 A screen for growth-restricting genes identifies lingerer.**

**Baumgartner, R**; Rottig, C; Stocker, H; Hafen, E
IMSB, ETH Zürich, 8093 Zürich Switzerland

Growth is a tightly regulated and essential process during animal development, determining the size and shape of organisms, organs and cells. Deregulated growth can result in overproliferation or size reduction, manifested in abnormal organ and body sizes or diseases like cancer. Despite extensive investigations on growth and size-controlling mechanisms, the picture of the growthregulatory genetic network has remained incomplete. We performed an unbiased genetic forward screen based on the ey-Flp technique to identify new growth-suppressing genes. In addition to components of the insulin/TOR pathway, our screen revealed the main components of the Hippo signalling pathway. Furthermore, we found mutations in lingerer (lig), which was originally identified in a screen for genes involved in copulation. Whereas lig mutants showed moderate overgrowth of the eye and head structures, an excess of Lig protein induced apoptosis in eye and wing imaginal discs of third instar larvae. We will present our genetic analysis of lig function as well as the results of an affinity purification/MS approach to identify binding partners of Lig.

**P-57 Quantification of tissue morphogenesis in proliferating Drosophila epithelial tissue.**

**Floris Bosveld**, Isabelle Bonnet, Boris Guirao, Fanny Serman, Francois Graner, Yohanns Bellaiche
INSTITUT CURIE, Biologie du Développement INSERM U934, 11-13, rue Pierre et Marie Curie, 75005 Paris, France

Tissue morphogenesis is fundamental to the formation of functional organs. Research into the mechanisms of morphogenesis will require quantitative tools to describe tissue morphogenesis. Using long-term live imaging of DE-cadherin-GFP in Drosophila epithelial tissues and in house developed software, we are able to quantify tissue morphogenesis from the cell level to the tissue level. The method employs a mathematical framework, which is based on the metric, "cell-texture", which characterizes cell geometry and topology. Here we describe cell morphogenesis, cell division, cell rearrangements and apoptosis within Drosophila dorsal thorax epithelia. We aim at analyzing how mutations that are known to affect tissue size and shape impair cell shape, cell division and cell rearrangements.

**P-58 A genetic screen in Drosophila identifies an antagonism between a splicing factor and a tumor suppressor gene.**

**Fernando,C** (1); Audibert, A (2); Gho, M (2); Tazi, J (1); and Juge, F (1)
(1) Institut de Génétique Moléculaire de Montpellier-UMR5535, 1919 route de Mende, 34293 MONTPELLIER; (2) UMR7622, Université Paris 6, Bâtiment C, 7ème étage, Case 24, 9 Quai Saint-Bernard, 75 Paris.

SR proteins are a family of splicing factors involved in both constitutive and alternative RNA splicing. Recent data show that they can be involved in cancer. Indeed, modifications in SR proteins activity change the expression of a lot of oncogenes and tumor suppressor genes by modulating their expression level or the nature of the proteic variant produced, and are able to transform cells. In Drosophila, B52 is the major SR protein and is required for viability. Targeted overexpression of B52 protein during Drosophila development induces lethality and morphological alterations. In order to better understand how the SR proteins activity is regulated in vivo, we performed a genetic screen by misregulation in Drosophila to identify negative regulators as well as antagonists of B52. We screened 800 random UY lines and recovered a dozen of candidates that rescue the B52-induced phenotypes in two different tissues. One of these lines contains an insertion in the brat gene (Brain tumor). Brat protein was characterized as a translational repressor in the embryo, and as a negative regulator of myc in larval neuroblasts. Brat also regulates cell growth, probably through its effect on myc. Remarkably we observed that varying B52 level in the salivary glands affects cell growth. We show by qRT-PCR that overexpression of B52 in salivary glands increases myc mRNA level. Conversely, in B52 mutant larvae, myc mRNA amount is reduced. These results show that B52 is directly or indirectly implicated in myc expression. Since Brat overexpression can rescue B52 overexpression-induced phenotypes, we hypothesize that the antagonism between B52 and Brat is due to opposite activities on myc. We are now investigating how B52 affects myc expression.

**P-59** **In Vitro cultivation of imaginal wing discs of Drosophila melanogaster**

**Handke, B** (1); Balbi, M (2); Baranek, S (3); Szabad, J (4); Hafen, E (2); Lehner, C (1);
(1)Institute of Zoology, University of Zurich; (2)Institute of Molecular Systems Biology, ETH Zurich; (3)Institute of Microengineering, EPFL Lausanne; (4)Department of Biology, University of Szeged, Hungary

Our current understanding of the molecular mechanisms controlling pattern formation and cell proliferation during animal development is based to a large extent on experimental analysis of Drosophila wing development. A wide range of sophisticated genetic methodology is available in this system (as for instance various types of clonal analyses). However, long term culture of Drosophila wing imaginal discs compatible with normal development in vitro has not been achieved so far. As wing development in vitro would further potentiate the accessibility of this important experimental system (including real time analyses down to the cellular and subcellular level), we are developing novel culture systems for imaginal wing discs based on microfluidic technology. Our initial goal is the identification of conditions allowing optimal growth of discs isolated from early third larval instar. On the one hand, we will evaluate the suitability of hemolymph as an optimal growth medium. It has been well established that imaginal wing disc fragments grow readily after transplantation into the hemolymph-filled abdomen of adult host flies. Moreover, after transfer into larvae such discs are able to progress through metamorphosis and give rise to adult wing structures. On the other hand, we will focus on medium conditioned by primary cultures of dissociated embryos. Additional long term goals include the efficient expansion of wing imaginal disc cells for subsequent disc reconstitution experiments where sorted cells from different lineage compartments are co-cultivated in vitro in micro-patterned cultures. Thereby, we want study conditions required for morphogen-driven pattern formation in vitro.

**P-60** **Wingless Promotes Proliferative Growth in a Gradient-Independent Manner.**

**Luis Alberto Baena-Lopez** 1 Xavi Franch-Marro 2 Jean-Paul Vincent 1
1Department of Developmental Neurobiology, NIMR, The Ridgeway, Mill Hill, London NW7 1AA, UK. 2Institut de Biologia Molecular de Barcelona (CSIC) and Institut de Recerca Biomèdica, Parc Científic de

Morphogens form concentration gradients that organize patterns and control growth. It has been suggested that, rather than the extent of morphogen signaling, it is its spatial derivative that is the relevant modulator of cell proliferation. According to this view, the ability of morphogens to regulate growth during development would crucially depend on their graded distribution. Here, we describe an experimental test of this model for Wingless, one of the key organizers of wing development in Drosophila. In this tissue, maximal Wingless signaling suppresses cellular proliferation. In contrast, we found that moderate and uniform amounts of exogenous Wingless, even in the absence of endogenous Wingless, stimulated proliferative growth. Beyond a few cell diameters from the source, Wingless was relatively constant in its abundance and thus contributed a homogeneous growth-promoting signal. Although morphogen signaling may act in combination with as yet uncharacterized graded growth promoting pathways, the graded nature of morphogen signaling is not required for proliferation, at least in the developing Drosophila wing.

**P-61 Nutrition controls mitochondrial biogenesis in the Drosophila adipose tissue through Delg and Cyclin D/C.**

**Claudia Baltzer**, Stefanie K. Tiefenböck, Mark Marti and Christian Frei

Mitochondria are cellular organelles that perform critical metabolic functions: they generate energy from nutrients but also provide metabolites for de novo synthesis of fatty acids and several amino acids. Thus mitochondrial mass and activity must be coordinated with nutrient availability, yet this remains poorly understood. Here, we demonstrate that Drosophila larvae grown in low yeast food have strong defects in mitochondrial abundance and respiration activity in the larval fat body. This correlates with reduced expression of genes encoding mitochondrial proteins, particularly genes involved in oxidative phosphorylation. Second, genes involved in glutamine metabolism are also expressed in a nutrient-dependent manner, suggesting a coordination of amino acid synthesis with mitochondrial abundance and activity. Moreover, we show that Delg (CG6338), the Drosophila homologue to the alpha subunit of mammalian transcription factor NRF-2/GABP, is required for proper expression of most genes encoding mitochondrial proteins. Our data demonstrate that Delg is critical to adjust mitochondrial abundance in respect to Cyclin D/Cdk4, a growth-promoting complex and glutamine metabolism according to nutrient availability. However, in contrast to nutrients, Delg is not involved in the regulation of mitochondrial activity in the fat body. These findings are the first genetic evidence that the regulation of mitochondrial mass can be uncoupled from mitochondrial activity.

**P-62 Characterisation of the role of GCN2 in nutrient sensing and growth.**

**Bjordal, M**; Leopold, P
Institute of Developmental Biology & Cancer, CNRS UMR 6543, Université Nice-Sophia Antipolis

In response to environmental stress, a family of kinases phosphorylates eIF-2α (eukaryotic Initiation Factor-2 alpha) and activates a cellular response via changes in transcription and translation. In mammals, there are four such kinases, PKR, HRI, GCN2 and PEK, while only one kinase, GCN2, is found in S. cerevisiae. In Drosophila two eIF-2α kinases have been identified, namely dGCN2 and dPEK. In yeast GCN2 is activated by limitation in both amino acid and glucose as well as by UV-irradiation. We are in the progress of characterising the role of dGCN2 in growth and nutrient sensing. Our study reveals that knockdown of GCN2 has dramatic effects on animal survival and cellular growth, even in normal food conditions. However, only small changes in the metabolic profiles of these animals are observed. We are currently investigating which signalling pathways and which tissues contribute to the physiology of the growth and nutrient response involving GCN2.

**P-63 Modifier genes of a fat body-directed starvation phenotyp.**

**Bourouis M**; Dussert A; Bjordal M; and Léopold P
Institut Biologie du Développement et Cancer -CNRS UMR 6543- Université de Nice, France

Drosophila fat body is an energy storage tissue that serves as metabolic centre. Previous work from our laboratory assigned a nutrient-sensing function to the larval fat body which involves the activity of the amino acid transporter slimfast (slif) coupled to that of the conserved nutritional regulator, TOR (Target of Rapamycin). This fat body sensor in turn, triggers peripheral growth via modulation of insulin/IGF signalling (Colombani et al. Cell, 2003). Such systemic regulation was recently proposed to imply retention of Drosophila insulin like proteins (Dilps) by secretory neurones of the brain (IPC) (Géminard et al. Cell Metab. In the press) We searched for new components of the fat body sensor including for its humoral relay part, by a combination of microarray analysis and functional screenings. Analysis of transcriptional output from fat bodies starved by slif inhibition and from fat bodies starved as the result of expression of the TOR inhibitor, TSC1/2, revealed extensive similarities. A set of common regulated genes as well as other selected sets of genes were then tested for the ability to modify the starvation phenotype caused by fat body-directed slif inhibition, using available misexpression strains and RNAi-based silencing strains. Secondary screenings of modifiers were conducted to refine the first hits, including the screening of enhancers of the slif induced starvation for suppression of a lethal phenotype caused by an excess function of TOR. A variety of modifiers were recovered including genes tied to lipid and energetic metabolism, presumed mediator of TOR function in the fat body including SREBP and Myc, and a possible regulator of TOR. Genes of predicted humoral roles were also recovered.

**P-64 The Drosophila PGC-1 Homologue Spargel Coordinates Mitochondrial Biogenesis to Insulin-signalling.**

Tiefenböck, SK; Baltzer, C; Egli, NA and **Frei, Ch**
ETH Zurich, Department of Biology, Zurich, Switzerland

Mitochondrial biogenesis must be adapted to tissue function, cell proliferation and growth, and nutrient availability. In mammals, the related transcriptional coactivators PGC-1?, PGC-1? and PRC regulate multiple metabolic functions, including mitochondrial biogenesis. However, we know relatively little about their respective roles in vivo. Here we show that the Drosophila PGC-1 family homologue Spargel promotes the expression of multiple genes encoding mitochondrial proteins. Spargel was not limiting for mitochondrial mass and OXPHOS activity and functions in parallel to Delg, the fly NRF-2?/GABP? homologue. Accordingly, the spargel delg double mutant showed strongly exacerbated mitochondrial defects compared to single mutants. More importantly, in the larval fat body, Spargel mediated mitochondrial biogenesis, cell growth and the transcriptional control of target genes in response to insulin-signalling. In this process, Spargel functioned in parallel to the insulin-responsive transcription factor dFoxo and provided a negative feedback loop to fine-tune insulin-signalling. Together, our data place Spargel at a nodal point for the integration of mitochondrial biogenesis to tissue and organismal metabolism and growth.

**P-65 micro-RNAs and growth control in the Drosophila wing.**

**Héctor Herranz** 2, Xin Hong 3, Lidia Pérez 2, Ana Ferreira 2, Daniel Olivieri 2, Stephen M. Cohen 3, 4 and Marco Milán 1, 2
1 ICREA and 2 Institute for Research in Biomedicine (IRB Barcelona), Parc Científic de Barcelona, Baldiri Reixac, 10-12, 08028 Barcelona, Spain 3 Temasek Life Sciences Laboratory and 4 Departmen

The regulation of gene expression at the transcriptional level plays a central role in development and physiology; however, the relevance of post-transcriptional gene regulation is increasingly recognized. miRNAs, endogenous small non-coding RNAs, 22 nucleotides long, that repress target transcripts, confer a novel layer of post-transcriptional regulation. microRNAs have been implicated in cell cycle regulation and in some cases shown to have a role in tissue growth control. In this work we have analyzed the role of micro-RNAs in the Drosophila wing, a highly proliferative epithelium.

**P-66 The SR protein B52 recruits DNA topoisomerase I during transcription to promote mRNA release and transcription shutdown.**

**Juge, F**; Fic, W; Fernando, C; Tazi, J
Institut de Génétique Moléculaire de Montpellier, 1919 route de Mende, 34293 Montpellier

Coupling between RNA transcription and splicing has emerged as a new level of regulation of gene expression, since transcription speed can affect splicing and reciprocally splicing factors such as SR proteins can influence transcription elongation. Among the proteins that lies at the boundary between transcription and splicing is the DNA topoisomerase I (TopoI). Indeed mammalian TopoI carries two enzymatic activities: a DNA topoisomerase activity that relaxes topological constraints during transcription and a kinase activity directed against the SR proteins family of splicing factors. To gain insight into TopoI function in vivo, we have investigated the relationship between TopoI and the SR protein B52/SRp55 in Drosophila. We show that the kinase activity of TopoI is conserved in Drosophila and that TopoI phosphorylates B52 in vivo. Analysis of TopoI and B52 distribution on polytene chromosomes reveals a striking colocalization of the two proteins. We demonstrate that B52 influences TopoI localization and that TopoI recruitment on chromatin involves the B52 protein. As a model we used the hsp70 gene on which TopoI and B52 are massively recruited after induction by heat shock. In the absence of B52, TopoI recruitment is compromised and hsp70 mRNA accumulates at its transcription site. Since hsp70 is not spliced, this result reveals a new, splicing-independent, role of B52 in RNA release from transcription sites. Remarkably, during the recovery phase after heat shock, hsp70 shutdown is dramatically delayed in B52– as well as in TopoI– mutant backgrounds, revealing a defect in gene deactivation. These results show that TopoI recruitment through B52 allows efficient RNA release from transcription sites and is necessary for transcription shutdown.

**P-67 IDGF3 as a prolifferative factor.**

**Kucerova, L**; Edgar, B; Zurovec, M.
LK, MZ: Institute of Entomology, Biological centre, v.v.i. and Faculty of Science, University of South Bohemia, Branisovska 31, 370 05 Ceske Budejovice, Czech Republic BE: Division of Basic Sciences

IDFGs are family of six Drosophila proteins expressed in the fat body and hemocytes, which show some similarity to chitinases. IDGF2 was shown earlier to collaborate with insulin in the stimulation of Drosophila wing disc cell proliferation in vitro. We studied the effects of IDGF3 overexpression in vivo and discovered that it works in cell nonautonomous way and it is able to induce cell proliferation in imaginal discs, while it does not influence the size of the organ.

**P-68 Systemic Hedgehog and its functional Implications.**

**Kumari, Veena** and Eaton, Suzanne.
Max Planck Institute for Cell Molecular Biology and Genetics, Dresden, Germany.

Hedgehog (Hh) is one of the important secretory proteins that control the patterning and development of tissues in various multicellular organisms. Signaling by Hh protein is crucial for proper embryogenesis and defects could lead to developmental anomalies ranging from cyclopia, holoproencephaly, cancers etc. Activation of Hh requires an intramolecular processing where the C-termius of the protein catalyzes the release of the N-terminal functionally active Hh protein (HhNp). During this process, HhNp is covalently modified with palmitoyl and cholesterol groups which explain the affinity of Hh for cell membranes. Despite this, HhN is secreted out in a paracrine manner and influences the transcription of target genes responsible for growth and patterning of various tissues and organs. Interestingly, the effect of HhNp sinalling can be observed in cells which are very distant from the source of production of HhNp. This long range effect is believed to be mediated by interaction of HhNp with lipophorins from the hemolymph in case of D. melanogaster. Our study on interaction of HhNp with lipophorin has led to the discovery of a new form of HhNp which systemically circulates in the hemolymph and also has the ability to interact with lipophorins. By replacing local Hh activity with that of systemeically circulating Hh we were successfully able to rescue growth but patterning in the wing imaginal disc. These results suggest that growth and patterning of wing disc incase of D.melanogaster can occur by a dual mechanism of HhNp signaling which can be uncoupled from each other.

**P-69 Coenzyme A links cellular metabolism to chromatin remodeling and DNA integrity.**

**Siudeja, K**; Rana, A; Lemstra, W; Sibon, OCM
Department of Cell Biology, Radiation & Stress Cell Biology, UMCG Groningen, The Netherlands

In a Drosophila screen aimed to identify genes required to survive DNA damage, several unexpected players involved in DNA damage responses were discovered. Three of these novel genes (dPANK, dPPCS and dPPAT-DPCK) code for enzymes required for de novo biosynthesis of Coenzyme A (CoA). Drosophila CoA mutants are highly sensitive to ionizing radiation, show increased levels of DNA damage and apoptosis, neurodegenerative phenotype and a reduced life span (Bosveld et al., Hum Mol Gen, 2008). With the use of this Drosophila model we show how a general metabolic pathway, namely CoA de novo biosynthesis, effects DNA integrity and sensitivity to genotoxic stress. CoA mutants show no obvious checkpoint or DNA repair dysfunction after exposure to ionizing radiation. Nevertheless, we demonstrate that in vitro reduced levels of CoA result in energy depravation and radiation sensitivity similar to one observed in starvation conditions. Moreover we show that CoA deficiency has an impact on chromatin remodeling. Specifically, we observe decreased levels of acetylated histones. dPANK, the rate limiting enzyme in CoA biosynthesis, is required for increase in histone acetylation in response to DNA damage inducing agents. Together these findings show how a general metabolite, CoA, links energy metabolism to chromatin regulation and maintenance of DNA integrity.

**P-70 Dilp6, a post-feeding Insulin-like peptide that controls pupal growth.**

**Maija Slaidina**[1], Rénald Delanoue[1], Sebastian Gronk[2], Linda Partridge[2] and Pierre Léopold[1].
1 Institute of Developmental Biology and Cancer, University of Nice-Sophia Antipolis/CNRS, Parc Valrose, 06108 Nice, France; 2 UCL Institute of Healthy Aging, GEE (Genetics, Evolution and Environmental

In vertebrates, the GH/IGF axis promotes growth in response to nutritional input. In Drosophila, seven insulin-like peptides (Dilps) carry both insulin and IGF functions. We find that amid all Dilps, Dilp6, structurally related to mammalian IGF1, is produced in the fat body (FB), the larval liver and fat. dilp6 expression is repressed in young and actively feeding larvae but strongly increases during non-feeding developmental stages – wandering and pupa. dilp6 knock out or its specific knock-down in the FB reduces animal growth, indicating that Dilp6-dependent growth cannot be fully compensated by the other Dilps. Using an inducible expression system, we demonstrate that dilp6 is not required during early feeding larval period, but that it regulates growth in post-feeding stage, consistent with its profile of expression in FB cells. The onset of Dilp6 expression coincides with the raise of ecdysone levels and we demonstrate that dilp6 transcription in the FB requires ecdysone signaling. Remarkably, we find that dilp6 expression can be strongly induced upon fasting during early larval development. We further demonstrate that the transcription factor dFOXO is required for fasting-induced dilp6 expression, therefore constituting a feedback regulation on insulin/IGF signaling. This study reveals the existence of a specialized class of insulin-like peptides promoting growth under low nutrients (starvation/fasting) or following developmentally-induced cessation of feeding.

**P-71 A New Gene Involved in Growth?**

**Jianbin, Wang**, Peter Smibert, Vicki Athanasopoulos and Robert Saint
ARC Special Research Centre for the Centre for the Molecular Genetics of Development (CMGD), Research School of Biological Sciences, The Australian National University, Canberra, ACT 0200, Australia

The agpat (1-Acylglycerol-3-phosphate O-acyltransferase) 3/4a gene was first discovered in Drosophila while working on the neighbouring gene roquin. Two roquin mutants, roqA2 and roqY2, were created by imprecise P-element excision. Of these deletion alleles, roqA2 takes out the first eleven coding amino acids of roquin while roqY2 takes out the 5'-UTR and part of the first intron of roquin, as well as some sequences of agpat 3/4a. Both alleles have very similar phenotypes in that they both produce small and highly translucent larvae compared to wild-type larvae, which have smaller and highly vesiculated fat body compared to wild-type larvae fat body. Clonal analysis of these alleles in the eye showed a cell autonomous growth defect for the roqY2 clones but not for roqA2 clones. Further clonal analysis using newly generated point mutants in roquin gave phenotypes consistent with those observed for the roqA2 allele, suggesting that the cell autonomous growth defect observed for the roqY2 allele is due to the disruption of AGPAT 3/4a. The agpat 3/4a gene encodes a 1-acylglycerol-3-phosphate O-acyltransferase gene. Orthologues of AGPATs are known to modulate cellular levels of phosphatidic acids (PA). It has recently been implicated in mediating cell signalling functions for example in the activation of the TOR pathway in mouse (Yu et. al., 2006). We are currently characterizing the function of the agpat 3/4a gene, and investigating its possible role in regulating cell growth, potentially through the TOR pathway. Tang et al., (2006) Identification of a Novel Human Lysophosphatidic Acid Acyltransferase, LPAAT-theta, Which Activates mTOR pathway. J Biochem Mol Biol. 39:626-35

**P-72 Growth MAPK signaling regulates insulin sensitivity in Drosophila**

**Zhang Wei**1,2,4, Ville Hietakangas1,4, Barry J. Thompson3 and Stephen M. Cohen1
1, Temasek Life Sciences Laboratory, National University of Singapore, Singapore 2, Department of Biological Sciences, National University of Singapore, Singapore 3, Cancer Research UK London Research

In Drosophila, insulin/PI3K signaling pathway is a potent regulator of tissue growth by activating the effector kinase AKT and inhibiting transcription factor FoxO. Adjusting the sensitivity of the pathway is a critical feature to coordinate cellular growth with nutritional conditions. In order to identify novel regulators of the pathway, we performed a genetic screen for modifiers of FoxO gain-of-function phenotype in Drosophila eye. From this screen, we found that the mitogen-activated protein kinase (MAPK) pathway plays an important role in adjusting the sensitivity of the insulin pathway to extracellular stimuli.

**P-73 Roles of the two isoforms of Lozenge and their interaction with Pointed during Drosophila**

**Augé, B**; Gobert, V; Haenlin, M; Waltzer, L
Université de Toulouse, UPS, CBD (Centre de Biologie du Développement), Bat4R3, 118 route de Narbonne, 31062 Toulouse, France CNRS, CBD UMR5547, 31062 Toulouse, France

RUNX transcription factors play important regulatory roles during haematopoiesis in different species. In Drosophila, the RUNX factor Lozenge (Lz), is required for the development of one of the two main blood cell lineages: the crystal cells. lz codes for two different protein isoforms generated by alternative splicing: one isoform (Lz3.5) contains an Ets interacting domain whereas the other (Lz?5) doesn't. Our results show that Lz3.5 is a stronger transactivator of Lz direct target genes than Lz?5. In addition, using a enhancer which recapitulates lz expression during embryonic haematopoiesis, we could show that Lz3.5 but not Lz?5 can rescue crystal cell formation in lz mutant embryos. In order to know if an Ets transcription factor is involved in haematopoiesis, we established the expression pattern of the different Drosophila Ets gene. pointed (pnt) is the only one with a consistent expression pattern in the hemocyte population. Interestingly, in a null pnt allele, we observed a decrease of 30% in crystal cells number and an absence of expression of the panhemocyte marker peroxidasin. Further experiments aiming at deciphering the modus operandi of Lz and Pnt during haematopoiesis will be presented.

**P-74 An in vivo RNAi screen identifies genes implicated in melanotic tumor formation in Drosophila.**

**Boyer,K**; and Avet-Rochex,A; Polesello,C; Osman,D; Augé,B; Gobert,V; Roch,F; Haenlin,M; and Waltzer,L.
Centre de Biologie du Développement UMR 5547, Bâtiment 4R3 118, Route de Narbonne 31062 Toulouse cedex 09

Drosophila blood cells (hemocytes) ensure the defence of the organism in particular by eliminating pathogens and abnormal cells. These hemocytes belong to three lineages : the plasmatocytes, the crystal cells, and the lamellocytes. Lamellocytes are normaly scarcely present but their differentiation is massively induced after an immune challenge such as the parasitisation of the larvae by wasp eggs around which they will form a melanotic capsule. Likewise, several zygotic mutations have been described to induce the production of melanotic capsules in the absence of infection. These mutations have been linked to misregulation in several processes including hemocyte differentiation, defect in self tissues recognition or tissue degeneration. Yet, little is known about the mechanisms controlling self versus non-self or alter-self recognition and how the subsequent immune cellular response is orchestrated. To identify new genes implicated in blood cell homeostasis, we used a collection of UAS-dsRNA transgenic lines to specifically induce loss of functions in the hemocytes (or in the hemocytes and the fat body) and we looked for melanotic tumour formation. Screening 10% of the Drosophila genes, we recovered around 50 melanotic tumour suppressors genes. This approach pinpointed several new pathways participating in blood cell homeostasis. Notably, results suggest that larval blood cell homeostasis is controlled by an intricate network of communications between the different immune tissues. Interestingly, we identified a set of genes that act cell-autonomously and we demonstrated that embryonic-derived plasmatocytes transform into lamellocytes. All together, our results shed new light on the control of Drosophila blood cell lineage development and plasticity.

**P-75 Unraveling the mechanisms of translation inhibition by Pseudomonas entomophila in** the gut of Drosophila melanogaster.

Sveta Chakrabarti, Nicolas Buchon, Peter Liehl, Bruno Lemaitre
Global Health Institute EPFL/SV/GHI, Building AI – Station 15, CH-1015 Lausanne, Switzerland.

Unraveling the mechanisms of translation inhibition by Pseudomonas entomophila in the gut of Drosophila melanogaster. Understanding the relationship between pathogens and their hosts is a major challenge and has important medical implications. During the course of evolution, pathogens have acquired multiple strategies that allow them to resist host immune responses and also to exploit host resources. To investigate how Drosophila deal with infectious agents in a natural context, we study the interaction between Drosophila and recenty identified entomopathogen, Pseudomonas entomophila (Vodovar. N, et al., 2005). Oral infection of Drosophila with P. entomophila increases the mortality rate of flies despite the induction of both local and systemic immune responses. This indicates that this bacterium has developed specific ways to escape the fly immune response. Our group has demonstrated that P.entomophila secretes a metalloprotease, AprA, that degrades the AMPs produced by the gut epithelium (Liehl. P, et al., 2006). However, knocking out aprA by transposon insertion did not result in complete loss of virulence. Ongoing studies in our lab further demonstrate that in addition to AMP degradation, P.entomophila is also able to inhibit the synthesis of some AMPs at the translational level. This study aims to unravel the mechanism of translation inhibition by exploring whether pathogen or host derived factors are responsible for this supression.

**P-76 Potassium channel activity in the drosophila heart modulates resistance against the cardiotrophic flock house virus.**

**Eleftherianos I**, Chtarbanova S, Squiban B, Ocorr K, Bodmer R, Hoffmann JA, Imler JL
UPR 9022 – CNRS, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France - Burnham Institute for Medical Research, La Jolla, California, USA

Drosophila has been established as an excellent genetic model for investigating host-microbe interactions including viral pathogens. The study of the response of Drosophila to RNA virus infections already identified two types of immune responses, which are evolutionary conserved. A first antiviral mechanism is degradation of viral RNA by RNA interference (RNAi). A second response to viral infection involves the induction of a large number of genes, some of which counter viral infection. We are currently investigating the role of a Drosophila homolog of the regulatory subunit of the mammalian potassium channel gene SUR2, called dSUR, which is expressed mainly in the heart. We find that silencing of dSUR in the heart, but not in other tissues, causes susceptibility to infection with the Flock House Virus (FHV). In contrast, dSUR knockeddown flies are as resistant as wild-type controls to the Drosophila C Virus (DCV), and to several bacterial and fungal pathogens. FHV, but not DCV, is detected in the heart after infection, where it causes swelling and an increased and irregular beat rate. dSUR expression decreases as flies age, and this correlates with declining heart performances and higher susceptibility to virus infection. Spectacularly, we demonstrate that pharmacological treatment of flies with agonists and antagonists of KATP channels modifies the resistance to infection. Finally, the genes Ir and Irk2, which encode the pore of the potassium channel regulated by dSUR, also exhibit protective effects against FHV infection. This effect is not restricted to stress-activated K+ channels. Indeed, flies mutant for the voltage-dependent K+ channel KCNQ involved in normal heart physiology also show increased lethality upon infection by FHV. A mechanism for the modulation of the resistance to FHV infection by potassium channels will be presented.

**P-77 Drosophila Ubiquitin specific protease 36: an enzyme to prevent improper immune signalling.**

Thevenon Dominique, Elodie Engel, Taillebourg Emmanuel and **Fauvarque Marie-Odile**
iRTSV/LTS U873 INSERM/CEA/UJF 17 rue des Martyrs 38054 Grenoble Cedex 9

Conjugation of ubiquitin monomers or polymers by ubiquitin ligases has emerged as a key mechanism for the control of the activity or the stability of proteins. Ubiquitination notably regulates signal transduction to Nuclear Factor NF-kappaB transcription factor controlling the expression of stress and immune response genes. In Drosophila, two evolutionary conserved NF-kB-like signalling pathways TOLL and IMD contribute to a robust immunity. As in mammals, a set of conserved ubiquitin ligases regulates signal transduction whereas to date, Drosophila CYLD is the sole ubiquitin protease known to down-regulate the IMD pathway. We identified the Drosophila Ubiquitin Specific Protease 36 (dUSP36) function in the negative regulation of the Immune deficiency (IMD) pathway. dUSP36 is an active ubiquitin protease which overexpression suppresses fly immunity against Gram-negative pathogens via its catalytic activity. In the opposite, silencing dUsp36 provokes IMD-dependent constitutive activation of IMDdownstream pathways, namely Jun kinase (JNK) and NF-kB, but not of the Toll pathway. This deregulation is lost in axenic flies indicating that dUSP36 permanently prevents immune signal activation by non pathogenic commensal bacteria. dUSP36 interacts with IMD and prevents K63polyubiquitinated IMD accumulation while promoting IMD degradation in vivo. Blocking the proteasome in dUsp36 expressing S2 cells enhances K48-polyubiquitinated IMD and prevents its degradation. Our findings identify dUSP36 as a novel repressor preventing unspecific NF-kB activation via IMD deubiquitination. This observation is a novel step in understanding how commensal bacteria are tolerated by the organism.

**P-78 Genome engineering-based analysis of the Imd pathway receptor PGRP-LC : role of individual isoformsp and expression pattern during bacterial signalling.**

**Mickaël Poidevin**1, Claudine Neyen2, Bruno Lemaître1,2
1CNRS Centre de Génétique Moléculaire, Gif-sur-Yvette, France 2EPFL, Global Health Institute, Lausanne, Switzerland

Innate immune recognition relies on a restricted number of Pattern Recognition Receptors, which recognize a set of structurally conserved, essential pathogen-associated molecular patterns. Downstream, the Toll and Imd pathways, which respond to Gram positive (G+) bacterial/fungal and Gram negative (G-) bacterial stimuli, respectively, lead to systemic production of antimicrobial peptides (AMPs) by the fat body. Genetic dissection has identified the highly conserved family of Peptidoglycan Recognition Proteins (PGRPs) as upstream sensors of Imd and Toll activation by bacteria. The Drosophila genome harbours 13 PGRP genes coding for both soluble and transmembrane receptors, with either signalling or enzymatic properties. Our study focuses on PGRP-LC, a G- sensing, transmembrane signalling receptor expressed on the surface of fat body cells. The PGRP-LC gene gives rise to 3 splice isoforms, LCx, y and a, that share an identical intracellular signalling domain but differ in their extracellular sensing domains. In vitro experiments suggested that homo- and heterodimeric combinations of these isoforms fine-tune the immune response to mono- or polymeric peptidoglycan. To dissect the specific roles of these isoforms in vivo, we have used recombineering and the ?31 integrase to generate transgenic flies lacking the PGRP-LC locus and selectively reconstituted with the PGRP isoforms LCx, y and a. Here we present preliminary data on survival, AMP production, and spatio-temporal expression patterns of PGRP-LC isoforms upon systemic and oral immune challenge with purified peptidoglycan products or G- bacteria. Using our transgenic model for further in vivo studies, we hope to add novel insights into the immune defence against G- infection in Drosophila.

**P-79 Drosophila immune response to parasitoids and the different ways to be an immune suppressive wasp.**

Colinet D., Dubuffet A., Schmitz A.1, Cazes D. and **Poirie M.**
Colinet D., Schmitz A., Cazes D. and Poirié M. : Institut National de la Recherche Agronomique, INRA Sophia Antipolis, UMR 1301 / Centre National de la Recherche Scientifique, CNRS, UMR 6243 / Unive

Endoparasitoid wasps have developed different strategies, mainly based on the use of virulence factors, to circumvent the immune defenses of their insect hosts. Toward parasitoid eggs, the insect immune response is encapsulation, which involved different categories of haemocytes as well as activation of the phenoloxidase cascade and production of cytotoxic radicals. Leptopilina wasps are parasitoids of Drosophila species, L. heterotoma being a generalist while L. boulardi is a specialist that only develop at the expense of some species of the melanogaster sub-group. Besides, two different types of L. boulardi females are available that have different virulence properties: the success of ISm females is host species-specific (success in D. melanogaster, failure in D. yakuba) while ISy females succeed in both host species depending on the genotype of the parasitized host. This model is then ideal to address the question of the basis of intra-specific and inter-specific variability of virulence in parasitoids and of the specificity of parasitoids toward their Drosophila hosts. Results regarding the characterization of a venom serpin and its effect on the regulation of the PO cascade in Drosophila species will be presented as well as data on a RacGAP multigenic family of venom toxins that target Drosophila haemocytes by targeting Rac GTPases. Data provided by a wide range analysis comparing cDNA sequences from libraries of venom apparatus of the two L. boulardi strains and L. heterotoma will be discussed together with the information they provide on the origin of the variability of virulence in parasitoid wasps and on new potential immune genes targeted in Drosophila species.

**P-80 Cactus localization during immune pathway activation.**

**Seyedoleslami Esfahani**, S; Holm, Å; Önfelt Tingvall, T; Björklund, G;& Engström, Y;
Department of Molecular Biology and Functional Genomics, Arrhenius Laboratories for Natural Sciences, Stockholm University, SE-106 91 Stockholm, Sweden; email: shiva@molbio.su.se

In Drosophila, the Toll signaling pathway is known as a regulator of dorsal-ventral patterning during embryogenesis, and it also has important roles in regulation of immunity. Activation of Toll pathway results in the nuclear accumulation of Dif and Dorsal and activation of many different target genes. The current model is that Cactus binds to Dorsal and Dif in the cytoplasm and thereby inhibits their nuclear translocation. Cactus has been considered a strictly cytoplasmic protein that is degraded upon Toll signaling. We investigated the sub-cellular distribution of Cactus, in larval fat body and Drosophila S2 cells in response to Toll signaling. Our results indicate a more differentiated regulation and role of Cactus. Immunostaining and Western blot analysis revealed that Cactus is distributed in both the cytoplasm and nucleus of fat body cells in uninfected larvae. Upon immune induction, the relative nucleo-cytoplasmic distribution of Cactus changes, revealing a primarily nuclear presence of Cactus 30 min post-induction. Similar results were obtained in Drosophila S2 cells. Detailed results and interpretations will be presented.

**P-81 Genetic dissection of tissue injury-induced cell death-related signaling in Drosophila.**

**Asuka Takeishi**1, Erina Kuranaga1,2, Ayako Tonoki1,2, Hirotaka Kanuka3, Masayuki Miura1,2
1Department of Genetics, Graduate School of Pharmaceutical Sciences, University of Tokyo, and 2CREST, JST, Tokyo, Japan and - 3National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan

Genetic analysis with Drosophila has contributed to reveal detail mechanisms of innate immunity response. Invaded microbial organisms activate various immune reactions in host, such as antimicrobial peptide secretion through Toll and IMD pathways. It has been indicated that these immune reactions are activated not only by pathogen-associated molecules, but also by endogenous molecules secreted from damaged cells or injured tissue. We found that flies with reduced cell death showed lethality after sticking with a microinjection glass needle, which suggests involvement of cell death-related signals in tissue injury response. Experiments using sterilized flies confirmed that the lethality of these compromised flies is not induced by bacterial infection, but by injury stress. We also observed the scab formation at the wound site occurs in these flies as similarly as in wild-type. These results suggest that cell death-related signals may play important roles to maintain homeostasis in the body after injury stress.

**P-82 In vivo analysis of phagocytosis of Mycobacterium marinum.**

**Yildirim, M**; Dionne, MS;
King's College London, London, United Kingdom

Mycobacterium marinum (M. marinum) is a pathogenic bacterium closely related to Mycobacterium tuberculosis, which causes human tuberculosis. We use Drosophila as a model host to study the pathogenesis caused by this organism. After the fly is infected, M. marinum survives being phagocytosed by plasmatocytes, immune cells responsible for phagocytosis and destruction of pathogens. The bacterium then goes on to reproduce, break free from infected plasmatocytes, spread, and eventually kill the host. This project focuses on the effect of M. marinum on plasmatocytes. Our preliminary data suggests that following a M. marinum infection, adult flies lose plasmatocytes as the bacteria spread. We are studying the infection time scale and processes that follow the infection of plasmatocytes with M. marinum. We inject M. marinum expressing dsRed into Drosophila with hemocytes co-expressing GFP and dsRNA targeting various molecules potentially involved in phagocytosis. The GFP expression and knockdown are driven by Hemolectin. We image the progress of the bacteria getting inside plasmatocytes, growing and forming colonies, and spreading. This has allowed us to analyze the contribution of various molecules and signals to the phagocytosis process and to defence against mycobacteria; some of these data will be discussed.

**P-83 Manipulation of Copper Levels in Neuronal Tissue Modifies Amyloid-Beta mediated neurodegeneration in Drosophila.**

**Allan, K**; Camakaris, J; Burke, R.
Department of Genetics, The University of Melbourne, Melbourne, Australia; School of Biological Sciences, Monash University, Melbourne, Australia.

Alzheimer's disease (AD) is characterised by extracellular senile plaques and intracellular neurofibrillary tangles. The major constituent of the plaques is amyloid-beta. It is currently thought that soluble oligomers of amyloid-beta are the toxic species resulting in neuronal degeneration rather than the plaques themselves. Copper (Cu) has been implicated in the etiology and/or progression of AD, however the exact role this essential trace element plays in the disease is unknown. Amyloid-beta has been shown to directly interact with Cu via a Cu-binding site. In vitro addition of Cu precipitates amyloid-beta into insoluble aggregates that are dissolvable with chelators. Amyloid-beta has also been shown to be capable of catalysing the reduction of Cu2+ to Cu1+ potentially releasing free radicals and increase oxidative stress. Thus it is hypothesised that disruption of Cu homeostasis plays an important role in AD and subsequent neurodegeneration. We have created a Drosophila model of amyloid-beta induced neurodegeneration using the GAL4/UAS system. Expression of human amyloid-beta in all neuronal tissue, via Elav-Gal4, leads to a marked decline in longevity and locomotion. The AD flies also display increased sensitivity to oxidative stress in an age-dependent manner. To explore the relationship between Cu and AD we have manipulated Cu levels in the AD fly using two approaches; genetically by over-expression or RNAi of specific Cu homeostasis genes or exogenously by feeding experiments. Here we show that raising the AD flies in a low Cu environment leads to a slight increase in longevity and delay in the onset in the decline of locomotion. This study should lead to an understanding of the role Cu plays in AD and a better insight into potential therapeutic strategies.

**P-84 Small molecule screen for potential Huntinton´s Disease therapeutics.**

**Byars, C**; Serras, F; Corominas, M
Departament de Genètica Facultat de Biologia Universitat de Barcelona

Huntington´s disease (HD) is a dominantly-inherited polyglutamine-expansion disorder that results in progressive neurodegeneration(1). One aspect of the disease is a global disruption of transcriptional regulation. The N-terminal polyglutamine-containing domain of the huntingtin protein (Htt) has been shown to bind to the acetyltransferase domains of a number of proteins(2) and to inhibit the acetyltransferase activity of others(3), thus interfering with histone acetylation and thereby disregulating gene transcription. Additionally, expression of the Htt exon 1 (Httex1p) in cultured cells reduces the histone H3 and H4 acetylation. This can be reversed with histone deacetylase inhibitors (HDACi). HDACi dosing arrests progressive neuronal degeneration in Drosophila models of HD and other polyglutamine-expansion disorders(4). Here we present a primary screen of biologically-active small molecules for their ability to rescue the mutant phenotype of HD model flies. The identified compounds will provide leads for therapeutic development for the polyglutamine diseases. Additionaly, a recent publication identified mammalian Rhes (Ras Homolog Enriched in Striatum) as a necessary co-factor for polyglutamine-Htt cytotoxicity in cell culture(5). We investigated several potential Drosophila Rhes homologs for genetic interaction with our HD model.

**P-85 Genome-wide identification of Atrophin direct targets.**

**Calamita, P**.; Napoletano, F.; Fanto, M.
Dulbecco Telethon Institute (DTI) San Raffaele Vita Salute University via Olgettina, 58 20132 Milano Italy

DRPLA (DentatoRubralPallidolouysian Atrophy) is a neurodegenerative genetic disease caused by the polyQ tract expansion in the Atrophin-1 protein, a transcriptional regulator. To understand if the degeneration due to Atrophin-1 mutation can be caused by transcriptional dysregulation we have monitored gene expression in a Drosophila transgenic model of the disease. Now we are going to carry out a study parallel to the previous gene expression analysis, aimed at identifying the direct targets of Atrophin. At the moment we have obtained different transgenic lines carrying the E.coli DNA Methyltransferase fused to the N-term or to the C-term of the wild type Drosophila Atrophin protein for a DamID experiment, and different transgenic lines carrying the Myc tag fused to the wild type Drosophila Atrophin for a Chip on Chip experiment. Characterization of all models is now in progress to choose the most efficient set up for our genome-wide analysis.

**P-86 A cytoplasmic suppressor of tko25t.**

**Shanjun Chen** and Howard T. Jacobs
Institute of Medical Technology, FI-33014 University of Tampere, Finland

Wolbachia is a widespread, maternally inherited, gram negative bacterium. In insect hosts it manipulates host reproduction by inducing parthenogenesis, feminization, male killing, and cytoplasmic incompatibility, all of which enhance its own transmission. We investigated genetic interactions between endosymbiotic Wolbachia and mitochondria, using the Drosophila mutant tko25t, harbouring a point mutation in a mitoribosomal protein gene. tko25t flies have impaired mitochondrial OXPHOS capacity and exhibit developmental delay, bang sensitivity, impaired male courtship, and defective response to sound [1]. Wolbachia cytoplasm partially compensates these mutant phenotypes, with cellular ATP level restored close to wild-type and mitochondrial DNA copy number approximately 2-fold elevated. This suppression was maintained after antibiotic treatment with tetracycline (0.03 mg/l), which eliminated the endosymbiont. The suppressor activity is therefore carried in the mtDNA of the 'Wolbachia' strain. We propose that the metabolic stress imposed by Wolbachia has acted as a natural selection system for improved mtDNA fitness.

**P-87 Study of the splicing factor prp8 in drosophila eyes.**

Giordano Ennio*, Cavaliere Daniela°, D'Amato Rocco°, Scialò Filippo°, Martìn Iker?, Polito L.C. and **Digilio Filomena Anna**°
°Institute of Genetics and Biophisics "A. Buzzati-Traverso???, Via P. Castellino 111, Naples, Italy *Dept of Biological Sciences, Section of Genetics and Molecular Biology, University of Naples

Retinitis Pigmentosa is a frequently observed, progressive degeneration of the retina that leads to blindness and that is at present neither preventable nor curable. The RP phenotype is very heterogeneous in term of date of onset of symptoms, degree of visual dysfunction, degree of respective rod and cone involvement even among affected members of the same family. This phenotypic heterogeneity finds its parallel in the genetic complexity of the disorder. The majority of known RP genes encodes for proteins involved in photoreception. Surprisingly, however, RP mutations have also been identified in housekeeping genes required for general cellular processes. Among these cases, a severe form of the disease, RP13, has been linked to mutations in the C-terminal portion of PRP8. PRP8 is a large U5 snRNP-specific protein that is highly conserved in both sequence and size from yeast to human. It has fundamental roles at several steps in spliceosomal assembly and function; although recent studies have contributed to our current understanding of PRP8 in terms of function and its relationship to RP, little is known about its role on the alternative splicing of endogenous genes and the cellular mechanisms that connect its mutations to eventual photoreceptor cell death by apoptosis, a process that can take decades in humans. The major goal of this study is to establish an animal model to elucidate how defects in a general splicing factor lead to cell-type specific RP phenotypes. Gene silencing of prp8 by RNAi into the eyes of transgenic flies leads to retina defects and irregular organization of the ommatidia. Our preliminary results will be presented.

**P-88 in vivo imaging of Drosophila adult mosaic photoreceptor neurons using double-labeling fluorescnece for neurodegeneration.**

Alexis Gambis1‡, **Pierre Dourlen**2‡, Bertrand Mollereau2* and Hermann Steller1* ‡ and * contributed equally to this work, respectively
1 Howard Hughes Medical Institute, Laboratory of Apoptosis and Cancer, The Rockefeller University, New York, NY, USA, 2 Laboratory of Molecular Biology of the Cell, UMR5239 CNRS/Ecole Normale Supérieure

At the end of development, drosophila retinal cells exhibit resistance to cell death that lasts during adulthood. Such survival mechanisms could be altered during neurodegenerative diseases. To study the survival of photoreceptor neurons in the adult, we have developed a new technique of in vivo double fluorescent labelling of mosaic adult photoreceptors. It consisted in generating homozygous mutant clones in a wild type or heterozygous background by mitotic recombination (FRT/FLP system) and visualising mosaic photoreceptor neurons expressing GFP/Tomato fluorescent proteins by cornea neutralisation microscopy. We performed a recessive screen on a collection of P-element lethal lines recombined on FRT chromosomes generated by the undergraduate research consortium at UCLA. Mutations inducing defects in photoreceptor survival were found despite a wild type eye surface as described by scanning microscopy. Moreover, in vivo imaging permitted to follow progressive neurodegeneration in the same animal, allowing to characterize late onset degenerative mechanisms. A wide range of other defects was also found affecting planar polarity, patterning or differentiation of photoreceptor neurons. A secondary screen with the eye-specific overexpression of p35 recovered candidates causing degeneration in a caspase-dependent manner. New mutations were found to better understand anti-apoptotic mechanisms in differentiated neurons.

**P-89 Identification of novel regulators of the JAK-STAT signalling pathway via genome-wide RNA-interference carried out at the University of Shefield RNAi Screening Facility.**

**Fisher K. H.**; Brown S.; Zeidler M. P.
MRC Centre for Developmental and Biomedical Genetics, Department of Biomedical Science, University of Sheffield, UK

The JAK/STAT pathway has been identified as an important mediator of tumourigenesis in a large number of human cancers and participates in the regulation of malignant processes. In comparison to mammalian systems, the Drosophila JAK/STAT pathway represents a lower complexity pathway that mediates multiple biological processes. High-throughput RNA-interference (RNAi) screens have identified regulators of the JAK/STAT pathway based on a STAT-transcriptional assay. A recent Wellcome Trust award has funded the establishment of a Drosophila RNAi screening facility at the University of Sheffield. This facility is open to external researchers who are invited to make use of this infrastructure, including integrated liquid handling robotics, cell culture facilities, and high content microscopy in an automated lab environment. The facility uses a second-generation genome-wide RNAi library originally developed by the Boutros lab (DKFZ, Heidelberg) and in-house bioinformatics support to undertake robust screens in Drosophila cells. Here we present an overview of the infrastructure available to external screening groups as well as preliminary screen data generated in the facility. One such screen, which is under development, is designed to investigate factors regulating the nuclear import/export of the STAT92E transcription factor both before and after pathway stimulation. Although canonical pathway members are relatively well characterised, it has recently been shown that STAT92E is present within the nucleus prior to pathway stimulation, and also accumulates there in its tyrosine phosphorylated form after stimulation. It is anticipated that this screen will identify novel factors involved in spatiotemporal control of these shuttling processes.

**P-90 Modelling mental retardation in flies: from systematic expression patterns to molecular networks and mechanisms undelying cognitive (dys)function.**

**Kochinke, K.**[1], Oti, M.[2], Zweier, C.[3,1], Mejstrik, P.[4], de Jong, E.K.[1], Oortveld, M.A.W.[1], Kramer, J.M.[1], , van Bokhoven, H.[1], Habermann, B.[4], Huynen, M.[2], Tomancák, P.[4]
[1]NCMLS & Donders Institute for Brain, Cognition and Behaviour & RUNMC, Nijmegen, the Netherlands [2]CMBI & NCMLS, Nijmegen, the Netherlands [3]Institute of Human Genetics, University of Erlangen

Mental Retardation (MR), characterized by an IQ of less than 70, affects 3% of the European population. It is a major, unsolved medical problem that has a strong genetic component. To date, more than 400 MR-causing genes have been identified. However, which of these genes are expressed and act directly in the nervous system and what is the role of the encoded proteins in brain development & function is poorly understood. To significantly advance our current knowledge, we use Drosophila as a model organism. Powerful genetic tools of Drosophila permit us to silence all MR-causing genes, one by one, specifically in the nervous system. In these functional MR gene screens, one of which I currently set up, we study landmarks of the nervous system that are also known to be affected in human MR patients, such as synaptic and dendritic morphology, neuronal (dys)function and behavior. Here, I present an approach that is complementary to our functional screens: the establishment of systematic expression patterns throughout embryogenesis by high throughput in-situ hybridizations. This will be the first complete dataset on genes implicated in MR. It will reveal which MR genes are expressed at the same time in the same place, a prerequisite for genes to fulfill a common function. Indeed, it is already evident that several MR genes act in common molecular pathways and with this approach we aim to contribute to the identification of further key modules and mechanisms that control nervous system development and/or cognitive (dys)function. The in situ data will also be used for extensive bioinformatics to investigate the biological coherence behind MR, knowledge that can subsequently facilitate human MR gene identification. I will present first data that arose from this approach.

**P-91 Human frataxin recovers pns phenotypes in a drosophila model of friedreich ataxia.**

**Llorens J.V.**1*, Navarro J.A.2, Soriano S.1, Blanco-Sobero L.1, Martínez-Sebastián M.J.1, Moltó M. D.1,3
1Department of Genetics. University of Valencia. C/ Dr. Moliner, 50, 46100. Burjassot. Valencia. Spain. 2Lehrstuhl für Entwicklungsbiologie. Universität Regensburg. Universitätstrasse 31, D -93040. Regensburg. Germany. 3 CIBERSAM

Friedreich ataxia (FA) is one of the most common forms of hereditary ataxia in humans. This disease is caused by a reduction of the nuclear encoded mitochondrial protein frataxin. The function of frataxin is still unclear, but there is no doubt about its involvement in mitochondrial iron homeostasis and the control of oxidative metabolism. Frataxin has been conserved in many organisms ranging from bacteria to humans. The models of FA in different organisms indicate a similar function of this protein. In a Drosophila model have been demonstrated oxidative damage and defects in iron-sulphur enzymes as humans, but whether Drosophila and human frataxin actually have interchangeable roles in mitochondria is unknown. Our objective was demonstrating the functional equivalence between human and fly frataxins. For that reason, flies interfering the expression of Drosophila frataxin homolog (fh) to undetectable levels (Anderson et al. 2005), flies overexpressing fh 9-fold (Llorens et al. 2007), and flies overexpressing the human frataxin gene (FXN) sixteen times the endogenous fh expression, have been used by means of the UAS-GAL4 system. All the three scenarios were lethal when the driver used is ubiquitous (actin-GAL4), while were viable with several nervous system drivers (neuralizedGAL4, C96-GAL4). First, we compared the defects observed in an excess of human and fly frataxin, obtaining similar phenotypes in both cases although always stronger in the first than in the latter case. In both the lethality was associated to several defects in muscle and PNS, which are relevant tissues in FA. The viable progeny obtained using the PNS driver, neuralized-GAL4, reach adulthood but flies showed reduction of lifespan and defects in behaviour as climbing. Second, we have generated flies combining the fh interference with the FXN overexpression to rescue the defects observed in both scenarios separately. Using the actin-GAL4 driver we couldn't rescue the lethality associated to systemic alterations of frataxin levels. However when we utilized the neuralized-GAL4, the flies showed recovery of the climbing capabilities and lifespan. In conclusion all these data suggest a functional equivalence between human and fly frataxins.

**P-92 Bug22 is a novel protein essential for ciliogenesis.**

**Teresa Maia**, Renata Basto
Institut Curie, Paris, France

Cilia are specialized organelles that are present in most organisms and that are involved in several processes such as motility, sensory perception and development. Ciliary defects have been linked to policystic kidney disease, some forms of deafness, situs inversus and sterility. A considerable effort has been put on understanding better the biology of these structures. bug22 is a novel gene whose protein product has been identified as a component of mature centrioles and as being recruited for the assembly of cilia or flagella. It belongs to a highly conserved eukaryotic protein family for which yet no member or function has been characterized. We are currently characterizing bug22 in Drosophila. Here we show the localization of Bug22 in several Drosophila tissues and provide the first evidences for its function. In terms of localization, Bug22 assumes a different location depending on the stage of cell cycle. Immunohistochemical characterization of endogenous and fluorescence protein-tagged versions of Bug22 shows that it localizes to the centrosomes and the mitotic spindle in dividing cells and that it can also assume a nuclear localization in interphase. Additionally, Bug22 is associated with the sperm tail, which provides a possible link to it playing an essential role in sperm maturation, as fly males depleted for Bug22 present a decreased fertility. Flies in which the levels of Bug22 have been depleted by RNAi also exhibit an unc (uncoordinated locomotion) phenotype, a perturbation associated with defects in cilia-containing sensory neurons. We are generating a null bug22 mutant to understand its function in development and its role in ciliogenesis.

**P-93 ER Stress Protects Against Retinal Degeneration.**

Clémence Levet1, César S. Mendes2, Gilles Chatelain1, Pierre Dourlen1, Antoine Fouillet1, Marie-Laure Dichtel-Danjoy1, Hermann Steller1 and **Bertrand Mollereau**1
1 Ecole Normale Supérieure de Lyon/CNRS. LBMC. Université de Lyon 1 France. 2 Howard Hughes Medical Institute, The Rockefeller University, New York, NY, USA

The unfolded protein response (UPR) enables cells to cope with an overload of unfolded/misfolded proteins in the endoplasmic reticulum (ER). ER stress is commonly associated with degenerative diseases, but its role in disease progression remains a matter of debate. We have shown that mild ER stress protects photoreceptor neurons against various death stimuli in adult Drosophila and Drosophila cell cultures. A specific ER-mediated signal promotes antioxidant defenses and inhibits caspase-dependent cell death (Mendes et al. 2009). Further analysis revealed that an autophagic response was triggered in cells subjected to apoptosis and ER stress signals. Autophagy may be protective in neurons, and an understanding of the molecular mechanisms directing cells towards an autophagic response is essential if we are to comprehend ER stress-mediated protection. In this study we focused on a new and important issue, the possibility that moderate ER stress protects not only against the deleterious effects of unfolded protein accumulation, but also against external apoptotic stimuli.

**P-94 The effects of Eugenol and Ferrulic Acid in relation to Drosophila models of Parkinson's Disease.**

**Sree Deepthi MuthuKrishnan**, Bharath Srinivasan and Mukesh Doble
Sree Deepthi MuthuKrishnan, Indian Institute of Technology-Madras Bharath Srinivasan, Principal Project Officer, Indian Institute of Technology-Madras Mukesh Doble, Facult, Indian Institute of Techn

Parkinson's Disease (PD) is one of the most common neurodegenerative movement disorders in humans, likely manifesting through complex interactions between environmental agents on the one hand and genetic susceptibility factors on the other. In recent years, Drosophila melanogaster has served as an excellent model system for investigating the molecular etiology underlying PD. Mutations relating to various forms of familial PD in humans have been successfully modeled in flies, such as those involving the Parkin, PINK1, DJ-1 and LRRK2 loci. In complementary approaches, fly models recapitulating definitive symptoms caused by PD-associated environmental toxins such as paraquat and rotenone, have been established in molecular and cellular terms. In addition, Drosophila is amenable to the testing of potentially therapeutic small molecules such as antioxidants, most often through simple dietary feeding. In view of all these, as well as the more conventional experimental advantages offered by Drosophila, we have undertaken to examine the effects of two phenolic phytochemicals, Eugenol and Ferrulic Acid, with respect to various fly PD models. Both compounds are known to possess potent antioxidant activities, thus paving the way for possible neuroprotective effects and therapeutic strategies. Meaningful results from our studies will be presented at the meeting.

**P-95 Transcriptional regulation by Atrophin in development and neurodegeneration.**

**Francesco Napoletano, FN**; Piera Calamita, PC; Manolis Fanto, MF
Dulbecco Telethon Institute (DTI), DiBiT-HSR, San Raffaele Scientific Institute, via Olgettina 58, I -20132 Milano, (Italy) MRC Centre for Developmental Neurobiology, King's College London, Guy's Camp

Atrophin (Atro) is the only Drosophila homolog of human Atrophin-1, the DRPLA disease gene. DRPLA (Dentatorubralpallidoluysian Atrophy) is a polyglutamine disease, caused by expansion of a polyQ tract in Atrophin-1, and characterized by brain specific neurons degeneration, together with psychiatric and motor symptoms. Primary causative events in polyQ diseases are unknown. Atrophins are ubiquitous transcriptional cofactors involved in neuronal development and survival. We have generated fly inducible models for DRPLA, to monitor the early transcriptional pathogenic alterations, by microarray based time-course gene expression profiling. Finally we are going to couple the transcriptional with chromatin profiling, by DamID, to identify the Atro direct targets. Our analysis reveals the dysregulation of key genes controlling neuronal differentiation and cell cycle, and a link between the neurodegeneration induced by polyQ-Atro and the Fat tumour suppressor pathway.

**P-96 Characterization of a small nuclear 7SK RNA-like which plays a role in proliferation and differentiation in Drosophila.**

**Nguyen, D**[1] ; Buisine, N[2] ; Trouslard, F[1] ; Lecorre, P[1] ; Michels, AA[3]* ; Bensaude, O[3] ; Uguen, P[1].
1 UMR CNRS 8080 Développement & Evolution – bât. 445 – Université Paris-Sud XI – Orsay -France. 2 MNHN - UMR CNRS 5166 Evolution des régulations endocriniennes - 75231 Paris -France. 3 ENS

It is increasingly recognised that non-coding RNAs play a critical role in normal and pathological cell metabolism. The 7SK RNA is the most abundant nuclear RNA in vertebrates and, surprisingly, its biological function has remained enigmatic for over 25 years. Recently, it has been found in human to be involved in regulation of transcription. The 7SK RNA, together with the factor called HEXIM, regulates transcription by modulating the kinase activity of the P-TEFb complex, which controls the phosphorylation level of the C-terminal domain of the RNA polymerase II. Biochemical data describe the interactions between the different partners of the P-TEFb/HEXIM/7SK complex. However, their role in cellular differentiation and proliferation, although strongly suspected, is difficult to assess in mammals. For these reasons, we undertook to functionaly characterised the 7SK RNA regulation pathway in Drosophila, during development. We have identified and characterised a putative 7SK RNA analog in Drosophila and collected evidences of its functional conservation between Drosophila and Vertebrates. In addition, expression profiling at different embryonic and larvae stages revealed a tissue-specific expression pattern of the 7SK RNA and its partners. Analyses of loss- and gain-of function mutants of HEXIM indicate a role in differentiation and proliferation processes. This supports the hypothesis that this RNA/protein complex would be a transcriptional regulator, involved in Drosophila development. We are now investigating the genes regulated by this complex to draw then the biological network of the 7SK RNA and identify its impact in cancer cells.

**P-97 ER stress and tumor formation in Drosophila.**

**Perez-Garijo, A;** Steller, H.
Rockefeller University.

ER stress is produced when misfolded proteins accumulate in the ER, which activates the unfolded protein response (UPR). This process has been associated to many diseases, such as diabetes, neurodegenerative diseases and cancer. ER stress can have a dual role in tumor formation. On one hand, it can help tumor cells to cope with stress and to survive. On the other hand, if the UPR is maintained for a long time it can trigger apoptosis. We analyze the expression of ER stress markers in different types of tumors in Drosophila and in most cases they are upregulated. Thus, Drosophila may be a good model to further study the role of ER stress in cancer.

**P-98 Drosophila as a model for Pantothenate-Kinase-Associated-Neurodegeneration.**

**Rana A**, Siudeja K, Seinen E, Srinivasan B, Muntendam R, Kayser O, Reijngoud DJ and Sibon OC
Department of Cell Biology, University Medical Center Groningen, The Netherlands

Pantothenate-kinase-associated-neurodegeneration (PKAN) is an autosomal recessive disorder which shows progressive impairment of locomotor function, cognition and early death. The pathophysiology of disease is not understood and there is no cure to revert, halt or even delay the neurodegeneration. PKAN is caused by the mutations in the PANK2 gene (Zhou B, Nat Genet., 2001), which encodes rate limiting enzyme for Coenzyme A biosynthesis. A mouse Pank2 knock out has been generated, however this mouse model did not show any signs of neurodegeneration (Kuo et al., 2005), thus leaving the key question, regarding the pathophysiology of the disorder, unexplored. Recently, we and others have demonstrated that CoA biosynthesis enzymes (such as PANK, PPCS and PPAT-DPCK) are conserved in Drosophila (Afshar et al., Bosveld et al., 2008; Zhou et al., 2009). Therefore, dPANK/fbl mutants can be used for identifying the underlying role of Pank gene disorder in PKAN pathogenesis. Moreover, Drosophila disease models are especially of value to identify compounds that are able to rescue disease associated phenotypes. In the present study, we used in vitro as well as in vivo approaches to address the following questions: 1) what are consequences of dPank depletion on the cell and organism? 2) Can restoration of Pank enzyme function rescue the disease phenotypes? 3) Can metabolic rescue of CoA reverse the disease phenotypes? Our results show that dPANK/fbl depletion in Drosophila indeed results in phenotypes similar to human PKAN disorder. In this study we are exploring metabolic compounds which can rescue the Pank enzyme defects and thus might be a potential cure for the PKAN disease.

**P-99 A mosaic genetic screen to identify downstream targets of the Ire1/Xbp1 pathway.**

**Rasheva V.I.** and Domingos P.M.
Instituto de Tecnologia Quimica e Biologica (ITQB), Universidade Nova de Lisboa, Av. Republica (EAN), 2780-157, Oeiras, Portugal

The endoplasmic reticulum (ER) is the cell organelle where secretory and membrane proteins are synthesized and folded. Proteins that fail to fold properly (misfolded proteins) accumulate in the ER, causing ER stress. The Unfolded Protein Response (UPR) includes several signalling pathways which have evolved to detect the accumulation of misfolded proteins in the ER and activate a cellular response that attempts to maintain homeostasis and a normal flux of proteins in the ER. If protective mechanisms activated by the UPR are not sufficient to restore normal ER function cells may die by apoptosis. How is the UPR regulated to change from a protective response to a pro-apoptotic one is still an open question. The genome of Drosophila contains many conserved homologues of UPR genes, including the Ire1/Xbp1 pathway. In order to identify downstream targets of this pathway, we are performing a mosaic genetic screen to identify suppressors of the “glossy??? eye phenotype caused by the overexpression of the active spliced form of Xbp1 in the eye. We have now the first mutants and next we will map these mutations and investigate their role in the UPR mechanisms.

**P-100 Drosophila melanogaster as a model system for studies of IAPP aggregation.**

**Schultz SW**1, Nilsson KPR2, Thor S1, and Westermark GT1
1 Department of Clinical and Experimental Medicine, Linkoping University, Linkoping SE-58183, Sweden. 2 IFM-Department of Chemistry, Linköping University, SE-581 83 Linköping, Sweden

Recent research supports that aggregation of islet amyloid polypeptide (IAPP) leads to cell death and makes it a plausible cause for the halving of beta cell mass that occurs in patients with type 2 diabetes. IAPP is produced as prohormones and processed into biological active hormones in the secretory granules by the convertases PC1/3 and PC2, prior to its stimulated secretion. In vitro, IAPP is one of the most amyloidogenic peptides known, while the presence of insulin can inhibit IAPP-fibril propagation. However little is known about the mechanism protecting non-diabetic patients from forming amyloid deposits and/or pathways involved in amyloid formation in patients with type 2 diabetes. We have constructed transgenic flies expressing proIAPP, IAPP and the non amyloidogenic mouseIAPP (mIAPP). Only flies expressing proIAPP with the Gal4 driver elavC155 showed a reduction in lifespan whereas neither expression of IAPP or mIAPP did influence survival. Even though IAPP expression was not related to a shorter lifespan, both IAPP and proIAPP expression in the CNS led to deposition in the fat body of the head. Congo red and pFTAA staining of brain sections revealed that these deposits were partly made up of amyloid. Our results suggest a promising potential for Drosophila melanogaster as a model system to study the connection of proIAPP and IAPP expression with subsequent amyloid formation and connected pathology.

**P-101 Effect of Deferiprone and Idebenone on a Friedreich ataxia model in Drosophila melanogaster.**

Laura B. Sobero1, **Sirena Soriano**1, José V. Llorens-Llorens1, M. Dolores Moltó1,2, M. José Martínez-Sebastián1*
1Department of Genetics, University of Valencia. Burjassot, Valencia, Spain - 2CIBERSAM

Friedreich ataxia (FA) is a hereditary and neurodegenerative disease affecting mainly the central and peripheral nervous system, as well as producing hypertrophic cardiomyopathy, which is the main cause of death in patients. Since iron overload and oxidative stress are among the main biochemical features observed, today's most advanced therapies are focused on alleviating these effects by intervention with an iron chelator or an antioxidant such as deferiprone or idebenone. It has been reported that these two drugs independently improve patient's motor coordination and decrease the frequency of death due to heart failure. We have studied the effect of these two drugs on an interference model of FA on Drosophila melanogaster, which reproduces several important aspects of this human pathology. We have tested the drugs by hatching embryos on treatment. Our data show that the effect of these two drugs parallel the clinic results, i.e., recovery of both motor coordination and mean-lifespan. We have also assessed the effect of deferiprone and idebenone on aconitase activity, one of the most affected enzymes in the disease, under an oxidative stress atmosphere. While idebenone recovered aconitase activity, deferiprone had no effect on that enzymatic activity. Therefore, this study demonstrate the validity of performing drug screenings for FA using Drosophila melanogaster as a model, since results obtained in the fruit fly are consistent with those obtained in preliminary clinical trials.

**P-102 Dominant mutations in the tyrosyl-tRNA synthetase gene recapitulate in Drosophila features of human Charcot-Marie-Tooth neuropathy.**

**Storkebaum,E** 1,*; Leitaõ-Gonçalves,R 1,2,*; Godenschwege, T 3; Nangle, L 4; Mejia, M 3; Bosmans, I 1; Ooms, T 2; Jacobs, A 2; Van Dijck, P 5; Yang X-L 4; Schimmel, P 4; Norga, K 1; Timmerman, V 2; C
1 Lab. of Developm. Genet., VIB & KUL, Leuven, Belgium 2 Dep. of Mol. Genet., VIB & UA, Antwerp, Belgium 3 Dep. of Biol. Sci., Florida Atlantic University, Boca Raton, USA 4 The Scripps Research In

Dominant-intermediate Charcot–Marie–Tooth neuropathy (DI-CMT) is characterized by axonal degeneration and demyelination of peripheral motor and sensory neurons. Three dominant mutations in the YARS gene, encoding tyrosyl-tRNA synthetase (TyrRS), have so far been associated with DI-CMT type C. The molecular mechanisms through which mutations in YARS lead to peripheral neuropathy are currently unknown, and animal models for DI-CMTC are not yet available. Here, we report the generation of a Drosophila model of DI-CMTC: expression of the 3 mutant —but not wild type— TyrRS in Drosophila recapitulates several hallmarks of the human disease, including a progressive deficit in motor performance, electrophysiological evidence of neuronal dysfunction and morphological signs of axonal degeneration. Not only ubiquitous, but also neuron-specific expression of mutant TyrRS, induces these phenotypes, indicating that the mutant enzyme has cell-autonomous effects in neurons. Furthermore, biochemical and genetic complementation experiments revealed that loss of enzymatic activity is not a common feature of DI-CMTC-associated mutations. Thus, the DI-CMTC phenotype is not due to haploinsufficiency of aminoacylation activity, but most likely to a gain-of-function alteration of the mutant TyrRS or interference with an unknown function of the WT protein. Our results also suggest that the molecular pathways leading to mutant TyrRS-associated neurodegeneration are conserved from flies to humans.

**P-103 Transactivation in Drosophila of human enhancers by human transcription factors involved in Congenital Heart Diseases.**

Maria Florencia Tevy (1), **Concetta Traina** (1), David Brook (2) and Maria Capovilla (1)
(1) Dulbecco Telethon Institute, Dept. of Biology and Evolution, University of Ferrara, Italy (2) School of Biology,? Institute of Genetics,? Queen's Medical Centre,? Nottingham, UK

Human GATA4, NKX2.5 and TBX5 are transcription factors (TFs) that form part of the core network necessary to build a heart. The human Atrial Natriuretic Factor (ANF) and alpha myosin heavy chain 6 (MYH6) genes are targets of such TFs, which activate these targets synergistically on well characterized enhancers. In order to develop a system to study the interactions between the human TFs and their target enhancers in vivo, we subcloned the ANF and MYH6 enhancers in the pH-Stinger vector upstream of the hs43 promoter driving nGFP and generated transgenic flies. No nGFP expression was observed in embryos carrying the reporter constructs, indicating that no Drosophila TF is able to regulate these enhancers in wild-type embryos. To determine whether the human GATA4, NKX2.5 and TBX5 TFs are able to transactivate these enhancers in flies, we obtained UAS>NKX2.5 stocks and generated UAS>GATA4 and UAS>TBX5 flies. When they are expressed in the whole mesoderm through the 24B-Gal4 driver, all three TFs are able to weakly transactivate the ANF and MYH6 enhancers starting from stage 11. Expression is clearly seen in the somatic and visceral mesoderm. Subsequently, each couple of UAS constructs was overexpressed using the 24B-Gal4 driver and we observed a higher level of nGFP expression than in embryos overexpressing the TFs individually, demonstrating for the first time in vivo the synergy between these TFs. We plan to determine whether this transactivation is tissue-specific and whether mutant forms of these TFs that have been shown to be involved in CHDs are defective in transactivation, setting up a powerful tool to clearly determine in vivo the genetic nature of the mutant human forms in order to unravel the underlying molecular genetic mechanisms that lead to CHDs.

**P-104 Pathways of pathogenesis in dominant expanded repeat diseases.**

**van Eyk, C.L.**; Samaraweera, S.; Lawlor, K.T.; O'Keefe, L.V.; McLeod, C.J.; Dayan, S.; Price, G.; Venter, D. and Richards, R.I.
Discipline of Genetics, School of Molecular and Biomedical Science, University of Adelaide, S.A., Australia. ARC Special Research Centre for the Molecular Genetics of Development. ARC-NHMRC Research N

Dominant expanded repeat diseases are caused by the expansion of a simple nucleotide repeat beyond a pathogenic threshold. They include neurodegenerative diseases caused by the expansion of a polyglutamine coding CAG repeat such as Huntington's Disease and a number of the Spinocerebellar Ataxias (SCA) 1,2,3,6,7 and 17. Expanded repeat sequences within noncoding regions can also give rise to neurodegenerative disease. Examples include SCA 8,10 and 12, Huntington's disease-like 2, Myotonic Dystrophy Type 1 and 2 (DM1 and DM2) and Fragile X Tremor Ataxia Syndrome. Since the untranslated and translated dominant expanded repeat diseases seem to share a number of clinical features and have a shared mutation mechanism we hypothesise that there may be common pathogenic pathways between the two disease classes. We are modelling these diseases using Drosophila to identify the molecular basis of pathogenesis and the contribution of hairpin RNA structure. We have employed a number of techniques, including microarray and proteomic analysis, to identify changes caused by the expression of these repeat tracts in the Drosophila nervous system with the aim of developing biomarkers for pathogenesis in these diseases. McLeod CJ, O'Keefe LV, RI Richards (2005) The pathogenic agent in Drosophila models of polyglutamine diseases. Hum Mol Genet., 14(8): 1041-8.

**P-105 Expression of Jaagsiekte Sheep Retrovirus (JSRV) envelope glycoprotein in Drosophila model.**

**Viginier, Barbara**; Terzian, C
UMR 754 « Rétrovirus et Pathologie Comparée », Université Lyon 1, INRA, ENVL, EPHE, 50 avenue Tony Garnier, FRANCE

Betaretrovirus Jaagsiekte Sheep Retrovirus (JSRV) is the etiological agent of ovine pulmonary adenocarcinoma (OPA), a contagious lung cancer of sheep. OPA shares morphological similarities with a human lung cancer: the bronchoalveolar carcinoma. The expression of JSRV envelope protein (JSRVEnv) alone can induce transformation of epithelial cells in culture (Palmarini et al, 2001). The transformation led to activation of PI3K/Akt and MAPK signaling pathways: these signalling pathways are involved in cellular proliferation and growth (Maeda et al, 2005). Therefore, elucidating the mechanism of oncogenesis by JSRV may provide insight into the development of a human epithelial cell lung cancer. Drosophila is a convenient model to investigate growth, proliferation and cell death mechanisms, because most signaling pathways are well conserved from invertebrates to mammalian organisms. In the present study, we generated transgenic lines encoding JSRV Env glycoproteins. Inducible and tissue-specific expression of JSRV Env were observed into eye and wing imaginal discs, as well as in follicle cells. The whole-wing size was significantly reduced when JSRV Env was expressed in imaginal discs of 3rd instar larvae, but cell density was not affected. In addition, JSRV Env expression using MS1096 driver induced ectopic wing vein. This phenotype is analysed with regards to the potential interaction between JSRV Env and EGFR signalling pathway.

**P-106 Protein Phosphatase 2A regulates self-renewal of Drosophila neural stem cells.**

**Cheng Wang**1, Kai Chen Chang2, Fengwei Yu2,and Hongyan Wang1
1Neuroscience & behavioral disorder program, Duke-NUS Graduate Medical School Singapore, 2 Jalan Bukit Merah, Singapore 169547. 2Temasek Life Sciences Laboratory, 1 Research Link, Singapore 117604.

Drosophila larval brain neural stem cells, neuroblasts, divide asymmetrically to generate a selfrenewing neuroblast and a Ganglion Mother Cell (GMC) that divides terminally to produce two differentiated neurons/glia. Failure of asymmetric cell division can result in hyperproliferation of neuroblasts, a phenotype resembling brain tumors. Here we have identified Drosophila Protein Phosphatase 2A (PP2A) as a brain tumor-suppressor that can inhibit self-renewal of neuroblasts. Supernumerary larval brain neuroblasts are generated at the expense of differentiated neurons in PP2A mutants. Neuroblast overgrowth was observed in both Dorsomedial (DM)/Posterior Asensenegative (PAN) and non-DM neuroblast lineages. The PP2A heterotrimeric complex composed of the catalytic subunit (Mts), scaffold subunit (PP2A-29B), and a B regulatory subunit (Tws), is required for the asymmetric cell division of neuroblasts. PP2A complex regulates asymmetric localization of Numb, Pon and atypical protein kinase C as well as proper mitotic spindle orientation. Interestingly, PP2A and Polo kinase enhance Numb and Pon phosphorylation. PP2A, like Polo, acts to prevent excess neuroblast self-renewal primarily by regulating asymmetric localization/activation of Numb. Reduction of PP2A function in larval brains or S2 cells causes a marked decrease in Polo transcript and protein abundance. Overexpression of Polo or Numb significantly suppresses neuroblast overgrowth in PP2A mutants, suggesting that PP2A inhibits excess neuroblast self-renewal in Polo/Numb pathway.

**P-107 Cell cluster migration requires crosstalk between TGF-B/BMP signaling and JAK/STAT signalling.**

Akiyama, T; **Wharton, K.A.**
Department of Molecular Biology, Cell Biology and Biochemistry Brown University

At critical times during development cells acquire migratory properties, often moving to distant sites to perform a new function. A key to understanding not only development but also homeostasis and cancer metastasis is elucidating the cellular and molecular mechanisms underlying the transition of cells into migratory or invasive behaviors. The specification and migration of cluster border cell cluster during Drosophila oogenesis provides an ideal system to study the mechanisms driving cell migration in vivo. Border cells are specified in response JAK-STAT ligand, Upd signals from adjacent polar cells at the anterior of each egg chamber. Together, border and polar cells then migrate through the nurse cells toward the oocyte. We have recently found that BMP signaling is also required for both the specification of border cells and their migration. The Drosophila BMP ligand encoded by glass bottom boat (gbb) is expressed in border cells and specific downregulation of BMP signaling in polar cells results in the specification of fewer border cells. Interestingly, the specific downregulation of BMP signaling in border cells does not affect cell number but impacts cluster migration and affects the distribution of DE-cadherin within the cluster. Based on our recent findings, we propose a model in which Gbb positively regulates the expression of upd in polar cells to maintain JAK-STAT activity, as well as provides BMP signaling in the border cells to influence cell migration. This study has identified crosstalk between BMP signaling and JAK/STAT signaling at multiple steps in the migratory process of the polar/border cell cluster that is important for proper coordination of cell fate specification with cluster migration.

**P-108 Transcriptional targets of Drosophila JAK/STAT pathway signalling as effectors of haematopoietic tumour formation.**

Samira Bina, Victoria Wright and **Martin P. Zeidler**
MRC Centre for Developmental & Biomedical Genetics, Department of Biomedical Science, University of Sheffield, UK

The JAK/STAT pathway has been identified as an important mediator of tumourigenesis in a large number of human cancers including both solid tumours and haematologically derived malignancies. Although multiple signal transduction pathways have been implicated in the development of human disease, identification of the pathway targets and biological processes that mediate disease progression is less experimentally tractable. Here we use transcript profiling of Drosophila haemocyte-like cells to identify JAK/STAT target genes that are both up and down regulated in response to stimulation by the pathway ligand Unpaired (Upd). We have extended this analysis in vivo using a model for JAK-induced blood cell over-proliferation caused by the gain-of-function allele hop[Tuml] and have identified key effectors required for haematopoietic tumour development. Potential effectors identified include Baz/PAR3 required for the establishment and maintenance of cellular polarity and G?73B, a G-protein subunit whose human homologue has been implicated as a disease marker in chronic lymphocytic leukaemia. Assays for signalling pathway-effectors in Drosophila therefore represent a potentially powerful tool for the identification of factors central to human disease development and progression.

**P-109 Drosophila bithorax complex genes regulates the spatial and temporal expression of castor, a neublast gene.**

**Ju Hyun Ahn**; Keuk Il Jung; Sang Hee Kim; Sang-Hak Jeon
Department of Biology Education, Seoul National University, Seoul 151-748, Korea. Department of Chemistry, Konkuk University, Seoul 143-701, Korea.

The ventral nerve cord (vNC) of Drosophila shows significant segmental-specific characteristics during embryonic development. Homeotic genes are expressed over long periods of time and confer identity to the different segments. Especially Bithorax-complex (BX-C) genes control the formation of segments of the posterior thorax and abdomen. castor (cas) is one of genes that are expressed in a segment-specific pattern at late embryonic stage. At stage 15, it appears in a few cells of each thoracic segments and terminal abdominal segments, but not in most of abdominal segments. Here we investigated whether the temporal and spatial expression of cas is regulated by the homeotic genes. In Utrabithorax (Ubx) and abdominal-A (abdA) loss-of-function mutant embryos, cas is ectopically expressed in the abdominal segments, but in Abdominal-B (AbdB) lossof-function mutant embryos cas disappeared. In ubx and abdA gain-of-function mutant embryos, cas expression was suppressed while in AbdB gain-of- function mutant embryos, cas was ectopically expressed in anterior abdominal segments. These results suggest that BX-C genes regulate expression of cas at late embryonic stage. More recent our studies suggest that this homeotic transformation appeared to be related to cell proliferation.

**P-110 Segmental specification of the embryonic neuroblast lineage 6-4 in the gnathal segments of Drosophila melanogaster.**

**Henrike Becker**1, Christian Berger2, L.S. Shashidhara3 and Gerhard M. Technau1
1 Institute of Genetics, Johannes Gutenberg University Mainz, Germany; 2 Institute of Molecular Biotechnology Vienna, Austria; 3 Indian Institute of Science Education and Research Pune, India

Along the anterior-posterior axis the pattern of NBs is segmentally repeated, with NBs born at the same position and time acquiring the same fate, and being called serial homologs. This is mirrored in specific cell lineages generated. Nevertheless, at least seven of the 30 NBs per hemisegment generate segment specific cell lineages, which differ in number and/or fate of particular subsets of progeny cells. Segment specific behaviour of NBs and their progeny cells is mediated by the function of the Hox proteins. We use the NB 6-4 lineage as a model to investigate the mechanism controlling segmental diversification of serially homologous lineages. We have shown that the labial and thoracic NB6-4 lineages consist of three glial and five to six neuronal cells. Instead, the abdominal as well as the maxillary lineage consists of glia cells exclusively. Genes of the Bithorax-Complex are expressed in the thoracic and abdominal NB6-4 lineages and genes of the Antennapedia-Complex (ANTP-C) mainly in the gnathal lineages. Loss of function experiments revealed that the thoracic/labial version represents the ground state of the NB6-4 lineage, which requires no homeotic gene function. In the maxillary segment Deformed and Sex Combs Reduced and in the abdominal segments Abdominal A and Abdominal B induce the generation of a pure glial lineage by repressing the cell cycle control gene CyclinE, which is necessary for generating the neuronal sub-lineage. Here we will focus on how segmental identities are generated in the gnathal region, which links the ventral nerve cord and the brain. Loss of function and expression analyses of the homeotic genes of the ANTP-C reveal that cofactors are necessary for mediating the different segmental fates of NB6-4 in this region.

**P-111 Genome wide analysis of self-renewal in Drosophila Neuroblasts.**

**Berger, C**; Harzer, H; Neumüller, R; Richter, C; Fischer, A; Knoblich, J
IMBA - Institute of Molecular Biotechnology of the Austrian Academy of Science Dr.-Bohr-Gasse Nr3 1030 Vienna Austria

Drosophila larval neuroblasts are a well-studied model system for stem cell division. Neuroblasts undergo multiple rounds of asymmetric cell division in which a highly conserved protein complex on the apical side of the cell directs the cell fate determinants Numb, Prospero and Brat to the basal side of the cell. As a consequence, cell fate determinants are inherited by the basal daughter cell where they induce cell cycle exit and neuronal differentiation. The apical daughter cell retains its neuroblast identity and continues to divide. Although we understand the mechanisms that result in the asymmetric segregation of cell fate determinants, the factors acting downstream regulating differentiation versus self-renewal are not known. For example, does a self-renewal program in the stem cell exist? How is growth linked to stem cell identity? To address these questions, our lab has conducted a large-scale genetic in vivo RNAi screen using the Vienna RNAi lines (VDRC). Using a neuroblast specific gal4 line more than 11 000 individual genes were knocked down and the resulting phenotypes were analyzed. Besides novel tumour suppressors that cause overproliferation of the neuroblasts, numerous factors were found that cause under-proliferation upon RNAi. We use this set of 904 genes to screen for potential factors required for stem cell maintenance and cell growth. To distinguish between these possibilities we are currently conducting a detailed immunofluorescence analysis of the RNAi brain phenotypes. This approach will be combined with a trancriptome analysis of neuroblasts. Based on the obtained data we will build up a network of novel and known genes involved in maintenance of stem cell identity and self-renewal.

**P-112 The protocadherin Flamingo collaborates with the axon guidance receptor Gogo.**

**Berger,S**; Hakeda-Suzuki,S; Tomasi,T; Suzuki,T
Max-Planck Institute of Neurobiology Am Klopferspitz 18 Martinsried Germany

In epithelial sheets, cells show a coordinated polarity in the plane of the epithelium, called planar cell polarity (PCP). In the retinal epithelium, photoreceptor cells form subunits called ommatidia, which contain eight different photoreceptor cells (R1-R8). The polarity of ommatidia is established in R cells before they extend their axons toward their specific target in the central nervous system. The protocadherin Flamingo (Fmi) is required for both PCP and axon guidance of photoreceptor cells, whereas other members of the core PCP complex (Frizzled, Vang Gogh and Dishevelled) are not involved in axon pathfinding. Therefore, it was posited that Fmi functions in two distinct pathways to mediate axon guidance and PCP. The axon guidance receptor protein Gogo shares striking phenotypical similarities with Fmi in R1-6 and R8 axon guidance, but Gogo is not required for PCP. We show that Gogo and Fmi interact genetically in the guidance of photoreceptor axons. Co-overpression of Gogo and Fmi causes mistargeting of R7 to the R8 target layer. In addition, Gogo colocalizes with Fmi at cell-cell borders in S2 cells, suggesting that they physically interact. Gogo expression starts after PCP is established. When Gogo was expressed precouciously in the eye, we observed polarity defects due to the mislocalization of Fmi. Taken together, our results show that Fmi act in different protein complexes to achieve its distinct function in axon guidance and PCP, and that Gogo serves as a guidance moiety of Fmi.

**P-113 Hedgehog signaling is required to restrict neuroblast proliferation in Drosophila post-embryonic brain..**

**Phing Chian CHAI**; Yu CAI; William CHIA
Temasek Life Sciences Laboratory, National University of Singapore, Singapore

In Drosophila, neural stem cells called neuroblasts divide asymmetrically to self renew and to generate differentiated neurons and/or glial cells. While it is known that this process is tightly controlled by a complex network of intrinsic signals, the involvement of extrinsic cues (extracellular signaling) remains unclear. In this study, we explored the roles of the Hedgehog (Hh) signaling pathway in the regulation of neuroblast proliferation and maintenance, as well as neuronal differentiation. Using clonal analysis, we found that loss of Hh signaling often leads to overproliferation of neuroblasts and clonal expansion. In contrast, excessive activation of the Hh signaling pathway results in partial loss of neuroblast identity and proliferative capacity. Using in situ hybridization and reporter assays, we were able to show that Hh is expressed transiently in the ganglion mother cells (GMCs) and mitotic neuroblasts. The signal is then perceived by both the neuroblast and GMCs, possibly in an autocrine and/or paracrine fashion. Our results suggest that Hh signalling is required to restrict the neuroblast from over-proliferating. Interestingly, the phenotype caused by aberrant activation of Hh signaling could be reverted by removing a copy of prospero, a key differentiation factor that is selectively segregated to the GMCs during asymmetric division. Hence, the Hh signaling pathway may function upstream of the intrinsic apparatus of asymmetric cell division.

**P-114 The sensory nervous system of Drosophila: mechanisms that corral axons into segmental bundles.**

**Caroline Fabre**, José Casal, and Peter Lawrence / Caroline Fabre, Laurent Arnoult, Nicolas Gompel, Benjamin Prud'homme
LMB, Department of Zoology, Cambridge, UK / IBDML, Marseille

The abdomen of adult Drosophila bears mechanosensory bristles whose axons connect directly to the CNS, each segment contributing a separate nerve bundle. Here, we alter the amount of Engrailed protein and manipulate the Hedgehog signalling pathway in clones of cells, studying the effects on nerve pathfinding. We find that high levels of Engrailed make the epidermal cells inhospitable to bristle neurons; any sensory axons too near these cells either avoid them or fail to extend properly or at all. We then searched for an engrailed-dependent agent responsible for these repellent properties. We found slit to be expressed in the P compartment and, using genetic mosaics, present evidence that Slit is the responsible agent. We conclude that, during normal development, gradients of Slit protein repel axons away from compartment boundaries — in consequence the bristles from each segment send their nerves to the CNS in separated sets.

**P-115 High-resolution transcriptional profiling to identify neural stem cell regulators in the optic lobe.**

**Gold, K.S.**; Egger, B; Brand, A.H.
Wellcome Trust/Cancer Research UK Gurdon Institute and Department of Physiology, Development and Neuroscience, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, UK

The Drosophila visual system comprises the optic lobe, which generates the visual processing ganglia of the brain, and the retina. During larval development, neural stem cells in the optic lobe undergo a transition from one division mode to another, as symmetrically diving neuroepithelial cells transform into asymmetrically dividing neuroblasts. In order to investigate the molecular mechanisms underlying this transition, the two distinct neural stem cell populations were genetically labeled and isolated in vivo. cDNA libraries from the two cell types were then compared directly on microarrays to generate high-resolution transcriptional profiles. This approach has led to the identification of numerous potential regulators of the neuroepithelial-to-neuroblast transition, including both genes that are known to play a role in neuroblast formation and novel candidates. We are currently analyzing the expression patterns of these genes and selecting candidates for further functional characterization. Strikingly, a number of genes involved in eye development were identified by the microarray as being significantly enriched in the neuroepithelium. These genes form part of the gene regulatory network required for compound eye development, known as the retinal determination network (RDN). Several core RDN genes are expressed in restricted domains within the larval optic lobe and we are now investigating their function in optic lobe development.

**P-116 The Drosophila formin dDAAM is required for axon growth in the adult brain.**

**Rita Gombos**, Tamás Matusek and József Mihály
Biological Research Center, Hungarian Academy of Sciences,Institute of Genetics, Szeged, Hungary

In the developing nervous system growth cones have an essential role in guiding axons to their correct target sites. Directed growth cone motility in response to extracellular cues is produced by the coordinated regulation of peripheral 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 Drosophila formin dDAAM in the embryonic CNS, where this protein shows a strong accumulation in the developing neurites. Genetic analysis suggested that this protein 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, we carried out a genetic interaction analysis. We demonstrated that dDAAM interacts with Ena and profilin. Moreover, we identified Rac as an 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. Specifically, in the developing mushroom body dDAAM is enriched in the newly born axons suggesting that dDAAM might be a general regulator of Drosophila axonal development. Consistently, by LOF 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.

**P-117 Putative role for the GEF protein Steppke in synaptogenesis.**

**Gündner A.L.**1, Zinke I.2, Fuss B.1, Becker T.1, Hahn I. 1 & Hoch M.1
1 LIMES-Institute, Program Unit Development, Genetics and Molecular Physiology, Laboratory for Molecular Developmental Biology, University of Bonn, Germany - 2 LIMES-Institute, Program Unit Development, Genetics and Molecular Physiology, Laboratory for Brain Physiology and Behaviour, University of Bonn, Germany

The steppke gene encodes a member of the cytohesin family of guanine nucleotide exchange factors (GEFs), which have been characterized as activators for ADP-ribosylation factor (ARF) GTPases. step mutants show a decrease in both cell and body size caused by impaired insulin signalling1. We have shown previously that Step acts upstream of PI(3)K and is required for the proper regulation of Akt and dFOXO. For downstream effectors of the insulin signalling cascade it has been shown that they affect synaptogenesis in Drosophila neurons2. It was demonstrated that overexpression of PI3K and Akt induces the formation of supernumerary, functional synapses in larval motor neurons. We are investigating the role of Step and other components of the insulin signalling pathway in neuron synaptogenesis at neuromuscular junctions by using mutant and overexpression studies.

**P-118 Putative upstream regulator of miR-279 expression and function in the development of the CO2 sensory circuitry in Drosophila.**

**Marion Hartl**[1], Laura Loschek[1], Pelin Cayirliglu[2], S.Larry Zirpursky[2] and Ilona Grunwald Kadow[1]
1 Max-Planck Institute of Neurobiology, Sensory Neurogenetics Group, Martinsried, Germany
2 HHMI/ UCLA, Department of Biological Chemistry, Los Angeles, U.S.A.

Among all the senses, olfaction plays a crucial role for insects in detecting food resources, in finding mating partners, and in avoiding stress factors. While most of the olfactory neurons are used for food detection, one class that expresses Gr21a receptor detects CO2. CO2 is the main component of the so called Drosophila stress odour that is emitted by flies during stress conditions, and leads to an immediate escape response. A robust behaviour among CO2 exposure is also found in Anopheles gambiae, the malaria vector, but in an opposite way: mosquitoes are attracted by CO2 as this molecule is used for host detection. These behavioural differences are also reflected on the anatomical level e.g. location of CO2 receptor expressing neurons. Previously, we have shown that a fly with a mutation in microRNA-279 shows mixed features of Drosophila and mosquito in the organisation of the CO2 circuit: similar to mosquito miR-279 mutant flies exhibit ectopic CO2 neurons on their maxillary palps (MP). We therefore proposed that miR-279 was involved in the divergence of flies and mosquitoes. Here, we present a transcription factor mutant that exhibits the same phenotype as loss of function of miR-279. We find that loss of function of this transcription factor leads to expression loss of miR-279 in MPs by directly binding to the putative regulatory sequences of miR-279. Interestingly, this transcription factor was proposed to be a positive regulator of one of the prime targets of miR-279 in this process. These data, together with our expression analysis in developing olfactory organs suggest that miR-279 is required for fine tuning the effect of transcription during developmental differentiation and evolutionary divergence

**P-119 The Drosophila LIM-only, dLMO transcription factor that controls sensory organ and wing develoment is regulated by mir-9a.**

Inna Biryukova, Joelle Asmar, Claudine Ackermann, Nadine Arbogast, **Pascal Heitzler**
IGBMC, Strasbourg, France

The Drosophila LIM only transcriptional factor dLMO, controls cell proliferation and apopotosis, and its human counterpart, LMO2, acts as a T-ALL oncogene. The dLMO gene encodes two isoforms, dLMO-RA and dLMO-RB but their respective biological contribution has never been investigated. We generated specific deletions and demonstrated that dLMO- and dLMO-RA- mutant flies have similar phenotypes, lacking some thoracic and wing margin sensory organs, while dLMO-RB- look normal, suggesting that dLMO-RA is the major isoform. dLMO-RA is expressed in proneural clusters at early stages of the developing peripheral nervous system, but is later excluded from the sensory organ precursors. We demonstrated that dLMO-PA functions as a coactivator of Pannier, the GATA transcription factor, to promote expression of the proneural Achaete expression. Interestingly, dLMO-RA but not dLMO-RB is regulated through common 3' untranslated region (3' UTR). We found that a small non-coding endogenous RNA, mir-9a, a microRNA from the mir-9 family, regulated steady level of dLMO during development. Deletions of the 3' UTR, including the mir-9a site, generate gain-of-function dLMO mutants (Beadex) associated with high levels of dLMO-RA mRNA and protein. Beadex mutants lack wing margins, a phenotype also observed in null mir-9a mutants. We found that mir-9a and dLMO interact genetically and are co-expressed in wing discs. Lack of mir-9a results in overexpression of dLMO, while gain-of-function mir-9a results in overexpression. Since dLMO is a repressor of Apterous, these data indicate that an important function of mir-9a is to ensure the appropriate stoichiometry of dLMO during Drosophila wing development.

**P-120 Analysis of kruppel repressor and activator protein domains in the context of temporal specification of Drosophila neuroblasts.**

**Jansen, S**; Urban, J
University of Mainz, Mainz, Germany

Neuroblasts (NBs) within the Drosophila ventral nerve cord produce specific cell lineages in which unique cell types are generated in a fixed temporal order. This birth-order dependent specification of NB progeny is controlled by a set of genes encoding transcription factors, which are sequentially and transiently expressed within the NBs, and whose expression is inherited by the ganglion mother cell (GMC) born at that time. One of these factors is the zinc finger protein Krüppel (Kr). Continuous expression of Kr beyond its normal time window arrests the NB in it's temporal development leading to the production of supernumerary cells with "middle aged??? cell fates (Isshiki et al., 2001). It was shown in vitro, that Kr acts as a transcriptional repressor after dimerization, while Kr monomers act as an activator and that dimerization is dosage dependent (Sauer, 1991 & 1995). Further, the co-repressor dCtBP can bind on one of the two Kr repressor domains and is required for certain aspects of their repressional activity (Nibu, 1998). To get an insight whether Kr acts as a transcriptional activator or repressor in the context of temporal NB specification, we are investigating which of the evolutionary conserved domains of this protein are functionally relevant by overexpressing mutated versions of Kr during NB 7-1 lineage development. Secondary, we are investigating whether Kr might form dimers or remains monomeric while specifying temporal state of the NB. The results of these experiments will be presented. This work is supported by the Deutsche Forschungsgemeinschaft (Ur 42/6-1). Isshiki, T. et al (2001), Cell 106, 511-21. Nibu, Y. et al (1998), EMBO 17 (23), 7009-20. Sauer, F. et al (1991), Nature 353, 563 -66. Sauer, F. et al (1995), Nature 375, 162-64.

**P-121 Yorkie and Scalloped regulate mutually exclusive rhodopsin expression in R8 photoreceptor neurons.**

**David Jukam**, Daniela Pistillo, Claude Desplan
Center for Developmental Genetics, Department of Biology, New York University, New York, NY United States

In many sensory systems, a neuron's functional identity depends upon the transcriptional choice among sensory receptors such that only one receptor is expressed per neuron. In the Drosophila eye, the two innermost photoreceptors, R7 and R8, respond to UV and colored wavelengths by expressing one of four Rhodopsins (Rh3, Rh4, Rh5, Rh6). Recently, we showed that a tumor suppressor, warts, and a growth regulator, melted, control the post-mitotic specification of R8 photoreceptor neuron subtypes. warts and melted repress each other's transcription to form a bistable feedback loop that directs expression of either Rhodopsin5 (Rh5) or Rhodopsin6 (Rh6). In growth control, Warts acts in a signaling pathway to negatively regulate the co-activator Yorkie (Yki), which would otherwise enter the nucleus, bind to the transcription factor Scalloped (Sd), and activate target genes. Yet whether Warts regulates rhodopsin transcription with its canonical signaling partners or an alternative downstream mechanism is unknown. Here we show that yki and sd are required to activate Rh5 and repress Rh6 downstream of the warts/melted feedback loop. yki and sd thus act in R8 as the transcriptional output of Warts signaling, similar to their role in growth. Transcriptional reporters for warts and melted reveal an additional function for yki in R8--it is required for melted expression and negatively regulates warts expression. Thus, yki is part of the warts/melted feedback loop and consequently can promote its own activity by repressing warts expression. This study reveals how the Warts signaling pathway is re-used in a post-mitotic context with new regulatory mechanisms appropriate for an all-or-nothing neuronal fate decision.

**P-122 Ioning and characterization of Drosophila Glial cell line-derived neurotrophic factor family receptor-like.**

**Kallijärvi, J**; Stratoulias, V; Heino, TI; Saarma, M
Institute of Biotechnology, P.O. Box 56, Viikki Biocenter, University of Helsinki, FIN-00014, Helsinki, Finland

GDNF family ligands (GFLs) are secreted growth factors distantly related to the TGF-beta superfamily. GFLs are crucial for the development and maintenance of distinct neuronal populations, as well as for kidney organogenesis and for spermatogenesis. Orthologs of GFLs, the RET receptor and the GFRalpha coreceptors exist in all vertebrates. In invertebrates, including C. elegans and D. melanogaster, homologs of RET and GFRalpha genes are found, but no obvious homolog of GDNF. In Drosophila, a partial cDNA sequence of a predicted GFRalpha homolog named munin has been identified. Here, we set out to uncover the full gene and transcript structure of the munin/dGfr-l (Drosophila GFR-like), to clone the cDNA, and to investigate spatial and temporal expression of dGfr-l during embryonic development. We found that the dGfr-l gene extends upstream and downstream of the original predicted locus and encompasses > 70 kb of genomic DNA. The experimentally assembled dGfr-l cDNA predicts a 1042-amino acid protein with a signal sequence, four GFR-like domains and a putative GPI anchoring sequence. In situ hybridization analysis revealed that dGfr-l is expressed in neurons of the ventral nerve cord and of the head sensory complex throughout late embryogenesis. Moreover, we found that dGfr-l and dRet are expressed in a similar pattern in the ventral nerve cord, but likely not in the same cells. When expressed in S2 cells, the dGfr-l protein is glycosylated and membrane associated. Deletion of the putative GPI anchoring sequence leads to secretion of the protein into the cell culture medium. Whether dGfr-l is a functional homolog of the mammalian GFRalpha receptors is a subject of our further studies.

**P-123 A genetic screen for axonal outgrowth and regeneration in the Drosophila Central Nervous System.**

**Koch, M** 1,2, Schlieder, M 1,2, Morgan, M 1,2, Yan, J 1,2, and Hassan, B 1,2,
1 Laboratory of Neurogenetics, Department of Molecular and Developmental Genetics-VIB11, VIB, Leuven, Belgium. 2 Department of Human Genetics, KU Leuven School of Medicine, Leuven, Belgium.

Lesioned axons of Central Nervous System (CNS) do not regenerate. For this reason, traumatic brain injury often results in devastating and irreversible deficits. Little information is available concerning the molecular mechanisms that control the success or failure of an injured axon to regrow and functionally regenerate. We are interested in the genetic mechanisms that regulate CNS axon growth and regeneration. We have shown that JNK activity is necessary and sufficient for axonal growth during Drosophila CNS development (Srahna et al, 2006). More recently, we have also demonstrated that JNK strongly induces the growth of novel axons after injury. This suggests that at least some signals required for developmental axonal outgrowth may regulate regeneration. We therefore undertook a systematic search for genes involved in axonal outgrowth and regeneration. A gain of function screen of over 400 genes uncovered approximately 20 genes that induced axonal outgrowth. A few of the genes identified in this screen have already been associated with neurite outgrowth, such as members of the Rho family of GTPases, as well as JNK interactors, validating the specificity and reliability of the screen. However, many of the genes we identified have not yet been associated with neurite outgrowth, nevermind regeneration. We tested these axonal-related genes for their capability of inducing neuronal regeneration following axotomy in the fly brain. Preliminary results show that overexpression of some of the genes that positively regulated physiological outgrowth is also able induce growth following injury to the CNS. Our data should lead to new insights into the molecular mechanisms of growth and regeneration after CNS injury.

**P-124 Origin of the mushroom body neuroblasts and composition of their embryonic lineages in Drosophila.**

**Kunz T**, Technau G M, and Urbach R
University of Mainz, Institute of Genetics, Mainz, Germany

The mushroom body (MB) of the arthropod brain is a prominent neuropile structure which is involved in olfactory learning and memory. In Drosophila, the MB is formed by the Kenyon cells which develop from four NBs (MBNBs) per hemisphere. The Kenyon cell axons fasciculate to constitute the pedunculus and lobes of the MB. Although much is known about the postembryonic development of the MB, the embryonic origin and development of the MBNBs, as well as their embryonic lineages, have remained elusive. Using ubi-GFPnls flies we undertook a 4D microscopic analysis of the procephalic neuroectoderm of the gastrulating embryo in order to establish early landmarks for in vivo labelling of putative neuroectodermal MBNB progenitor cells and tracing of their lineages with the lipophilic marker DiI. We show that all four MBNBs delaminate from the neuroectoderm of mitotic domain B, and belong to the earliest born brain NBs. Each MBNB develops from a separate neuroectodermal progenitor cell, excluding that they arise by division from a common precursor cell. Moreover, we present for the first time the complete embryonic lineage of each individual MBNB. Remarkably, each lineage does not only contain the MB intrinsic Kenyon cells, but in addition neurons which do not contribute to the MB. These non-intrinsic MBNB neurons reveal, in a lineage-specific manner, descending axonal projections to the VNC and/or axonal projections into the contralateral brain hemisphere.

**P-125 Rescue and gene silencing experiments to dissect the DmMANF mutant lethality.**

**Lindström, R** (1); Palgi, M (1,2); Saarma, M (2); Heino, TI (1)
(1) Department of Biological and Environmental Sciences, University of Helsinki (2) Institute of Biotechnology, University of Helsinki

A novel mammalian neurotrophic factor, MANF (Mesencephalic Astrocyte-derived Neurotrophic Factor) (Petrova et al. 2003, J Mol Neurosci 20:173–188), selectively promotes the survival of DA neurons in vitro. A paralogous gene for MANF was identified in vertebrate genomes, CDNF (Conserved Dopaminergic Neurotrophic Factor), and shown to promote and repair nigrostriatal dopaminergic system in a rat model of Parkinson's disease. Thus, CDNF represents a potential drug for treatment of PD (Lindholm et al. 2007, Nature 448:73–77). These proteins form the first family of neurotrophic factors with well conserved protein sequences through evolution among the multicellular organisms from C. elegans to human. We have identified the fly homologue to mammalian MANF and CDNF, DmMANF, and generated genetically null mutant alleles of the DmMANF gene. DmMANF mutants die as early second instar larvae. This lethality was rescued by ubiquitous expression of DmMANF transgenes (Palgi et al. 2009, PNAS 106:2429–2434). Our rescue experiments also showed that both human MANF and CDNF are able to substitute the lack of endogenous DmMANF protein. We designed several rescue and RNAi experiments based on the expression pattern of DmMANF to find out the critical tissues for survival. Here we report how different ectopic expression of DmMANF rescues the lack of endogenous DmMANF and how gene silencing using RNAi fly line (Dieztl et al. 2007, Nature 448:151–156) in broad range of expression patterns alters the fly development.

**P-126 Cellular and molecular mechanism of dendrite pruning in Drosophila.**

**Nicolas Loncle** and Darren W. Williams
MRC Centre for Developmental Neurobiology; King's College London; New Hunt's House, 4th Floor; Guy's Hospital Campus; London SE1 1UL; United Kingdom

Pruning, the selective elimination of synapses, axons or dendrites, is essential for the matching of network components during nervous system development. We are using Drosophila dendritic arborizing (da) sensory neurons as a model to study the phenomenon of large-scale pruning. Da neurons form large, complex dendritic arbors under the larval epidermis, which are then completely removed during early metamorphosis. We, and others, have shown that the apoptotic machinery is required for the efficient removal of da neuron dendrites during pruning. Using a genetically encoded caspase reporter CD8::PARP::Venus, we have shown that caspases are activated in the dendritic compartment of pruning neurons and not in the soma or axon. This probe suggests that caspases are activated in severed branches to mark them for engulfment by phagocytes. We would like to know which upstream components of the pathway are required during pruning and how the cell is able to survive after the activation of the apototic machinery.

**P-127 Origin and specification of a motor control centre in Drosophila.**

**Zoe Ludlow**, Katherine E. White, and Frank Hirth
MRC Centre for Neurodegeneration Research, King's College London

The Drosophila central complex has been described as a centre for the higher control of locomotion. It comprises four neuropils: the ellipsoid body (eb), fan-shaped body, paired noduli and protocerebral bridge. The eb is involved in flight control, spatial memory and walking behaviour. We have used a tool to locate and trace eb neurons throughout development into adulthood. Using this genetic tracing system, we identified neural lineages in each hemisphere of the adult brain which project via the lateral triangles to the eb; these cells include ring neurons R1-R4. Genetic tracing marks two clusters of cells that are already present in the embryonic brain, where they coincide with neuromere boundaries. We have been able to identify the stem cells from which these two neuronal clusters derive. To gain insights into development and specification of eb neurons, we have identified Fgf8 signalling and other factors that are involved in the formation and maintenance of these neural lineages. RNAi knockdown experiments as well as cell inactivation experiments suggest that these lineages modulate adult walking behaviour.

**P-128 Wnk – a novel conserved kinase involved in axon guidance in Drosophila.**

**Mann K.**; Suzuki T.
Axonal Guidance and Neuronal Connectivity, Max Planck Institute of Neurobiology

The interpretation of the visual stimulus requires the correct axon pathfinding of the photoreceptor cells. We identified Wnk - a novel, conserved among plants and animals Ser/Thr kinase involved in guidance of Drosophila photoreceptor axons. Wnk's are involved in the ion transport and regulation of Synaptotagmin (Syt) activity. Our phenotypic analysis of wnk revealed its requirement for the lamina cartridge and medulla formation. In vitro and in vivo overexpressed Wnk is present in the cell body and in the axon as well. We identified dSERCA as a binding partner for Wnk. dSERCA is a Ca2+-ATPase that transports Ca2+ from the cytoplasm into ER and is expressed at a high level in neurons. dSERCA shows a similar to Wnk axon guidance phenotype in the lamina revealing the importance of Ca2+ homeostasis for axonal pathfinding. The interaction of human Wnk1 with Syt2 suggest that Drosophila Wnk can also play a role in modulation of Syt activity. This is supported by our finding that in both wnk and syt mutant there is a decreased number of synaptic vesicles in photoreceptors which suggests that the synaptic vesicle recycling is altered. The similarity of wnk and syt phenotypes supports our model that Wnk modulates Ca2+ sensing by Syt. The involvement of Wnk in axon guidance and Syt activity gives the opportunity to link these two processes.

**P-129 Role of Poxn in Drosophila ellipsoid body development.**

**Shilpi Minocha**1,2, Werner Boll1, and Markus Noll1
1Institute for Molecular Biology, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland; 2Ph.D. Program in Molecular Life Sciences, Zürich, Switzerland

The Drosophila brain is characterized by an enormous diversity and specificity of neurons and offers an ideal model to study neuronal connections. During development, Drosophila passes through two successive forms of life, larva and adult, which are linked by metamorphosis. Many larval neurons are conserved through metamorphosis but exhibit pronounced remodeling of central and peripheral processes. Pox neuro (Poxn), a member of the Drosophila Pax gene family, encodes a transcription factor with a DNA-binding paired domain. It is expressed in discrete domains throughout brain development. A large fraction of the cells acquire a Poxn fate during larval development but are already present in the embryonic brain since there is little mitotic activity in the Poxn expression domain during larval life. All cells of these Poxn expression domains represent post-mitotic neurons that are not fully differentiated. There is only a slight increase in the number of Poxnexpressing cells during metamorphosis.
Lineage analysis suggests that the Poxn-expressing immature neurons observed in the brain of the late third-instar larva survive metamorphosis and seem to form a scaffold of pioneering tracts during larval stages along which the pupal and adult Poxn-expressing cells extend their respective neurites. Thus, the projection patterns of the larval Poxn-expressing neurons appear to represent a pattern analogous to that of the adult Poxn-expressing neurons, as midline commissures, antennal lobes, and lateral regions are already targeted. Our results indicate that the majority of the dorsal Poxn-expressing cells represent large-field neurons whose neurites contribute mainly to the formation of the ellipsoid body (eb). In the late third instar larva, these processes start to form the eb which can be recognized by 45 hrs APF as the typical donut-shaped structure that is prominent in the central complex of the adult fly brain. In Poxn mutant larvae and pupae, the neuronal projection pattern is highly aberrant, and neurites seem to stall before turning towards the midline and lateral regions of the brain. Accordingly, in the adult mutant brain the projections of these neurons are highly disturbed and the ellipsoid body is transformed into a degenerate structure.

**P-130 Fascin is necessary for the promotion of actin-rich spikes and the suppression of bona-fide banching in Drosophila sensory dendrites.**

**Negele, J**[1]; Delandre, C[2]; Moore, AW[2] and Tavosanis, G[1]
[1]Dendrite Differentiation Group, MPI of Neurobiology, Am Klopferspitz 18, Martinsried, Germany [2]Disease Mechanism Research Core, RIKEN Brain Science Institute, 2-1 Hirosawa, Wako-shi, Saitama, Japa

What mechanisms mediate the difference between filopodia formation and maturation into a short spine-like structure versus maturation into to a bona-fide dendritic branch? We have addressed this question in Drosophila dendritic arborization (da) sensory neurons where class III da neurons have short straight actin-rich spikes along their main dendrite shafts while the dendrites of highcomplexity class IV neurons terminate with high order branches of various lengths. We first examined a conserved molecule required for filopodium formation, the actin bundling protein fascin. singed is the sole fascin gene in Drosophila and loss of singed/fascin strongly reduced the number of class III spikes, but did not affect the number of high order branches of class IV neurons. In addition, the overall complexity of class III neurons was increased in singed/fascin mutants. Class III neurons appeared to undergo a partial transformation towards class IV neurons; hence we investigated a connection between Singed/Fascin and spike promotion by the transcription factor Cut. Ectopic expression of Cut in the simple class I neurons that do not have spikes induces an increase in dendrite complexity and the formation of spikes. Singed and the small GTPase Cdc42 are essential effectors of this program since loss of singed/fascin or expression of a dominantnegative Cdc42 construct in Cut-overexpressing class I neurons eliminated the spikes. Furthermore, fluorescently-tagged Singed/Fascin was recruited at sites of Cdc42 mediated filopodia formation. We hypothesize that class III spikes are stabilized by the invasion of Singed/Fascin, and in the absence of this molecule primary filopodia can instead mature into bone fide dendritic branches.

**P-131 Signal strength and signal duration define two distinct aspects of JNK-dependent axon stabilization.**

Andrew Rallis, Coralie Moore, **Julian Ng**
MRC Centre for Developmental Neurobiology, King's College London, Guy's Campus, London SE1 1UL

The mechanisms that explain how signaling proteins control multiple aspects of neuronal morphogenesis are unclear. Here we show activity levels and timing mechanisms determine distinct aspects of Jun N-terminal kinase (JNK) pathway dependent axonal morphogenesis in Drosophila mushroom body (MB) neurons. In the complete absence of Drosophila JNK (Basket), MB axons fail to stabilize, leading to their subsequent degeneration. However, with a partial loss of Basket (Bsk), or of one of the upstream JNK kinases, Hemipterous or Mkk4, these axons overextend. This suggests that Bsk activity prevents axons from degenerating and from overextending beyond their terminal targets. These distinct phenotypes require different threshold activities involving the convergent action of two distinct JNK kinases. We show that sustained Bsk signals are essential throughout development and act additively but are dispensable at adulthood. We also suggest that graded Bsk inputs are translated into AP-1 transcriptional outputs consisting of Fos and Jun proteins.

**P-132 Drosophila optic medulla progenitors turn their epithelial structure into neuroblastic one along with the cell cycle progression and Notch down-regulation.**

**Orihara-Ono, M.**, Nakao, K., Okano, H.
Dept. Physiol., Sch. Med., Keio Univ. SORST, JST Dept. Physiol., Saitama Medical Univ.

Diverse types of neural cells with various maturities are shown one after another in the course of development depending on many extrinsic signals and intrinsic events. The neuroepithelia first come into being and then some types of asymmetrically dividing progenitors follow. The optic medulla neuron primordia of Drosophila are observed as neuroepithelia in their early stages. The medulla precursors come to expand during the first half of development. Subsequently more mature neuroblasts are increasing to bring about progenies. However, few studies had demonstrated the details of neither the observed morphological processes nor the required cellular events. Here we show that neuroepithelial cells near the border of the optic primordia converted individually into neuroblastic cells during G1. As the cell cycle progresses from G1 to the S phase, the transition must be completed judging by the expression of progenitor gene Ase. During this transitional G1 period, a cell that used to be a neuroepithelium became committed to differentiate. Then it altered its cell phase control and changed its morphology from a columnar to a drop-like shape by reorganizing the adherens junctions that had linked it to the flanking neuroepithelial cells. Finally, it got becoming a neuroblast to commence asymmetric division. Moreover, the clonal reduction of Notch caused the precocious Delta accumulation that forced the neural progenitor maturation. The depletion of Notch did not induce the expression of progenitor gene Ase directly suggesting some other factors are required in addition to Notch to complete the transition. This study unveils the multi-step transition that would push progenitors status to progress sequentially.

**P-133 Characterization of genes involved in photoreceptor differentiation.**

**Öztürk A**; Sayin S; Çelik A
Bogazici University Department of Molecular Biology and Genetics 34342 Bebek, Istanbul Turkey

The one receptor – one neuron rule is a common phenomenon observed in sensory organs. In the Drosophila eye, each PR expresses one of six different rhodopsin genes. The expression patterns of inner photoreceptors divide ommatidia into mainly two distinct subtypes: pale and yellow. In the pale subtype R7 and R8 cells express rh3 and rh5 and the yellow subtype R7 and R8 cells express rh4 and rh6, respectively. Photoreceptor specification is a step-wise process that requires activation of specific genes. Although certain transcription factors like spalt, prospero, senseless, ortodenticle and spineless have been identified so far, the pathways leading to mutual exclusive and coordinated expression of rhodopsins are not fully understood. In order to identify novel genes that play a role in PR specification, a piggyBac element based enhancer trap screen was performed. Eleven genes have been selected for further analysis according to their molecular functions and expression patterns. We will present data on the analysis of these genes.

**P-134 Ectopic Repo suppresses expression of castor gene in Drosophila central nervous system.**

**Ki-Seok Park**, a; Su-In Moon,a; Sang Hee Kim, b; Sang-Hak Jeon, a,*
a Department of Biology Education, Seoul National University, Seoul 151-742, Republic of Korea b Department of Chemistry, Kunkuk University, Seoul 143-701, Republic of Korea

The ventral nerve cord (VNC) of the Drosophila embryo is derived from neuroblasts (NBs). NBs divide in a stem cell lineage to generate the series of ganglion mother cells (GMCs), each of which divides once to produce a pair of neurons or glial cells. One of the NB genes, castor (cas), is expressed in a subset of NBs and has never been identified in neurons and the peripheral nervous system; cas plays a role in axonogenesis. But its limited expression along the dorsal–.ventral axis within the central nervous system has not been investigated yet. In the present study, we examined the expression patterns of both genes using confocal microscopy to determine the effects of repo mutation on cas expression. Cas was mainly expressed in layers different from repo-expressed layers during early embryogenesis: repo was expressed mostly from deep to mid layers, while cas, from mid to superficial layers. Loss-of-function of repo did not result in an ectopic expression of cas, but rather, a scattering of cas-expressing cells. However, repo gain-of-function mutation caused repression of cas. In addition, repo-expressing cells seemed to block the migration of casexpressing cells.

**P-135 Identification and genetic-molecular anális of new genes required for the control of cell proliferation and neural differentiation.**

**Beatriz, Pérez San Juan**; Antonio, Baonza Cuenca
Centro de Biología Molecular “Severo Ochoa” and University Autónoma de Madrid, Madrid 28049, Spain

The definition of size and pattern of an organ largely depends on the control of cell proliferation ad differentiation. In order to better understand how these processes are regulated, it is critical to identify as many genes as posible involved in their regulation. Most of the screenings carried out for searching mutation that affect cell proliferation have been focused in the identification of loss of function alleles. One of the problems of this approach is that the lack of function of genes required for proliferation, usually induce cell death. In addition, it has been previously reported that, at least in Drosophila, the functions of some of the genes required for the control of cell proliferation are redundant. Both problems, the redundancy and the cell lethality effect, can be avoid in an overexpression screening. Our screening allow us to identify genes by their over-expression phenotype. We have used a Gal4 line (GMR-Gal4) to drive the expression in the eye of a collection of about 500 P-UAS elements (EP) inserted randomly throughout the genome. Our screening is aimed to identify genes that affect the patteern of cell proliferation and neuronal differentiation in the second mitotic wave (SMW) during eye development. We have identified several genes that specifically affect one of these processes or both at the same time.

**P-136 Genetic interactions between roughest and hibris during Drosophila embryonic CNS development.**

1 Moda,LMR 2 Costa, MSA; **Ramos, RGP**;
1 Departments of Genetics,FFCRP, University of São Paulo 2 Department of Cell & Molecular Biology and Patogenic Bioagents, Faculty of Medicine of Ribeirão Preto, University of São Paulo – Ribei

The rst and hbs loci of D. melanogaster both encode Ig-like superfamily members whose direct interaction is critical for the final steps of ommatidial morphogenesis and are also required, either independently or together, in a variety of embryonic and post embryonic developmental processes such as axonal pathfinding in the optic lobe, myoblast fusion, sensory organ patterning and salivary gland autophagy. Previous findings of our group demonstrated that overexpression of the Rst ectodomain (RstECD) under a heat shock inducible promoter between embryonic stages 5 and 8 cause a high percentage of embryos to display a specific neural phenotype characterized by longitudinal separation, in several points, of the two symmetrical halves of the ventral cord around the midline and by commissural abnormalities. Here we show that lowering hbs dosage in these individuals allows this phenotype to be observed even in non heatshock conditions while additional defects, such as the expansion of the CNS area in some embryos, start to be observed. Immunocytochemical analysis showed that Rst and Hbs proteins are present in partially complementary subsets of cells in the CNS midline. Taken together these data suggest that, similarly to what occurs in the developing retina, heterophilic binding between these two proteins might be required for at least some of the cell adhesion events essential to ventral cord formation.

**P-137 A Catalogue of Interneuronal Cell Types in the Late Embryonic Nervous System of Drosophila.**

**Rickert, C.**(1), Kunz, T.(1), Harris, K.-L.(2), Whitington, P.(2), and Technau, G. M.(1)
(1) Institut für Genetik, Universität Mainz, Becherweg 32, 55099 Mainz, Germany - (2) Dept. of Anatomy and Cell Biology, University of Melbourne, Victoria 3010, Australia

In the Drosophila embryonic ventral nervous system each hemineuromere derives from a set of 30 neuroblasts and comprises about 350 neurons and 30 glial cells. While the entire lineage of each neuroblast is known and all glial cells and all motoneurons have been identified individually, the vast amount of the more than 300 interneurons are not described on a single cell level. The work presented here attempts to describe the interneuronal morphology in the late Drosophila embryonic nerve chord in a quantitative way. About 1000 random single cells have been labelled and grouped on the basis of morphological similarity. This led to a set of 268 groups that we believe to represent a good first approximation to a full hemisegmental set of abdominal interneurons. This set allows to look for principles of neuromere organization e.g. correlations between cell body position and axonal projection or between different aspects of axonal morphology. In addition we suggest the one (or few) most probable parental NB(s) for all cell types and provide an easy to use interactive database that will help to identify cells within expression patterns or search for potential interaction partners of identified neurons.

**P-138 Investigating the role of Abnormal Spindle Protein Asp in brain development.**

**Rujano. MA**; Le-Dez, G; Basto, R.
Institut Curie, UMR 144, Paris, France

Drosophila melanogaster Abnormal Spindle Protein (Asp) was first identified by phenotypic characterization of a late larval-pupae lethal mutation that displays defects in spindle morphology, mitosis and meiosis. Interestingly, the human homologue of Asp, ASPM, is one of the genes implicated in primary microcephaly, a congenital disorder characterized by reduced brain size, with no other abnormalities either within or outside of the nervous system. In addition, the mouse homologue is known to be necessary for the symmetric divisions of neuroepithelial cells during brain development. Thus, Asp function likely resides in the regulation of proliferation of neural progenitors during neurogenesis. We utilized the larval Drosophila brain as a model system to ascertain the role of Asp during neurogenesis. Analysis of the dynamics of the protein in vivo showed that Asp has a cell cycle-dependent localization. However, during mitosis, it specifically localizes to spindle microtubules and spindle poles. In the larval mutant brain, the dividing neuroblasts of the central nervous system fail to complete asymmetric cell division and are arrested in prometaphase-like state with highly condensed chromosomes, mitotic spindles are unfocused and disorganized and cells have aberrant centrosome numbers. Intriguingly, while all other structures are properly developed, asp mutant larvae display reduced brain size, with extensive lost of cells in the optic lobe and in the eye imaginal disc. Likewise, despite reduced head size and severely impaired eye morphology, asp pharate adults appear morphologically normal. Overall, our results suggest that Asp is a neurogenic gene with a major role in the regulation of neural stem cell proliferation and differentiation during brain development.

**P-139 sadari, formally called CG11902, is involved in glial differentiation and axogenesis during Drosophila Embryogenesis.**

Ruy, Kum-Bok
1Department of Biology Education, Seoul National University, Seoul 151-748, Korea 2Department of Biochemistry and Molecular Biology, Yonsei University School of Medicine, Seoul

The nervous system consists of two principal types of cells, neurons and glia. Glial cells play important roles in axonal development as well as nourishment, homeostasis, and insulation of neurons. Although more data on the significance of the glia is constantly being generated, only a relatively small number of genes have been characterized. In the present study, we examined the expression and roles of sadari, which was formally called CG11902. sadari transcripts begin to appear from stage 11 and decrease from stage 15. The anti-Sadari antibody directed against amino acids 71 to 210 detected the same expression pattern of sadari that was shown by the antisense RNA probes. Sadari is expressed in the nucleus. The double staining of embryos with anti-Repo antibody and sadari antisense RNA probes showed that sadari was expressed in a subset of cells in which repo was also expressed. Minos insertion into the sadari exon caused embryonic sub-lethality, partial loss and abnormal localization of glial cells, as well as abnormal longitudinal axon tracks and commissures. These results suggest that sadari is involved in glial and axonal development.

**P-140 The role of the Angelman Syndrome E3-ubiquitin ligase Ube3A in regulation of synaptic platicity.**

**Siller, K.H.**; Piper, J.; Zhang, Z.; Chen, L.
Department of Molecular and Cell Biology and Helen Wills Neuroscience Institute, University of California, Berkeley, CA 94720-3200

Proper function of neuronal circuits requires dynamic adjustment of the strength of synaptic connections. These adjustments, collectively referred to as synaptic plasticity, serve two purposes: a) maintenance of synaptic input and output in a physiologically meaningful range and b) information processing that underlies memory and learning. In humans, elimination of the E3ubiquitin ligase Ube3A in hippocampus and cerebellum results in seizures as well as severe cognitive and locomotory deficits, categorized as Angelman Syndrome (AS). It is thought that these deficits arise from impaired synaptic plasticity, yet the precise molecular mechanism is unknown. The Drosophila genome encodes a single Ube3A homolog and we use the Drosophila neuromuscular junction (NMJ) as a model glutamatergic synapse to investigate Ube3A function. Ube3A mutants or animals overexpressing Ube3A show altered accumulation of distinct sets of vesicle markers. In addition, presynaptic overactivity of Ube3A induces sprouting of numerous ectopic but smaller synaptic boutons leading to increased synaptic area. Together these data suggest that Ube3A function may be tightly regulated at the NMJ. Currently, we are analyzing the electrophysiological properties of NMJ synapses in Ube3A mutants and Ube3A-overexpressing animals, and screening for genes that functionally interact with Ube3A. These experiments are expected to reveal more details of Ube3A function, and to identify potential upstream regulators and downstream effectors of Ube3A at the synapse.

**P-141 Enhanced microtubule bundling through a c-terminal region of the Drosophila spectraplakin short stop is essential in vivo.**

**J. Alves-Silva**, N. Sanchez-Soriano, I. Hahn, W. Bottenberg, K. Vrenken, H. Bellen, R. Kammerer, C. Ballestrem, A. Prokop
Faculty of Life Sciences, The University of Manchester, UK

F-actin and microtubule (MT) networks are fundamental components of all cells. Their dynamics are interdependently regulated, but the molecular mechanisms mediating this linkage are little understood. A candidate molecule with high potential to shed light into these fundamental processes is the actin-MT linking factor Short stop (Shot), which is crucially required in a number of developmental contexts and represents a genetically amenable paradigm for its clinically relevant mammalian homologues ACF7 (wound healing) and BPAG1 (skin blistering, nerve degeneration). One demonstrated role of spectraplakins is to organise MT networks in an F-actin dependent manner. However, so far the molecular basis of their MT interaction is little understood. We report here that the MT-binding Gas2 domain of Shot is insufficient to mediate appropriate MT interaction. Additional activity of the C-tail is required - a region of low complexity at the very C-terminus of Shot. Using shot mutant alleles and rescue experiments with deletion constructs we demonstrate that the C-tail is absolutely required for tendon cell integrity and partially required for the organisation of neuronal MT networks. Structure function analyses in fibroblasts reveal that both Gas2 and C-tail display low affinity for MTs, but in combination they show dramatically increased affinity and MT bundling capacity. These data are supported by localisation data of ?Gas2 and ?Ctail constructs of Shot in Drosophila neurons. We furthermore demonstrate EB1-binding capability of the C-tail and have identified motifs in the C-tail mediating MT plus end association. The in vivo relevance for this latter interaction is currently being investigated and results will be presented.
Support: BBSRC, Wellcome Trust, EU

**P-142 Transcriptional networks determining Drosophila neural stem cell fate.**

**Tony D. Southall** and Andrea H. Brand
The Wellcome CRUK Gurdon Institute and Department of Physiology, Development and Neuroscience, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, United Kingdom

Drosophila neural stem cells (neuroblasts) divide in an asymmetric self-renewing manner. At each division they produce another neuroblast and a ganglion mother cell (GMC), which divides only once more to give rise to 2 neurons and/or glial cells. Prospero is an atypical homeodomain transcription factor that is asymmetrically segregated from the neuroblast to the GMC. Previously we have shown that Prospero acts as a binary switch between self-renewal and differentiation. However, little is known about the transcriptional networks that Prospero contributes to, in controlling neural stem cell maintenance and neuronal differentiation. Amongst its targets are three neural stem cell transcription factors, Asense, Deadpan and Snail, of which Asense and Deadpan are repressed by Prospero. Using DamID, we have identified the binding sites of these 3 factors and compared them to that of Prospero. This revealed many genes multiply bound by these four transcription factors, suggesting that there is a core set of genes crucial for the switch between self-renewal and differentiation. Furthermore, we show that multiply bound loci are enriched for genes previously linked to nervous system phenotypes, thereby providing a short-cut to identifying genes important for nervous system development. Prospero has the ability to both repress and activate gene expression. Therefore it likely works with co-factors to perform its function. We have used bioinformatics tools to identify putative Prospero co-factor binding sites from our DamID data. Using a yeast one-hybrid system we have identified a transcription factor which binds to one of these sites. Preliminary analysis shows that this factor is expressed in GMCs and their progeny. Functional characterisation of this factor is underway.

**P-143 Direct cell-cell communication in the central nervous system of Drosophila**

**Spéder, P.** ; Brand AH
The Wellcome Trust/Cancer Research UK Gurdon Institute University of Cambridge Cambridge CB2 1QN United Kingdom

A mature functional nervous system is the result of dynamic interactions within the complex network of neural cells. The immense variety of cell types is generated through the regulated division of self-renewing neural precursors and the appropriate differentiation of the progeny. Neural stem cell homeostasis has thus to be a highly regulated process throughout development. Interestingly, several studies in mammalian cultured cells and vertebrate gene knock-outs have pinpointed the role of connexins, the building unit of gap junctions, in maintaining stem cell pluripotency. Nevertheless, the identity of the cells connected through these junctions, as well as the mechanisms underlying connexin function in stem cells, remain merely unknown. Here we propose to use Drosophila neuroblasts as a model to decipher connexin function in neural stem cells. We looked at the function of the Drosophila innexin family, homologue of the vertebrate connexins, in the larval optic lobe, which will give rise to the visual processing center. A RNAi screen targeting the eight members of the innexin family in three different cell types, the neuroblast, the neuroepithelium and the glia has identified two candidates : Inx1, on which we focus here, and Inx3. In inx1 mutants the optic lobe is totally lost. Preliminary experiments suggest that, surprisingly, inx1 function seems required in a restricted subpopulation of glial cells to promote the appropriate regulation of neuroblasts leading to the formation of the visual system. Ongoing experiments involving temporal mapping of inx1 function, subcellular compartimentalisation of the protein as well as identification of potential interactors will shed light on how a gap junction protein is able to regulate neural stem cell homeostasis.

**P-144 New player in the axon targeting of distinct classes of Drosophila olfactory receptor neurons.**

**Stephan D.;** Kadow I.
Sensory Neurogenetics Research Group, MPI of Neurobiology, Am Klopferspitz 18, Martinsried, Germany

Olfactory receptor neurons (ORNs) expressing the same olfactory receptor (OR) target a specific glomerulus in the antennal lobe (AL) in a stereotyped manner. We used the olfactory system of Drosophila to study axon guidance and neuronal connectivity. In a large EMS screen, we have identified a new player that appears to have an important role in axon targeting of distinct ORN classes. Using MARCM analysis, we identified three ORN classes (OR47a, OR59c and OR42a) that show a strong mistargeting phenotype. Mutant OR47a neurons not only form an ectopic glomerulus in the AL, but they also have a different axonal growth pattern. Wildtype OR47a axons usually grow towards their wildtype glomerulus using two main projections routes. We observed that mutant neurons deviate from this pattern and project along a third route eventually innervating the ectopic glomerulus. For the maxillary palp (MP) neurons OR59c and OR42a we observed that axons of mutant neurons are no longer able to recognize their innate glomerulus, but instead innervate in a random pattern across the AL. We next investigated, if axons form misprojections on their contralateral side after establishing the ipsilateral connections. Removal of one antenna/MP shortly after hatching showed that the mistargeting was exclusively on the ipsilateral side and thus possibly a consequence of the aberrant ingrowth route of the ORNs. To further reveal the requirement of the gene we used reverse MARCM. Some classes require the gene cellautonomously whereas other classes require it non-autonomously. These observations led us to hypothesize that the gene is involved in several steps of axon targeting of distinct ORN classes and determines their axon growth route together with their ability to recognize the target glomeruli.

**P-145 A genome wide deficiency screen to identify upstream regulators of DmManf and Prospero in the embryonic garland cells of Drosophila.**

**Vassileios Stratoulias**; Tapio I. Heino
Department of Biological and Environmental Sciences, University of Helsinki, Finland

The Drosophila Mesencephalic Astrocyte-derived Neurotrophic Factor, DmMANF, encodes the ortholog of a recently identified novel family of neurotrophic factors in mammals. This novel family of neurotrophic factors have been shown to protect and rescue midbrain dopaminergic neurons and represent promising candidates for treatment of neurodegenerative diseases, such as Parkinson's disease. Up to day, there is little functional data and limited knowledge about DmMANF regulation and the pathways it is involved. Here we study the upstream regulatory mechanisms that control the expression pattern of DmMANF during late embryogenesis. Using immunofluorescence and an antibody that specifically recognises DmMANF protein, we have screened the Bloomington Deficiency Kit for embryos homozygous that bear large chromosomal deletions. DmMANF is prominently expressed in garland cells in addition to its expression in the nervous system. We are interested in mutants, in which this DmMANF protein distribution is significantly altered. In parallel, a second gene, prospero, that is also expressed in garland cells is used as a control for the screen. Therefore the screen identifies also upstream regulators for prospero. These large chromosomal deletions are narrowed by using smaller deficiencies and with the help of web-based data mining, single candidate genes are tested.

**P-146 A RalA and exocyst dependent pathway for the growth of postsynaptic membrane specializations at the neuromuscular junction.**

**Teodoro, RO**; Schwarz, TL
F.M. Kirby Neurobiology Center, Children's Hospital Boston and Department of Neurobiology, Harvard Medical School, Boston, MA 02115, USA

Neurons and muscle cells require tight spatial control of membrane traffic in order to form and maintain specialized domains within the cell, including the synapse. Postsynaptic membranes in particular are plastic structures that can grow and retract as synaptic strength is modified. We have investigated the role of membrane traffic and of a protein complex known as the exocyst in the formation of postsynaptic specializations at the NMJ. The exocyst complex is involved in the tethering, docking and fusion of post-Golgi vesicles with the plasma membrane and it can be regulated by its interactions with several small GTPases, including RalA. We found that overexpression of a constitutively active (CA) form of Ral induces a strong recruitment of Sec5, a Ralbinding exocyst subunit, to the NMJ postsynapse. The increase in postsynaptic Sec5 does not correlate with a change the levels of three major components of the postsynaptic membrane: GluRIIA, GluRIIC and Dlg. Instead, there is an increase in the F-bar protein Syndapin and the expansion of the sub-synaptic reticulum (SSR), the convoluted membrane specialization of the postsynapse. Expression of RasCA does not mimic this phenotype, but increased intracellular Ca2 +, recruits Sec5 to the synapse. This response to Ca2+ is blocked in Ral mutants. Our findings suggest the following pathway: Ca2+ influx at the NMJ activates Ral, which binds to Sec5, induces exocyst recruitment to the NMJ, and targets membrane vesicles for delivery to the postsynapse, thereby expanding the SSR. Our studies have identified a novel pathway that contributes to the development of the postsynapse and that can, potentially, represent a form of activity-dependent plasticity that leads to membrane growth in response to synaptic Ca2+ influx.

**P-147 R8 specific genes involved in photoreceptor differentiation.**

**Terzioglu Kara, E**; Talay, M, Çelik, A
Bogazici University Department of Molecular Biology and Genetics 34342 Bebek Istanbul Turkey

A common phenomenon observed in sensory epithelia is the mutually exclusive expression of individual receptor molecules in a given receptor cell. In the Drosophila retina, only one of 6 rhodopsin genes is expressed in a given photoreceptor cell. Two stochastically distributed ommatidial subtypes can be distinguished based on the selective expression of sets of rhodopsin genes in the inner photoreceptors R7 and R8. In the pale subtype R7 cells express Rh3, and R8 cells express Rh5. In the yellow subtype R7 cells express Rh4 and R8 cells express Rh6. This rhodopsin expression is established in late pupation through stochastic developmental mechanisms. To understand the mechanisms underlying this exclusive and coordinated expression a piggyBac based enhancer-trap screen has been performed. We are currently analyzing candidate genes that have been identified in this screen that show restricted expression among photoreceptors using gain-of-function and loss-of-function analyses. In particular we are working on genes that show R8 specific expression and are analyzing their possible role in rhodopsin gene regulation.

**P-148 Neuronal Sub-type Specification within an Identified Lineage by Temporal and Sub-temporal Feed-forward Loops.**

Magnus Baumgardt1, Daniel Karlsson1, Javier Terriente2*, Fernando J. DíazBenjumea2 and **Stefan Thor**1
1) Department of Clinical and Experimental Medicine, Linkoping University, SE-581 85 Linkoping, SWEDEN - 2) Centro de Biología Molecular-Severo Ochoa/C.S.I.C., Universidad Autónoma-Cantoblanco, Madrid, SPAIN

Neural progenitors generate distinct cell types at different stages, but the mechanisms controlling these temporal transitions are poorly understood. In the Drosophila CNS, a cascade of transcription factors, the ‘temporal gene cascade’, has been identified, that acts to alter progenitor competence over time. However, many CNS lineages display broad temporal windows, and it is unclear how broad windows progress into sub-windows that generate unique cell types. We have addressed this issue in an identifiable Drosophila CNS lineage, the neuroblast 5-6T, and find that a broad castor temporal window is sub-divided by two different feed-forward loops, both of which are triggered by castor itself. The first loop acts to specify a unique cell fate, while the second loop suppresses the first loop, thereby allowing for the generation of alternate cell fates. This mechanism of temporal and ‘sub-temporal’ genes acting in opposing feed-forward loops may be used by many stem cell lineages to generate diversity.

**P-149 Two types of cell death occur in the developing Drosophila optic lobe: Naturally occurring cell death and denervation induced cell death.**

**Yu Togane** 1),2); Yusuke Hara 1),2); Rie Ayukawa 1); Keiichiro Hirai 1); Kengo Beppu 1); Tatsuro Enomoto 1); Hiromi Akagawa 1); Takashi Takahashi 1); Kikuo Iwabuchi 2) and Hidenobu Tsujimura 1)
1) Developmental Biology, Tokyo University of Agri. and Tech., Japan 2) Applied Entomology, Tokyo University of Agri. and Tech., Japan

Enormous cells die naturally during the CNS development. This naturally occurring cell death (NOCD) eliminates surplus neurons when the neural network is formed. Moreover, if normal innervation is interrupted at the time neurons naturally die, more cells can die and we call this type of cell death denervation induced cell death. We are studying the development of Drosophila optic lobes to define the mechanisms in which these two types of cell death occur and their role for the formation of neural network. Enormous neuronal cells die naturally in the developing adult optic lobes during metamorphosis. The number of dying cells starts to increase at APF12h and peaked at APF24h. Pro-apoptotic genes hid and/or grim play an essential role in this cell death but another gene, rpr, does not. A steroid hormone, ecdysone, controls timing of the cell death and EcR is required. Histological observation using some molecular markers showed an alteration in the structure of developing optic lobes from the early phase of development to the adult one at this time. To study denervation induced cell death, we are using mutants, such as soD, that have no adult eyes or no ordered retinal innervation. Experiments show that denervation induced cell death in the developing optic lobe occur at the same time as NOCD. Notably, it requires rpr as well as hid and/or grim for the death, in contrast to NOCD. Ecdysone signal enhances the cell death here, too.

**P-150 Rhodopsin 6 protein maintains transcriptional repression of rhodopsin 5 in R8 photoreceptors.**

**Daniel Vasiliauskas** (1), Esteban Mazzoni (2), Claude Desplan (1)
1) Department of Biology, New York University, New York, NY; 2) Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, NY.

Individual sensory neurons generally express single sensory receptors from a repertoire that can run into the thousands. This is accomplished by a cell's choice to express only one receptor while excluding expression of the rest. Studies of olfaction in mice raised an intriguing possibility that a feedback signal from the sensory receptor protein itself plays a role in directing repressed state of alternative receptors genes. Photoreceptor cells (PR) in the adult Drosophila eye express five different rhodopsins. R8 PRs express either Rh5 (in pR8 subset) or Rh6 (in yR8 subset), but never both. Most R7 PRs express either Rh3 or Rh4. This expression is established by the end of pupation through stochastic and deterministic developmental mechanisms. We asked whether feedback signals from rhodopsin proteins participate in exclusion of other rhodopsin genes. We tested mutants of rhodopsins 3, 4, 5 and 6 for inappropriate expression of alternative members of the family. In young flies that lack Rh6, yR8s are empty of rhodopsin proteins. However, in older adults, these cells start to express Rh5. A rh6-lacZ reporter correctly marks yR8 cells in mutants, suggesting that the absence of Rh6 leads to de-repression of Rh5 in the context of proper specification of yR8. Thus, Rh6 signal acts to maintain repression of Rh5 expression in the adult. Loss of function of other rhodopsins does not lead to de-repression of alternative members of the family, yet they can substitute for Rh6 and repress Rh5 in yR8s. Thus, rhodopsin proteins, in addition to their main function as light receptors that initiate the phototransduction cascade, can also direct transcriptional repression and maintain functional identity of yR8 PR throughout the life of the fly.

**P-151 Neuropeptides in the Drosophila antennal lobe.**

**Mikael A Carlsson**1, Max Diesner2, Joachim Schachtner2, Dick R Nässel1
1Department of Zoology, Stockholm University, Stockholm, Sweden 2Department of Biology, Animal Physiology, Philipps University, Marburg, Germany

The antennal lobe (AL) is the major olfactory processing neuropil in insects. The AL is innervated by olfactory receptor neurons (ORN), which synapse with local interneurons (LN) and projection neurons (PN) in olfactory glomeruli, the functional subunits of the AL. The primary neurotransmitter of ORNs and PNs is acetylcholine (ACh), whereas a large proportion of the LNs are GABAergic. Centrifugal innervation of the AL is primarily aminergic and has a likely modulatory function. In addition to these classical transmitters, neuropeptides have been identified in neurons of the AL. We here present data on the distribution and colocalisation of neuropeptides in the Drosophila AL identified by a combination of mass spectrometry (MS), immunohistochemistry and promoter-Gal4 lines. Peptides of seven different neuropeptide precursors were unambiguously identified by MS in micro dissected ALs. Five of these could be confirmed by antisera to be expressed in different types of AL neurons. Short neuropeptide F (sNPF) is expressed in a subpopulation of cholinergic ORNs, while MIP, tachykinin and allatostatin A were found to be colocated with either ACh or GABA in different subpopulations of LNs. A fifth peptide, SIFamide is expressed in two pairs of neurons with processes widely arborizing all over the brain including the antennal lobe. A sixth peptide, IPNamide, showed high abundance in MS, whereas immunohistochemistry revealed that the antennal lobe is tightly surrounded by IPNamide positive axon processes, but none of these innervate the lobe. The abundance of different neuropeptides in the AL indicates neuronal substrates for a high degree of plasticity and modulation and future experiments will hopefully reveal the physiological and behavioural significance of these peptides.

**P-152 Bioinformatic approach of the role of Drosophila histone acetyltransferase Chameau in neural development and neuroendocrine control.**

**Hainaut,M**; Rodriguez, R; Bérenger, H; Graba, Y; Pradel, J
IBDML

Chameau (Chm) is involved in distinct epigenetic mechanisms of transcription control: i) Maintenance of Hox gene transcription ; ii) Modulation of Fos/Jun transcriptional activity by histone H4 acetylation at target gene loci; iii) Control of replication origins. The genetic evidences for Chm neuroendocrine functions are : On the one hand, chm inactivation causes phenotypes reminiscent of hormonal signaling defects : null mutation prevent hatching and abdominal differentiation ,related to an excess of circulating juvenile hormone (JH) -; chm late inactivation allows the emergence of adults (deprived of Chm) highly susceptible to different types of stress (starvation, oxidative, infections) ,related to a defect in insulin signaling. On the other hand, rescue experiments showed that the early phenotypes are rescued by chm re-expression in the 6 neurons (CA-LPs) of the pars lateralis that innervate the CA (JH productive gland) during development; and the late phenotypes by re-expression in the 12 neurons (IPCs) of the pars intercerabralis that produce insulin-like peptides and inervate the CA/CC complex in adults. DNA chip experiments showed that Chm mutation affects 6 functional classes of genes witch perfectly match the above-mentioned phenotypes. Furthermore, a significant fraction (160) of these genes is similarly affected by the mutation of chm or by the inactivation of CA-LP and IPC neurons. Fourteen of these target genes code for peptidases or peptidase inhibitors (serpins). We hypothesize that Chm controls the transcription of peptidase and serpin genes required for neuropeptide production in CA-LP/IPC neurons , thus their addressing to neuroendocrine glands, and thereby hormone synthesis and secretion. Chm would be a major regulator of signaling by JH and insulin.

**P-153 A role for miRNAs on circadian rhythms in Drosophila.**

Weissbein U.(1); Davidov S.(1); Ferber A.(1); Menet J.(2); Rosbash M.(2) and **Kadener S.**(1).
(1) The Dept. of Biological Chemistry, Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel (2) Hughes Medical Institute, National Center for Behavioral

The existence of circadian clocks, which allow organisms to predict daily changes in their environments, have been recognized for centuries, yet only recently has the molecular machinery responsible for their generation been uncovered. The current model in animals posits that interlocked feedback loops of transcription-translation produce these 24-hour rhythms. However, little is known about the contribution of translational control to circadian rhythms. To address this issue and in particular translational control by miRNAs, we knocked down the miRNA-biogenesis pathway in Drosophila circadian tissues. In combination with an increase in circadian-mediated transcription, this severely affected Drosophila behavioral rhythms, indicating that miRNAs function in circadian timekeeping. To identify miRNA-mRNA pairs important for this regulation, immunoprecipitation of AGO1 followed by microarray analysis identified mRNAs under miRNAmediated control. They included three core clock mRNAs, clock (clk), vrille (vri) and clockworkorange (cwo). To identify miRNAs involved in circadian timekeeping, we exploited circadian cell-specific inhibition of the miRNA biogenesis pathway followed by tiling array analysis. This approach identified miRNAs expressed in fly head circadian tissue. Behavioral and molecular experiments show that one of these miRNAs, the developmental regulator bantam, has a role in the core circadian pacemaker. S2 cell biochemical experiments indicate that bantam regulates the translation of clk through an association with three target sites located within the clk 3? UTR. Moreover, clk transgenes harboring mutated bantam sites in their 3?UTR rescue rhythms of clk mutant flies much less well than wild-type CLK transgenes.

**P-154 Metabolic stress responses are modulated by brain neurosecretory cells that produce multiple neuropeptides.**

**Lily Kahsai**, Neval Kapan, Heinrich Dircksen, Åsa M. E. Winther, Dick R. Nässel
Department of Zoology, Stockholm University, S-10691 Stockholm

In Drosophila, neurosecretory cells that release peptide hormones play a prominent role in the regulation of development, growth, metabolism, and reproduction. Previous studies have identified several types of peptidergic neurosecretory cells in the brain of Drosophila with release sites in the corpora cardiaca and anterior aorta. We show here the colocalization of products of three neuropeptide precursors in four pairs of large protocerebral neurosecretory cells (designated ipc-1): Drosophila tachykinin (DTK), short neuropeptide F (sNPF) and ion transport peptide (ITP). These peptides were detected by immunocytochemistry in combination with GFP expression driven by the enhancer trap Gal4 lines c929 and Kurs 6. The c929 driver is known to specify secretory peptidergic neurons that express the transcription factor DIMMED. This mix of colocalized peptides is remarkable considering what is known so far about DTKs, sNPFs and ITPs in insects and prompted us to analyze functional roles of the 8 ipc-1 neurons producing the cocktail. We investigated the role of peptide signaling from ipc-1 cells in stress responses by monitoring the effect of starvation and desiccation after knockdown of DTKs and sNPF in the ipc-1 neurons by targeted RNA interference (Gal4-UAS system). Flies with reduced DTK or sNPF levels displayed decreased survival time at starvation, suggesting that ipc-1s and their peptides are important during metabolic stress. Knockdown of sNPF in ipc-1s (DTK not yet tested) led also to strongly decreased resistance to desiccation. Further assays are clearly required for an understanding of the role of ipc-1s in physiology, but so far it seems that homeostasis during stress requires intact ipc-1 signaling.

**P-155 Differential regulation of ferritin and iron in neurons and glia of Drosophila.**

**Stylianos Kosmidis** [1], Efthimios A. Skoulakis [1] and Fanis Missirlis [2]
[1] Institute of Molecular Biology and Genetics BSRC ‘‘Alexander Fleming’’ [2] School of Biological and Chemical Sciences, Queen Mary University of London

Cellular and organismal iron storage depends on the function of the ferritin protein complex in insects and mammals alike. Mammalian ferritin shows enhanced expression in oligodendrocytes and has recently been implicated in iron trafficking across the mammalian blood brain barrier. Here we show that the blood-brain barrier of Drosophila also functions to protect the insect’s brain from direct exposure to systemic iron fluctuations. We have assessed the consequences of transgenederived expression of fly ferritin subunits in neuronal and glial cells of the adult fly brain. We demonstrate that most neurons maintain a strong post-transcriptional homeostatic mechanism that prevents excessive ferritin accumulation whereas particular neurons show subunit-specific enrichment. Conversely, we identify a subpopulation of glia that accumulate iron-loaded ferritin inclusions, microscopically visible as crystal-like structures in the lamina. Flies carrying such ferritin iron deposits are surprisingly healthy yet demonstrate a characteristic late onset behavioural decline. Our results suggest that Drosophila ferritin can participate in brain iron trafficking and that ferritin accumulation is regulated in a cell type specific manner.

**P-156 Abstract not available**

**P-157 The function of dRFX in central brain neurons.**

**Laurencon, A.** ;Grenier,G. ;Durand B.
CGMC, UMR 5534 CNRS Université Lyon 1, Villeurbanne, France

Transcription factors of the RFX family are conserved from yeast to mammals and have been shown to control genes involved in ciliogenesis in metazoans. In Drosophila, dRfx mutants present defects in chemosensory and mechanosensory behaviors correlated with abnormal function and structure of neuronal sensory cilia. This predominant phenotype corresponds to dRfx expression in ths PNS which is restricted to type I sensory neurons. dRfx is also expressed in the brain throughout development. In order to investigate the function linked to this brain expression, we developed tools to uncouple the PNS and the CNS dRFX function. We show that dRFX in these central neurons contribute to a developmental program governing larval growth and specific feeding behaviors. Indeed, amongst the 250 neurons expressing dRFX per lobe, we could identify small groups of neurons that do innervate the ring gland. More precisely, they are four neurons innervating the Corpora Allata and the two PTTH expressing neurons which innervate the Prothoracic Gland. The cellular function of dRFX in these neurons is under investigation: in the mutant flies, the global arborization of these neurons is dimmed, revealing a defect in neurite formation. To identify these defects more clearly at the level of single neurons, we either use MARCM technique or the PTTH-Gal4 driver to follow these two dRFX expressing neurons only. To understand if these defects are dependant on basal body or cilia assembly, we are comparing these dRFX expressing neurons in mutants affecting totally (sas-4) or partially (oseg-1, oseg-2, nompB, btv) the ciliogenesis process. Interestingly, we can reproduce the cellular phenotypes observed in dRfx mutants in some of these mutants.

**P-158 Modelling of potential resistance mutations in Rdl and their verification in vivo.**

**Emily Remnant** (1), Phillip Daborn (1), Michael Parker (2), Philip Batterham (1)
1) Department of Genetics, Bio21 Molecular and Biotechnology Institute, The University of Melbourne, Victoria, Australia; 2) Biota Structural Biology Laboratory, St Vincent's Institute, Melbourne,

The GABA receptor gene Rdl encodes a ligand-gated chloride channel subunit that is implicated as a target for cyclodiene and phenylpyrazole insecticides. Mutations substituting alanine to serine at position 301 of the protein are found in many insect species and result in high levels of resistance to the cyclodiene dieldrin. The same substitution provides low levels of resistance to phenylpyrazoles such as fipronil, however a second substitution, threonine to methionine at position 350, was found in a highly resistant strain of Drosophila simulans. This change was shown to further reduce fipronil activity in vitro. The A301S change confers resistance without abolishing the endogenous role of RDL. Functional restraints preventing the mutation of other residues in the channel pore have never been fully explored. A homology model of RDL and chemical autodocking has implicated other residues in this region in phenylpyrazole binding. We wanted to examine effects of changes at these predicted sites on the function of the protein in vivo, and resistance at the whole organism level when incorporating the contributions other targets and detoxification processes. The Rdl locus spans 50kb, with transcripts subject to alternate splicing and RNA editing. To maintain all aspects of this complexity in our model, we are using recombineering and site-specific integration to explore the impact of a variety of amino acid substitutions on phenylpyrazole resistance. This allows manipulation of a major insecticide target gene and in vivo validation of predictions based on homology modelling. It also provides a controlled genetic background to examine the behavioural consequences of mutations in Rdl, including its roles in processes such as sleep and olfaction.

**P-159 Genetic and environmental factors influencing pupation site selection in D. melanogaster.**

**Ringo, John M**
Laboratory of Ethological Genetics, Jerusalem, Israel University of Maine, Orono, ME, USA

In Drosophila melanogaster, some larvae wander away from the food to pupate, often in the soil. Other larvae "stay at home,??? pupating directly on the food. The proportions of wanderers and stay-at-homes were observed in a survey of 102 wild strains of D. melanogaster collected from five continents, nearly all of them isofemale strains. For each replicate, 40 eggs were transferred to a 1.7-ml polypropylene tube containing cornmeal-molasses medium; the tube stood on moist tissue paper inside a 35-ml vial; this was cultured at 25 C under LD12:12. Strains varied significantly in the proportion of wanderers, which pupated on the moist paper or the walls of the culture vial, vs. stay-at-homes, which pupated on the medium or on the surface of the tube containing the medium. From the survey, ten strains were chosen for further study. An experiment measuring percentage of wanderers was performed using three larval densities (10, 20, or 40 eggs per tube), two kinds of container (one described above and the other a 1.8-ml polystyrene food cup; there were 3 replicates per treatment combination (balanced factorial design, randomized blocks). The percentage of wanderers varied significantly by strain ($P < .001$), by larval density ($P < .001$; the higher the density, the more wanderers), and by food container ($P < .001$); no interactions between variables were detected. The significant effect of two environmental factors highlights the need for careful control of the environment in genetic studies of this phenotype. The significant variation among strains suggests that it will be possible to analyze genetically the wandering trait described here, as has been done successfully for pupation site selection using a different method (Riedl et al. 2007; Fly 1:23-32).

**P-160 Novel mitochondrial fission gene PM1 is required for neuronal activity in Drosophila.**

**Rival T***, Macchi M*, Maillet F*, Lievens JC#, Fatmi A*, Royet J*
* Institut de Biologie du Développement de Marseille Luminy, Marseille, France # Centre de Recherche en Neurobiologie et Neurophysiologie de Marseille, Marseille, France

The shape and number of mitochondria within cells are determined by fusion and fission events. Fusion of mitochondria builds large interconnected networks, whereas fission releases numerous individual mitochondria of smaller size. Mitochondrial dynamics is a universal process relying on specific dynamin-related GTPases. First identified in fly and yeast these components are conserved in mammals. The mitofusins and OPA1 promote fusion, whereas DRP1 is required for fission. We report here the characterization of PM1 a novel mitochondrial fission gene. PM1 encodes a mitochondrial protein. It has no sequence similarities to known protein families but its amino acid sequence is highly conserved in the animal kingdom. Using homologous recombination we generated a PM1 nul mutant. In cells deficient for PM1 we observed a dramatic increase in mitochondrial size together with a reduced number of mitochondria. PM1 mutant flies developed to adulthood but show shortened lifespan and severe neurological defects including bang sensitivity. Neurological phenotypes and surprisingly the early lethality were rescued by targeting the expression of PM1 in neurons only. Indeed we determined that PM1 knock out not only impairs mitochondrial function but massively decreases mitochondrial export to synapses, and finally results in neurotransmission defects. Taking together our results i) describe a novel gene involved in mitochondrial fission, ii) illustrate the essential role of mitochondrial remodeling in neuronal activity, iii) provide an attractive in vivo model to explore the roles of mitochondrial fission in the physiology of cells.

**P-161 Characterization of RFX target genes required for ciliogenesis in Drosophila.**

**Soulavie,F**; Laurencon,A; Thomas,J; Enjolras,C; Durand,B
CGMC UMR 5534 CNRS Université Lyon 1

Cilia and flagella are microtubular structures conserved throughout species from algae to mammals. They play major functions in physiology and development of these organisms. In human, ciliary dysfunctions are responsible for several diseases called ciliopathies. RFX transcription factors regulate genes involved in cilia assembly from C. elegans to mammals. In Drosophila dRfx is involved in sensory cilia development. Putative RFX transcription targets have been identified in our lab by an in silico screen (Laurencon et al, 2007). I have compared this screen and several screens in the literature and selected good candidates for functional studies in Drosophila. For each selected gene, I have created tagged reporter constructs to follow their expression profile and protein localization. CG13125 is a very interesting candidate; its expression correlates with dRfx expression in the PNS and it is downregulated in dRfx deficient Drosophila. Furthermore it has a very good mammalian ortholog of unknown function. It has been involved in flagellar motility in protozoa and our observations raise the question of a non motile function in sensory cilia. Another good candidate present in our screen is expressed in sensory chordotonal organs of the fly embryo and its expression is lost in dRfx deficient Drosophila. It has an ortholog of unknown function in humans. Mutations for these two genes are under construction. Several other genes have been cloned to determine their expression profile in drosophila embryo. This project will provide a global understanding of the function of dRfx in the ciliogenesis and in the cilia function.

**P-162 ASH2 interacts with trxG SET proteins and is required for H3K4me3.**

**Carbonell, A**; Serras, F; Corominas, M.
Departament de Genètica, Universitat de Barcelona and Institut de Biomedicina de la Universitat de Barcelona (IBUB), Av. Diagonal, 645, 08028, Barcelona, Spain.

Cell fate decisions can be maintained during long periods of developmental time by stable states of gene expression. Epigenetic regulation is essential for the inheritance of the gene expression program of each cell. Polycomb (PcG) and trithorax group (trxG) proteins are key components of this epigenetic machinery. Both act in multimeric complexes changing the chromatin structure in order to regulate, repressing or activating respectively, the transcriptional state. Although the exact mechanism still remains unclear, histones post-translational modifications appear to play an important role. An epigenetic signature generally associated with transcriptionally active genes is the trimethylation of the lysine 4 of histone H3 (H3K4me3). Among trxG proteins, Trithorax (TRX), Trithorax related (TRR) and Absent, small or homeotic discs 1 (ASH1) contain the SET domain necessary for the deposition of the H3K4me3 mark. Absent, small or homeotic discs 2 (ASH2) does not have any SET domain but is required for H3K4me3. In order to understand the function and putative relationships between these trxG proteins we have set up several approaches. Co-immunoprecipitation studies have demonstrated that ASH2 interacts with TRX, TRR and ASH1. Immunolocalization on polytene chromosomes has confirmed this interaction and has suggested that the ASH2 interaction with these proteins is highly dynamic. To stablish the function of ASH2, we have performed immunolocalization experiments on polytene chromosomes using different mutant backgrounds. Results we have obtained suggest that ASH2 does not act only as a cofactor of the H3K4me3 activity but is also involved in maintaining the complex stability. Current experiments are underway to shed light into the functional relationship of these proteins.

**P-163 Drosophila RecQ5 functions preferentially in SSA mediated DSB repair and suppresses inter-homologous recombination in vivo.**

**Yixu Chen**#, Wen Dui# and Renjie Jiao* # authors contributed equally * corresponding author
Institute of Biophysics, Chinese Academy of Sciences, Datun Road 15, Beijing 100101, China

RecQ5 in mammalian cells has been suggested to suppress inappropriate homologous recombination. However, the underlining mechanism(s) is still not well understood. Therefore we took the advantage of powerful Drosophila genetics to investigate into how Drosophila RecQ5 (dRecQ5) functions in vivo to regulate homologous recombination mediated double strand breaks (DSBs) repair. For this purpose, we first generated null alleles of dRecQ5 using targeted recombination technique. The mutant animals are homozygous viable, but with growth retardation during development. The homozygous mutants are more sensitive than wild type to both exogenous DSB inducing reagents such as gamma-irradiation, and endogenously induced DSBs by I-Sce I enzyme. Several models of in vivo repair assays suggest that dRecQ5 is predominantly required for intra-chromosomal homologous recombinational repair, rather than inter-chromosomal homozygous recombination. These models include in vivo reconstitutions of the yellow gene and the white gene which are judged by the body color, eye color and animal lethality. In the absence of dRecQ5, the flies use more inter-homologous gene conversion rather than SSA to repair DSBs while NHEJs remain unchanged. However, if there is no inter-homolog available, flies use more NHEJs than SSA when dRecQ5 function is abolished. mwh experiment indicates that dRecQ5 is important for maintaining genome stability. Altogether, our data demonstrate that dRecQ5 functions preferentially in SSA mediated DSB repair and suppresses inter-homologous recombination in Drosophila.

**P-164 DEAD Box Helicase Rm62 is a novel Enhancer of trithorax and Polycomb.**

**Lamiable, Olivier**; Locker, Daniel; Decoville, Martine;
Centre de Biophysique Moléculaire CNRS, rue Charles Sadron 45071 Orléans, France

During development, cells differentiate under the influence of many factors playing on genes expression. Once established, the gene expression pattern is transmitted to daughter cells. This involves epigenetic mechanisms that locally reshape the structure of chromatin. In Drosophila melanogaster, Polycomb (PcG) and trithorax (trxG) group genes are involved in maintaining the profile of gene expression during development. PcG proteins maintain genes in repressed state whereas TrxG maintain genes active. trxG mutation suppress PcG mutation and vice versa. A screen to identify enhancers of trxG genes allowed isolation of genes that were previously identified as PcG. These genes were then called Enhancers of Trithorax and Polycomb (ETPs). Mutations in ETP genes enhance phenotype due to PcG and trxG mutations. Dorsal Switch Protein 1 (DSP1) is a High Mobilty Group B (HMG) protein acting as an ETP. PcG and TrxG proteins are involved in multimeric complexes. But DSP1 has not yet been identified in PcG or TrxG complexes. On the basis of gel filtration analysis of protein complexes in embryo nuclear extracts, it appears that the majority of DSP1 is present in complex(es) from 100 kDa to 1MDa. We used immunopurification to identified DSP1 proteins partners from drosophila embryonic nuclear extracts. The polypeptides co-purified with DSP1 were characterized through sequencing of peptides from individual protein bands by nanoelectrospray tandem mass spectrometry. Among identified proteins, we focused on Rm62 which is a drosophila homolog of mammalian DEAD Box RNA Helicase P68. We have shown that DSP1 and Rm62 are together in a 300 kDa complex only in 3 -12 h old embryo but not later in embryogenesis. Genetic studies presented suggest that Rm62 is a novel ETP.

**P-165 The polycomb protein SU(Z)12´s function in gene silencing.**

**Larsson, AL;** Tegeling, E; Chen, S; Rasmuson-Lestander Å
Department of Molecular Biology, Umeå University, Umeå, Sweden

Polycomb group proteins are important gene regulators that maintain genes in a repressed state by modification of histones. They act by specifically binding to promoter regions and polycomb response elements (PRE) which regulate important transcription factor and homeotic genes. Polycomb group proteins are organised in three different complexes. Suppressor of zeste 12 (SU (Z)12) is a core component of the PRC2 complex together with Enhancer of zeste (E(Z)), Extra sex comb (ESC) and Nucleosome Remodelling Factor-55 (NURF-55). SU(Z)12 is crucial for PRC2´s methyltransferase activity as the H3K27 trimethylation activity is abrogated when SU(Z)12 is absent. The Su(z)12 gene is spliced into two different transcripts; Su(z)12A and Su(z)12B. These transcripts are then translated into two different isoforms; a 95 kDa SU(Z)A-protein and a 100 kDa SU(Z)12B-protein. These two isoforms are differently expressed during development indicating different functions for SU(Z)12A and SU(Z)12B. GST-pulldowns with in vitro expressed proteins show that the isoforms interact differentially with the other core components in the PRC2 complex. Ubiquitous over-expression of SU(Z)12 during late larval/ pupal stage leads to lethality or homeotic transformations due to misexpression of the Hox genes.

**P-166 Genome-wide analyses confirm the association of ASH2 and H3K4me3 in Drosophila wing imaginal disc.**

**Sílvia Pérez-Lluch**, Enrique Blanco, Andrea Mateo, Florenci Serras and Montserrat Corominas.
Departament de Genètica, Universitat de Barcelona and Institut de Biomedicina de la Universitat de Barcelona (IBUB), Av. Diagonal, 645, 08028, Barcelona, Spain.

Epigenetic regulation is essential for the maintenance of gene expression along cell divisions and, thus, for cell fate. Trithorax (trxG) and Polycomb Group (PcG) proteins are responsible for the maintenance of the transcriptional activity and silencing throughout development, respectively. PcG and trxG proteins act in a multimeric complex manner. It has been shown that some of these proteins exhibit histone methyltransferase activity directed against specific lysines of the histones H3 and H4, whereas others are capable of interpreting these marks and translate them into changes on the chromatin structure and, finally, on gene expression. ASH2 (absent, small or hometic discs 2), a member of the trxG involved in wing development, does not have any SET domain but is essential for the deposition of the H3K4me3 mark. In other systems, ASH2 has been associated to different histone methyltransferases. However, the mechanism by which these proteins regulate gene expression is still unknown. In our group, we have performed chromatin immunoprecipitation assays followed by high-throughput sequencing (ChIPseq) of late larvae wing imaginal discs using antibodies against tagged ASH2 and several histone marks. We have seen that, whereas the H3K4me3 mark is mostly present at transcription start sites, the silencing mark H3K27me3 spreads over large regions of chromatin. Besides, in this context, there is little overlap between genes showing both H3K4me3 and H3K27me3, suggesting that the presence of bivalent domains is probably restricted to other species. Finally, we have also seen that ASH2 is present at gene promoter regions and is highly associated to the H3K4me3 mark. Further studies are currently in progress to analyze the role of ASH2 in the deposition of this activating mark.

**P-167 The farnesylated proteins LaminDm0 and Kugelkern affect the structure of phospholipid bilayers.**

**Polychronidou M** and Grosshans J
ZMBH, University of Heidelberg, Zentrum Biochemie und Molekulare Zellbiologie, Universitätsmedizin, Universität Göttingen

Changes in nuclear shape are observed under physiological conditions, as well as in various pathologies such as nucleopathies and progeroid syndromes. The mechanisms that underlie the alterations in nuclear shape and size in the above situations have remained mostly unknown. Kugelkern (Kuk) is a farnesylated protein of the inner nuclear membrane (INM) that shares structural and functional similarities with Lamins. Kuk regulates nuclear elongation in the Drosophila embryo. Kuk overexpression results in the formation of large, abnormally shaped nuclei, in a similar way as overexpression of permanently farnesylated variants of Lamins. The localization of Kuk and its effect on the NM depends on farnesylation as we observed by treatments with farnesylation inhibitors and a non-farnesylatable mutant. We found that localization of Kuk to the INM does not depend on other lamina proteins and that overexpression of Kuk changes nuclear shape in different cell types, even in yeast cells, despite the absence of a nuclear lamina. Taking into account these observations, that suggest a lamina independent mechanism for the function of INM farnesylated proteins, we examined the effect of Kuk and LaminDm0 on protein free liposomes. We used LaminDm0-?N and Kuk?N constructs, consisting of the C-terminal protein part that contains the NLS and the CaaX box. By performing binding assays we found that farnesylation promotes the binding of Kuk-?N and LaminDm0-?N to the liposomes. Most importantly, farnesylated LaminDm0-?N was able to tubulate spheric liposomes, suggesting an asymmetric insertion of Lamin into the lipid bilayer. Unraveling the mechanism through which Kuk and Lamin affect nuclear morphology can provide insight into the analysis of pathologies related to nuclear shape changes.

**P-168 Regulation of gene expression in primary spermatocytes by meiotic arrest genes.**
Jiang, J; Doggett, K; Benson, E; **White-Cooper, H.**
Cardiff University; Peter Macallum Cancer Centre; Journal of Biology; Cardiff University.

In Drosophila spermatogenesis, meiotic cell cycle progression is linked to spermatid differentiation by the function of the “meiotic arrest??? genes. aly-class meiotic arrest mutants (aly, comr, topi, tomb and achi/vis) fail to enter both the meiotic divisions and spermatid differentiation. The proteins encoded by aly-class genes form a complex termed TMAC. can-class meiotic arrest proteins, the testis TAFs (tTAF) form a distinct complex. Using microarray analysis we found that aly-class mutants fail to express many testis specific genes. RNA in situ hybridisation revealed that most of the aly-class target genes are normally transcribed in primary spermatocytes, and are likely to function in spermatid differentiation. The can-class mutants also show significant defects in transcriptional activation in testes; they regulate a significant subset of the aly-class target genes. Protein purification and yeast-2-hybrid strategies have revealed additional proteins that physically interact with the aly-class gene complex. We have focussed on two genes, mip40 and wake-up-call (wuc). mip40 mutants are viable, but male sterile, with a meiotic arrest phenotype. Similarly, RNAi against wuc in testes reveals a weak meiotic arrest phenotype. Unexpectedly, neither mutant falls into the aly-class, in terms of the gene expression defects seen in the mutant testes. Instead these two genes appear to define a novel meiotic arrest class, whose target gene set only partially overlaps with that of aly and can. To further understand how TMAC regulates gene expression we are comparing the gene expression changes in tomb and mip40 testes to the patterns of Tomb and Mip40 binding to chromatin by DAM-id, hybridised to genomic tiling arrays.

**P-169 The SUUR protein plays key role in DNA late replication and underreplication in polytene chromosomes of Drosophila melanogaster.**
**Zhimulev I.**, Andreyeva E., Babenko V., Belyaeva E., Belyakin S., Boldyreva L., Kolesnikova T., Koryakov D., Makunin I., Pindyurin A., Pokholkova G., Yurlova A., Zykov I.
Department of Molecular and Cellular Biology, Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, RUSSIA, e-mail: zhimulev@bionet.nsc.ru

Mutation of the SuUR gene makes late replicating regions replicate early and produces an increase in the polytenization level of heterochromatin in polytene chromosomes. The SuUR gene is active most strongly in embryos and in the nurse cells. The SUUR protein preferentially binds transcriptionally silent and late-replicated genes. The deduced length of the SUUR protein is 962 aa. The N-terminal part has similarity to the ATPase motif of the SNF2/SWI2 protein family. Central part of the protein has positively and negatively charged clusters and NLS. Mutations in the N-terminal region impairs binding of SUUR to late replicating regions of polytene chromosomes and reduces ability of the protein to cause DNA underreplication. The SuUR orthologues are present in all sequenced Drosophilids. Amino acid substitutions are distributed non-uniformly: N-terminal part of SUUR displays the highest level of conservation, middle part is the least conserved, C-terminal part has several short conserved regions. SuUR belongs to fast evolving genes and its distribution is restricted to the Drosophila lineage. The domain organization, charge and size of SUUR are largely maintained though evolution of Drosophililds. YTH screen identified HP1 as a strong interactor of the SUUR. The central region of SUUR is necessary and sufficient for interaction with the C-terminal part of HP1 containing the hinge and chromoshadow domains. Recruitment of SUUR to ectopic HP1 sites on chromosomes provides evidence for their association in vivo. The SUUR protein does not directly interact with SU(VAR)3-9 in YTH system, but the mutation SuUR influences binding of the SU(VAR)3-9 protein to polytene chromosomes. The SuUR mutation also influences methylation of the residues K9 and K27 in histone H3.

**P-170 Myosin requirements during wing disc eversion.**

**Aldaz S.**, Escudero LM., Freeman M.
Laboratory of Molecular Biology-MRC, Cambridge, U.K.

Cell reorganization is an essential event during the morphogenesis. Inside of a tissue, the change of cell shape of a selected group of cells is able to generate a force over the surrounding cells. These cells respond to this force suffer with a deformation that depends on their physical properties. This balance between generated force and response, gives as a result a net movement, and produce a change of shape of the whole tissue/organ. Different Drosophila model systems have been used to analyze tissue rearrangements. We have approached this problem using a new model system: The wing disc during the eversion. The eversion is an active process that drives the wing disc outside the larval epidermis during the metamorphosis. We have developed and ex-vivo culture system where the disc reproduces the movements of the metamorphosis. With this culture system we are able to follow the whole process using confocal microscopy. The wing disc is bag of epithelia formed by two layers. The squamous cells form mainly one of the layers, the peripodial epithelia, PE, and columnar cells form the opposite one, Disc proper, DP. Connecting both there are a margin cells that gradually change their shape from squamous to columnar. We have studied what is the contribution of the PE and margin cells for the first step of the eversion. The folding of the disc. Previous the folding the disc is a flat structure and suffers a folding that generates an angle of 270º of the organ. We have analyzed the different forces responsible of this folding and the contribution of each of the three cell types. We have carefully dissected the Myosin requirements in both, PE and margin cells. We developed a model that explains how the folding is produced. This model shows how during a tissue rearrangement is not only important to generate a force inside the epithelia but the response of the surrounding cells to this force.

**P-171 Spatial regulation of an E3 ubiquitin ligase modulates caspase activation during sperm differentiation in Drosophila.**

Kaplan, Y; Gibbs, L; Feinstein-Rotkopf, Y; **Arama, E**
Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel

In both insects and mammals, the final stage of spermatid terminal differentiation involves the removal of their bulk cytoplasm and organelles. In Drosophila, this process requires apoptotic proteins, including caspases. However, little is known about how spermatids avoid excessive caspase activation and undesirable death. We show here that the small protein Soti can bind to and negatively regulate a Cullin-3-based ubiquitin ligase complex required for caspase activation in this system. Soti is expressed in a distal-to-proximal gradient, promoting an inverse gradient of caspase activation. Another interactor of this complex, the giant inhibitor of apoptosis-like protein dBruce, is also distributed in a gradient of the same direction as that of Soti. Finally, dBruce distribution is modulated by the actions of both Soti and the Cullin-3-based complex, and mutations in dbruce cause infertility phenotypes reminiscent of soti inactivation. These findings reveal a novel mechanism for modulation of caspase activation in a nonapoptotic process. Importantly, a similar Cullin-3-Klhl10 complex is also required for proper late spermatogenesis in human. Therefore, our findings may shed light on the etiology of some forms of human male infertility.

**P-172 The role of apoptosis in shaping the tracheal system in the Drosophila embryo.**

**Baer,MM**; Bilstein,A; Caussinus,E; Csiszar,A; Affolter,M; Leptin,M
Biozentrum, University of Basel, Klingelbergstr 50/70, CH-4056 Basel, Switzerland Institute of Genetics, University of Cologne, Zülpicher Str.47, D-50674 Cologne, Germany

The tracheal system in the Drosophila embryo arises from a set of epithelial placodes that, upon cell migration, rearrangements, fusions and shape changes, form a tubular network. A designated number of cells is initially allocated to each branch of the system. We show here that the final cell number in the dorsal branches is not only determined by early patterning events and subsequent cell rearrangements but also by elimination of cells from developing branches – an event not reported so far in the embryonic tracheal system. Extruded cells die and are engulfed by macrophages. Our results suggest that the pattern of cell extrusion and death is not hard-wired, but is determined by environmental cues, possibly by mechanical stress.

**P-173 Functional links between Mediator complex subunits and GATA transcription factors during Drosophila development.**

**Boube M.**, Gobert V., Augé B., Immarigeon C., Bernat-Fabre S., Osman D, Klapholz B., Cavelier C., Cribbs D.L., Haenlin M., Waltzer L. and Bourbon H.M
Centre de Biologie du Développement, UMR 5547/CNRS, Toulouse

Mediator (MED), a conserved ~30 subunit modular complex, plays a pivotal role by bridging sequence-specific transcription factors (STF) to the PolII transcriptional machinery. We are using Drosophila as a model to analyze the functional specificity of MED subunits in vivo. We previously showed that the four MED subunits of the regulatory CDK8 module (Med12, Med13 Cdk8 and CycC ) share some functions but also have distinct roles in developmental gene regulation. Here, we analyzed the functional relationships between the CDK8 module subunits and the core Med1 subunit, whose mammalian counterpart is a direct interactor of GATA-type STFs and nuclear hormone receptors. Our recent generation of Med1 null mutations and the availability of dsRNAs transgenic lines showed that like the Cdk8 module subunits, Med1 is required for normal development but not for cell viability. Loss of function phenotypes indicate that Med1 is required for leg, wing and thorax development, as Med12-13, suggesting a functional link between Med1 and Med12 as previously shown in C. elegans. Nevertheless, the absence of eye phenotypes indicates that Med1 does not share all Med12-13 functions. Given that thoracic closure depends on the GATA transcription factor Pnr, we analyzed at which level(s) the Med1 Med12 and Med13 subunits functionally interact. In parallel, we studied the role of MED subunits during embryonic haematopoiesis, a process depending on Serpent, another GATA transcription factor.

**P-174 A developmentally regulated two-step process generates a non-centrosomal microtubule network during tracheal morphogenesis.**

**Brodu V.** 1, Baffet A.D. 1, Casanova J. 2,3, Guichet A.1
1Institut Jacques Monod- CNRS, Paris, France 2Institut de Biologia Molecular de Barcelona-CSIC and 3 Institut de Recerca Biomèdica de Barcelona, Barcelona, Spain.

Microtubules (MTs) are essential for many cell features, such as polarity, shape, motility and vesicle trafficking. Therefore, in a multicellular organism, their organisation differs between cell types and during development. MTs form at distinct structures termed MT organising centres (MTOCs) and, in most animal cells, centrosomes act as the major MTOCs. However in many cell types, MTs are not associated with the centrosome, but it is unclear how these arrays of noncentrosomal MTs are generated and what controls this different polarisation of the MT cytoskeleton. Here we addressed the control of MT reorganisation during trachea formation in Drosophila. We show that MT reorganisation is coupled to relocalisation of the MTOC components from the centrosome to the apical cell domain from where MTs then grow. We reveal that this process is controlled in a two-step mechanism by Trachealess, a transcription factor specifying tracheal fate. First, MTOC components are released from the centrosome in a process that requires the activity of the MT-severing protein Spastin. And second, the transmembrane protein Piopio contributes to anchoring MTOC components apically. We further show that mutants that interfere with the relocalisation of MTOC components impair proper tracheal morphogenesis thus stressing the functional relevance of MT reorganisation. Given the widespread apical organisation of MTs in various cell types, this two-step process may be a general regulatory mechanism to control the relocalisation of MTOC components and MT reorganisation in many organisms.

**P-175 Genetic and genomic analysis of the novel role of Tramtrack in embryonic myogenesis.**

**Lucia Ciglar**, Bartosz Wilczynski, Eileen E. M. Furlong
European Molecular Biology Laboratory, D-69117 Heidelberg, Germany

The development of Drosophila embryonic musculature is regulated by intricate transcriptional regulatory networks, our understanding of which has been greatly enhanced by recent genomewide studies. Yet the contribution of repression to the establishment of precise spatio-temporal expression patterns of myogenic genes has remained ill-defined. We have uncovered a novel role for a well-established transcriptional repressor, Tramtrack (Ttk), in the development of body wall musculature. Phenotypic analysis, complemented by a molecular dissection of Ttk's direct target genes in the developing muscle, has revealed its involvement in the regulation of myoblast fusion. Myoblast fusion is the process giving rise to multinucleated muscle syncytia from single founder cell (FC) and multiple fusion competent myoblasts (FCMs). This finding was further supported by a ChIP-on-chip experiment which showed a preferential binding of Ttk to cis-regulatory modules (CRMs) associated with FC-specific genes compared to genes expressed only in FCMs or in both types of myoblasts. In addition, a substantial number of CRMs co-bound by mesodermal transcription factors early in embryogenesis are later occupied by Ttk. As Ttk itself is a target of these regulators, it implies Ttk's involvement in an incoherent feed forward loop. Finally, we have investigated whether Ttk and Lame duck, a key regulator of FCM specification, regulate the same battery of target genes and thus have an overlapping function in this process.

**P-176 The serine protease homolog gene scarface is a new transcriptional target of JNK signalling that regulates epithelial morphogenesis in Drosophila.**

**Raphaël Rousset**, Sophie Bono-Lauriol, Melanie Gettings, Magali Suzanne, Pauline Spéder and Stéphane Noselli
Institute of Developmental Biology & Cancer - University of Nice Sophia-Antipolis - UMR 6543 CNRS - Parc Valrose - 06108 NICE cedex 2 - France

In Drosophila, dorsal closure is a model of tissue sealing consisting in the migration of ectodermal sheets dorsally to insure continuity of the embryonic epidermis. Activation of JNK signalling in the leading edge is essential. We identified new JNK target genes in a genome-wide micro-array screen during dorsal closure. One of them is the gene scarface (scaf), which belongs to the vast family of Chymotrypsin-like serine proteases. Some proteins of this family, like Scaf, bear an inactive catalytic site, representing a subgroup of serine protease homologs (SPH), whose functions are poorly understood. Here we report the characterization of scaf during JNK-dependent epithelium morphogenesis in Drosophila. We show that scaf is a general transcriptional target of the JNK pathway. Scaf is a secreted protein and scaf loss of function induces defects in JNKcontrolled morphogenetic events such as embryonic dorsal closure and adult male terminalia formation and rotation. Live imaging of the latter process reveals that, like for dorsal closure of the embryo, JNK directs the fusion of two epithelial layers in the pupal genital disc. We show that, in both events, scaf loss of function mimics JNK over-activity. Moreover, scaf ectopic expression aggravates the effect of the negative regulator puc on male genitalia rotation, and reduced scaf function in JNK mutant rescues dorsal closure phenotypes in the embryo. Overall our results identify the SPH scaf as new JNK target gene that restrains JNK-regulated epithelium sealing for proper Drosophila development and open new perspectives on mechanisms that could influence collective cell movements through the regulation of extracellular activities.

**P-177 Larval haematopoiesis and the cellular immune response to wasp parasitisation.**

Rami Makki1, Delphine Pennetier1, Justine Oyallon1, Joanna Krzemien1, Marie Meister2 and Alain Vincent1.
1-Centre de Biologie du Développement, UMR 5547/CNRS, Toulouse - 2-Institut de Biologie Moléculaire et Cellulaire, UPR 9022/CNRS, Strasbourg

Larval hematopoiesis takes place in the lymph gland (LG). The LG is composed of the “Posterior Signalling Center” (PSC) which express Collier, a medullary zone (MZ) containing progenitors and a cortical zone (CZ) composed of differentiated hemocytes: plasmatocytes (macrophages) and crystal cells (melanisation). In normal conditions, only plasmatocytes and crystal cells differentiate. A third type of hemocytes, the lamellocytes devoted to encapsulation of foreign bodies too large to be phagocytised differentiate only under specific immune conditions such as wasp infestation. We established that the PSC plays a key role in controlling the maintenance of a pool of multipotent progenitors in the LG. The role of the PSC is reminiscent of the hematopoietic “niche” of vertebrates, a micro-environment required for survival and self-renewing of Hematopoietic Stem Cells (HSC). The maintenance of a pool of multipotent progenitors which is a prerequisite for lamellocyte differentiation in response to wasp parasitisation requires to maintain JAK/STAT signalling on in progenitor cells. We show that CG14225/latran, which encodes a short cytokine receptor, is required for efficiently switching off JAK/STAT signalling in prohemocytes and allowing massive differentiation of lamellocytes following wasp parasitization. Latran antagonises the function of Domeless, the Drosophila type I cytokine-related receptor in a dose-dependent manner, via the formation of inactive heterodimers. The specific role of latran in controlling a dedicated cellular immune response via the repression of JAK/STAT signalling raises the question of whether short, non signalling receptors could also control specific aspects of vertebrate immunity.

**P-178 Regulation of Arf activity during myoblast fusion.**

**Dottermusch, Christine**; Renkawitz-Pohl, Renate; Önel, Susanne-Filiz
Philipps-University Marburg, Developmental Biology, Karl-von-Frisch-Str 8, 35043 Marburg, Germany

The bodywall musculature of Drosophila consists of 30 multinucleated muscles per hemisegment, which arise through sequential fusion of mono-nucleated myoblasts. Fusion takes thereby place between two types of myoblasts, Founder cells (FCs) and Fusion competent myoblasts (FCMs). The FCs determine the fate of the resulting muscle, e.g. shape, size and attachment site. In contrast, FCMs represent a more uniformly myoblast population which provide the mass to the muscles. During the last years, a variety of components have been identified to play a role in myoblast fusion in Drosophila. There are members of the Ig-domain containing transmembrane receptors which mediate the recognition and adhesion of myoblast. Interestingly, the receptor proteins Duf/Kirre and Sns are organized in a ring-like structure at points of cell-cell contact. The center of this ring contains F-actin. This adhesive structure has been previously named Fusion-Restricted Myogenic Adhesive Structure (FuRMAS, Kesper et al, 2007). Another component, which is essential for myoblast fusion is the Arf-Guanine nucleotide Exchange Factor (Arf-GEF) Schizo/Loner. Arf-GTPases are known to be important regulators of membrane trafficking and actin cytoskeleton dynamics. In Drosophila, there exist three Arf-GTPases, D-Arf1, D-Arf2 and D-Arf6. Here, we have analyzed the function of Schizo/Loner in the activation of these Arf-GTPases and discuss their relevance for myoblast fusion.

**P-179 Specification of muscle identity in Drosophila: Role of the, Col/EBF and D- MyoD transcription factors.**

Jonathan Enriquez, **Laurence Dubois**, Hadi Boukhatmi, Mathilde de Taffin, Michèle Crozatier and Alain Vincent
Centre de Biologie du Développement, UMR 5547/CNRS, Toulouse

At the onset of germ band retraction, a complex pattern of around 30 muscles starts to appear in each hemi segment of Drosophila embryo. Each muscle can be distinguished from its neighbours by, its position, size, attachment site and ultimately the innervations it received. The earliest sign of muscle diversification is the specific expression of transcription factors characteristic of each muscle or subset of muscles. It has been proposed that the distinctive characteristics of each muscle must be regulated by specific combination of these transcription factors. While many such combination have been now documented, how they control muscle specific identity remains largely unknown. The transcription factor Collier (Col), the single Drosophila ortholog of mammalian Early-B Cell Factor (EBF) and the transcription factor Nautilus the only Drosophila ortholog of the MyoD family are both expressed in muscle DA3 (Dorsal Acute 3). Within this framework, we have studied how the combinatorial activity of both Col and Nautilus control the formation of muscle DA3 and the expression of its distinctive characteristics like choice of attachment site or number of nuclei incorporated in the fibre. Finally we have studied how the combinatorial activity of Col and Nau is modulated in a segment specific way by Hox genes.

**P-180 How do muscles find and attach to their tendons?**

**Estrada Martin Beatriz**
Centro Andaluz de Biología del Desarrollo (CABD) CSIC-Universidad Pablo de Olavide Carretera de Utrera Km. 1 41013 Sevilla, Spain

The molecular mechanisms underlying muscle guidance, migration and attachment to their tendon cells are poorly understood both in vertebrates and Drosophila. I am using the development of the embryonic Drosophila myotendinous junction as an in vivo model system to study these mechanisms. During muscle attachment formation tendon cells serve not only as site of adhesion but also as signaling cells, therefore there is a reciprocal communication between these two cell types and coupling of their development. I am developing an in vivo high resolution microscopic assay to visualize myotendinous junction morphogenesis in real time both in wild type and in different genetic backgrounds. In particular, I am interested in analyzing the dynamics of muscle filopodia which may be important for the muscles to interact with their environment. One of these backgrounds being mutant muscles for the gene perdido, (perd), which we recently identified to be essential for the formation of proper muscle projections and stable attachments to the epidermal tendon cells. Perd encodes a conserved single-pass transmembrane cell adhesion protein that contains laminin globular domains and a small intracellular domain with a C-terminal PDZ-binding consensus sequence. We proposed that Perd regulates projection of myotube processes and subsequent engagement with the tendon cells by priming formation of a protein complex at the muscle membrane. I am currently investigating the interaction between perd and the different integrin subunits which are also involved in the morphogenesis of the myotendinous junction.of the embryonic Drosophila myotendinous junction as an in vivo model system to study these mechanisms.

**P-181 Drosophila Mig-10/Riam/Lamellipodin regulates actin cable formation during oogenesis.**

**Huelsmann, S** (1); Johnson, CB (2); Beckingham, KM (2); Brown, NH (1)
(1) Gurdon Institute, University of Cambridge, UK (2) Dept. Biochemistry and Cell Biology, Rice University, USA

Cells control their cell shape during their lifetime and this control is important during development as well as during homeostasis of an organism. The actin cytoskeleton, working with cell adhesion receptors, has a crucial role in specifying cell shapes. The mechanisms by which cells regulate their different actin structures are largely unknown. We are using the formation of actin cables during Drosophila oogenesis to uncover the mechanisms by which a new actin structure is formed: in nurse cells, actin cables arise just before 'dumping', when nurse cells start to expel their content rapidly into the oocyte, and they are thought to hold the nucleus in the centers of contracting nurse cells during dumping. We found that Riam, sole homologue of MRL (Mig-10, Riam, Lamellipodin) proteins in Drosophila, is a key player regulating the formation of actin cables in nurse cells. Nurse cells mutant for riam lack actin cables, their nuclei clog the ring canals during dumping, and they fail to complete dumping. In wild type, RiamGFP localises at the membrane of nurse cells at the barbed end of each actin cable. These findings indicate that Riam is involved in the organisation of actin cables. Consistent with this, the actin elongation factor Enabled (Ena) colocalises with Riam at the barbed ends and Ena's localisation depends on Riam function but not vice versa. These data suggest that Riam provides a mechanism to specify the sites where actin cables form. In other cells, this mechanism of localised actin polymerisation may regulate cellular processes, such as formation of filopodia or lamellipodia during e.g. axon outgrowth. Currently we are analysing how Riam becomes localised, and examining whether regulating Riam triggers the precisely timed appearance of actin cables.

**P-182 Regulation of toy, a Drosophila Pax6 gene.**

**Jacobsson, L**; Skottheim-Honn, J; Rasmuson-Lestander, Å
Department of Molecular Biology, Umeå University, S-901 87 Umeå, Sweden

There are two Pax6 genes in Drosophila, eyeless (ey) and twin-of-eyeless (toy). They encode transcription factors that are important for the correct development of head structures of flies, a function that is conserved also in humans. Heterozygosity in humans leads to eye abnormalities known as aniridia, homozygous mice are lacking eyes and in severe mutants in Drosophila, the whole head is missing. This indicates that Pax6 is not only involved in eye formation and development but in formation of the central nervous system as well. Pax proteins also play important roles in the development of the peripheral nervous system and sensory organs. It has been shown that Toy regulates Ey transcription and three Toy binding sites in an eye-specific enhancer have been identified within the second intron of the ey gene. In this study, our focus is on how the toy gene is regulated since that is still an open question. Toy is expressed very early during development (stage 5, 2-3 hrs after egg laying) and it is conceivable that early transcription factors, like maternally deposited proteins or gap gene products are involved. By the use of a betagalactosidase reporter, transgenic fly lines carrying intron sequences of the toy gene have been investigated. By using a Toy specific antibody, we also study the expression of Toy in various mutant backgrounds to find out which other genes are involved in the temporal and spatial regulation of Toy.

**P-183 Specific activities of epithelial complexes and junctions during morphogenesis: the contribution of crb and the SAR to tracheal development.**

**Letizia, A.** and Llimargas, M.
Department of Developmental Biology Institut de Biologia Molecular de Barcelona (IBMB), CSIC

The tracheal tree is a pre-polarised tissue with an ectodermic origin. Tracheal morphogenesis involves a dramatic remodelling of the tissue, but remarkably, during this remodelling, the tracheal tissue maintains its integrity and polarity. Polarity and adhesion in ectodermal cells depend on protein complexes that accumulate at the apical part of the membrane. In particular, there is a Zonula Adherens (ZA), composed of Adherens Junctions (AJs), which forms a circumferential adhesion belt. Basal to ZA, there are the Septate Junctions (SJs), which establish and maintain a transepithelial diffusion barrier. Apical to the ZA there is a region known as Subapical Region (SAR), required for the establishment of apico-basal polarity The maintenance of tracheal polarity and epithelial integrity during morphogenesis poses at least two interesting questions. On the one hand, whether and how these cell complexes play specific roles during tracheal development. On the other hand, how are these cell complexes maintained and remodelled during tracheal development. We have been approaching these questions in our lab. The results we and other labs have obtained indicate that the epithelial junctions play specific roles in tracheal development: SJs play a role in controlling organ size; and AJs maintain epithelial integrity and their modulation plays a role in cell rearrangements. Less is known about SAR function in tracheal development. To overcome this lack of information we are analysing 1)the pattern of gene expression and protein accumulation of SAR components to determine a possible modulation, 2)the tracheal requirements of selected SAR proteins (namely crb), and 3)genetic interactions with other genes affecting similar tracheal processes in order to integrate the function of SAR

**P-184 Sec24-dependent secretion drives cell-autonomous expansion of tracheal tubes by promoting apical membrane growth.**

Förster, D; Armbruster, K; **Luschnig, S**
Institute of Zoology, University of Zurich, Winterthurerstr 190, CH-8057 Zurich, Switzerland
Life Science Zurich Graduate School, University of Zurich, CH-8057 Zurich, Switzerland

The dimensions and shapes of epithelial tubes are tightly controlled to ensure the functions of many organs. However, despite the significance of these processes for development and disease, the cellular mechanisms controlling tube size and shape are only beginning to be understood. We are using the Drosophila tracheae as an accessible model to study tube morphogenesis at the cellular level. The embryonic tracheae expand rapidly in length and diameter to give rise to tubes of stereotyped size and shape. Secretory activity of tracheal cells was previously shown to be essential for this expansion and for the deposition of an apical extracellular matrix (aECM) inside the tracheal lumen. However, the relevant secreted molecules and their mode of action, as well as the nature of forces acting in tube expansion are not known. In an EMS mutagenesis screen for genes involved in embryonic tracheal morphogenesis we identified several components of the early secretory apparatus, including proteins involved in trafficking between the ER and the Golgi apparatus. We used mutations in the sec24 gene stenosis (sten), encoding a cargo-binding subunit of the COPII complex, to dissect the cellular mechanism of tracheal tube expansion. We show that sten-dependent secretion drives tube expansion in a cell-autonomous fashion by promoting apical membrane growth, while luminal components are not required. Thus, tube expansion is driven by a cell-intrinsic morphogenetic program rather than by extrinsic cues. These novel findings suggest a key role of membrane-associated proteins and imply a mechanism that coordinates individual cells during epithelial tube expansion.

**P-185 The dynamics of flight muscle sarcomere development.**

**Zacharias Orfanos** and John C. Sparrow
University of York, UK

Differentiation of striated muscle involves the organisation of muscle proteins into the myofibrils. These are serial assemblies of the large macromolecular contractile complexes known as sarcomeres. The Drosophila indirect flight muscle (IFM) sarcomeres have an extremely regular structure, providing a unique system for studying striated muscle differentiation. Using a GFP protein trap of a Z-disc protein we followed the dynamics of sarcomere assembly in the IFM, confirming earlier EM studies that once the initial sarcomeric precursors appear in the muscles at 30-36 hours after puparium formation (APF), all sarcomeres develop in synchrony across all the indirect flight muscle fibers (both DLM and DVM). Having described the dynamics of the process in the wild-type, we investigated the development of mutant phenotypes; we have shown that in the absence of IFM-specific ACT88F actin (Act88F6 null mutation) the Z-disk precursor arrays appear early on, suggesting that another actin isoform may be responsible, but then disappear, arguing that expression of ACT88F actin is normally required for further sarcomere development. In the absence of sarcomeric myosin heavy chain (Mhc7; IFM-specific Mhc null) some early, delayed, sarcomere elongation occurs, and although mature sarcomere lengths are never achieved the widening of Z-discs and myofibrils does occur. This argues that thick filament assembly is required for sarcomere elongation, but not for widening. Using antibodies (immunofluorescence) and sarcomeric protein GFP traps we have investigated the dynamics of flightin and myosin assembly into developing sarcomeres. Our results support and add to the premyofibril model of sarcomere assembly.

**P-186 The translational repressor Cup promotes germ plasm accumulation and germ cell development at the posterior pole of Drosophila embryo.**

**Ottone C** a,b, Giangrande A c, Graziani F d, Verrotti AC a,e
a CEINGE-Biotecnologie Avanzate/ b SEMM-European School of Molecular Medicine, Naples, Italy; c Institute of Genetics and Molecular and Cellular Biology, IGBMC/CNRS/ULP/INSERM, Strasbourg, France; d I

In metazoans, germ cell specification, formation, and maintenance are essential processes that begin early in development. Drosophila germ cell specification commences during oogenesis when a specialized cytoplasm, named germ plasm and containing maternal mRNAs and proteins necessary for abdomen induction and germ-line formation, is assembled at the posterior tip of the developing oocyte. Staufen and Oskar proteins are key germ plasm components: Staufen directs the localization, stabilization, and translation of oskar mRNA to the posterior pole of the oocyte during stage 8-9, while Oskar recruits all the downstream germ plasm components. Our work shows that the translational regulator Cup is a new germ plasm component capable to associate with Oskar and Staufen. In particular, Cup interacts with Oskar in yeast, by performing two-hybrid system analysis, and in vitro, by performing co-immunoprecipitation experiments. We further demonstrate that Cup co-localizes with Oskar and Staufen in vivo. Moreover, cup mutant ovaries have reduced levels of Oskar and Staufen at the posterior pole of stage 10 oocytes. Taken together our data suggest a novel role of Cup during germ plasm assembly in the Drosophila egg chamber.

**P-187 Control of larval haematopoiesis: cell lineages of the lymph gland.**

**Justine Oyallon**[1], Joanna Krzemien[2], Delphine Pennetier[1], Michèle Crozatier[1] and Alain Vincent[1]
1-Centre de Biologie du Développement, UMR 5547/CNRS, Toulouse, France 2 Present address: Department of Zoology, University of Cambridge, Cambridge

Larval hematopoiesis takes place in a specialised organ, the lymph gland. Two types of hemocytes differentiate in 3rd instar larvae in normal conditions: plasmatocytes (macrophages) and crystal cells (melanisation). A third type of hemocytes, the lamellocytes, are devoted to encapsulation of foreign bodies too large to be phagocytised and only differentiate under specific immune conditions such as wasp parasitism. The lymph gland is composed of a cortical zone (CZ) where hemocytes differentiate, a medullary zone (MZ) containing immature pro-hemocytes and a “Posterior Signalling Center” (PSC). We have previously shown that the PSC plays a key role in the maintenance of a pool of multipotent progenitors, which is a prerequisite for lamellocyte differentiation in response to wasp parasitism. The role of the PSC is reminiscent of the hematopoietic “niche” of vertebrates, a micro-environment required for survival and self-renewing of Hematopoietic Stem Cells (HSC). To better understand the communication between progenitors and their micro-environment, we performed lineage analyses. Our results show that distinct pools of progenitors are restricted to a plasmatocyte or crystal cell fate early during larval development while the lineage restriction between PSC cells and other LG cells is already established in embryos. A genome-wide analysis is in progress to identify new genes expressed in the medullary zone and/or the PSC and better characterise the mechanisms involved in the maintenance of pro-hemocytes and the segregation of the different hemocyte lineages.

**P-188 Wing heart muscle differentiation depends on the Drosophila bHLH transcription factor Hand.**

Tögel1, M; Lehmacher1, M., Pass, G.; **Paululat, A.**
1 University of Osnabrueck, Department of Zoology, Barbarastrasse 11, D-49069, Osnabrück, Germany 2 University of Vienna, Department of Evolutionary Biology, Althanstrasse 14, A-1090, Vienna, Austria

The Drosophila wing hearts are two small pumps that are essential for hemolymph circulation in the wings. They are located in the lateral angles of the scutellum and consist of parallel muscle cells as well as a thin non-muscular cell layer. In a previous study (1), we identified the embryonic progenitors of the wing hearts which are eight cells arising in the cardiac mesoderm. By a combination of genetic methods, live cell iamging and laser assisted cell ablation we were able to demonstrate, that wing hearts are crucial for the maturation process of wings. Essentially, animals lacking wing hearts, or lacking wing heart functionality exhibit severe wing malformations that finally results in flightlessness of the manipulated animals (1). Each wing heart consist of parallel syncytial muscle cells and a thin layer of non-muscular cells (2). Both tissues are characterized by the continuous expression of the cardiogenic bHLH transcription factor Hand throughout their entire development (3). Here we show that Hand is required for proper wing heart formation during metamorphosis. (1) Tögel, M., Pass, G. and Paululat, A. (2008) The Drosophila wing hearts originate from pericardial cells and are essential for wing maturation. Developmental Biology 318: 29-37. (2) Lehmacher, C., Tögel, M., Pass, G. and Paululat, A. (2009) The Drosophila wing hearts consist of syncytial muscle cells that resemble adult somatic muscles. Arthropod Structure and Development 38: 111-123. (3) Sellin, S., Albrecht, S., Kölsch, V. ? and Paululat, A. (2006) Dynamics of heart differentiation, ? visualized utilizing enhancer elements of the Drosophila ? melanogaster bHLH transcrition factor Hand. Gene Expression Patterns 6(4): 360-375.

**P-189 Genomics analysis of Sox100B during embryogenesis and testis development.**

**Phochanukul, N**; Meadows, L; Russell, S;
Department of Genetics and Cambridge Systems Biology Centre, University of Cambridge, Downing Street, Cambridge, CB2 3EH, UK

The transcription factor, Sox9, is involved in sex determination and testis development in mammals. Recent work reveals that Drosophila Sox100B, the fly homolog of Sox9, is expressed in male-specific cell types during late embryogenesis. Later, the expression of Sox100B is detected in larval gonads and is maintained throughout the post-embryonic stages. In Sox100B mutants the testis is malformed and fails to undergo proper morphogenesis, which is likely due to the loss of testis pigment cells; however the ovary is comparatively normal. It is worth noting that an excessive amount of larval fat body is found in both male and female mutants hinting at a defect in fat metamorphosis. These observations suggest that Sox100B and Sox9 may have related functions in testis development. Although Sox100B mutants do not have detectable embryonic defects, using genome-wide approaches we have identified a set of genes transcriptionally altered in Sox100B loss and gain of function conditions. In addition, we have generated tissue-specific gene expression profiles from testis and fat body of Sox100B mutants, identifying genes implicated in sexual development and lipid metabolisms. Complementing these studies, we have performed chromatin immunoprecipitation (ChIP)-array experiments in embryo and in 0-hr pupal testis. The ChIP-array data demonstrate that Sox100B occupies hundreds of regions in the genome. Interestingly, we observe that Sox100B binds at different regions of some genes in embryo and testis, suggesting activity at tissue-specific enhancers. Combining results from the gene expression profiles and ChIP-array experiments provides insights into Sox100B functions, which contribute to an understanding of the evolutionally conserved roles of the vertebrate Sox9 and other Sox genes.

**P-190 FuRMAS: signalling and restrictinf myoblast fusion in Drosophila.**

**Renate Renkawitz-Pohl**, Susanne Önel
Philipps-Universität Marburg, Fachbereich Biologie, Entwicklungsbiologie Karl-von-Frisch-Str. 8 D -35043 Marburg Email: renkawit@staff.uni-marburg.de

In higher organisms, myoblast fusion is essential for muscle development and repair (in vertebrates). In Drosophila, chemoattraction and heterologous cell adhesion between founder/growing myotubes and fusion competent myoblast leads to formation of complex structure which we named FuRMASs (Fusion Restricted Myogenic Adhesive Structures). We proposed that these dynamic and transient FuRMASs are pivotal in linking cell adhesion to local signaling and Factin accumulation. Upon successful cell adhesion, actin plugs/foci accumulate at the sites of contact. Blow, Kette/HEM, WASp, WAVE, Verpolin and the Arp2/3 complex as has been shown by several groups regulate actin dynamics. At the ultrastructural level, characteristic features are paired vesicles (accumulate in an area of 1_m2) and electron dense plaques (up to 500 nm) along the apposed cell membranes (Doberstein et al., 1997). Multiple small fusion pores are formed, residual membrane vesicles disappear and a large fusion pore is formed, the FCM is then integrated into the growing myotube. We will present a model, which integrates the FuRMASs and the features observed at the ultrastructural level. Ongoing work addresses the central question how membrane breakdown is initiated and controlled. We further will discuss that the FuRMAS share structural and regulatory features with other transient adhesive structures such as the immunological synapses, podosomes and invadopodia.

**P-191 The Drosophila melanogaster homolog of the LDL receptor Megalin functions in the formation of the adult cuticle and is required for the cellular uptake of Yellow.**

**Riedel, Falko**; Vorkel, Daniela; Eaton, Suzanne
Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307 Dresden, Germany

The various structures of the insect cuticle are formed by the processing and assembly of cuticle components, which are secreted apically by epithelial cells. We study the function of the Drosophila melanogaster homolog of Megalin (Mgl), an endocytic receptor belonging to the LDL receptor family, in the formation of the adult wing cuticle. In the cells of the developing wing, Mgl localizes to apical punctate structures. RNAi-mediated knock-down of mgl causes reduced pigment formation of wild type, but also of pigmentation mutant wings. In contrast, the levels of Yellow, a secreted protein required for the formation of black cuticle pigments, are increased in mgl RNAi wings. We show that Yellow can be taken up by wing epithelial cells. It localizes to intracellular punctate structures only in cells expressing mgl. Furthermore, mgl and yellow genetically interact, suggesting that both function in the same cuticle-associated process. In addition to the pigmentation defect, mgl RNAi causes wing fragility and splitting of the procuticle indicating a sclerotization defect. Both sclerotization and pigmentation are extracellular processes, which depend on the oxidation of the catecholamine dopamine catalyzed by a phenoloxidase. We present evidence that the levels of dopamine are not reduced in mgl RNAi wings. Therefore, to study a possible function of mgl in the utilization of dopamine, we identify the phenoloxidase required for sclerotization and pigmentation of the fly cuticle. We show that this enzyme localizes to the cuticle, but that its secretion does not depend on mgl. These data suggest that mgl functions in cuticle formation by regulating the levels of extracellular Yellow and the activity of either the phenoloxidase or another as yet unidentified factor.

**P-192 dNAB interacts with Brinker to eliminate cells with reduced Dpp signaling.**

Oren Ziv*1, **Yaron Suissa*1,** Hadar Neuman1, Tama Dinur1, Peter Geuking2, Christa Rhiner3, Marta Portela3, Fidel Lolo3, Eduardo Moreno3 and Offer Gerlitz1
1Developmental Biology and Cancer Research, IMRIC, The Hebrew University-Hadassah Medical School, Jerusalem, Israel. 2Institut fur Molekularbiologie, Universitat Zurich, Switzerland. 3Spanish Nationa

The proper development of tissues requires morphogen activity dictating appropriate growth and differentiation of each cell according to its position within a developing field. Elimination of underperforming cells less efficient in receiving /transducing the morphogenetic signal is thought to provide a general fail-safe mechanism to avoid developmental misspecification. In the developing Drosophila wing, the morphogen Dpp provides cells with growth and survival cues. Much of Dpp's regulation of transcriptional output is mediated through repression of the transcriptional repressor Brinker (Brk) and therefore activation of target genes. Mutant cells impaired for Dpp reception or transduction are lost from the wing epithelium. At the molecular level, reduced Dpp signaling results in Brk upregulation that triggers apoptosis through activation of the JNK pathway. Here we show that the transcription coregulator dNAB is a Dpp target in the developing wing that interacts with Brk to eliminate cells with reduced Dpp signaling through the JNK pathway. We furtherer show that both dNAB and Brk are required for cell elimination induced by differential dMyc expression, a process that was shown to depend on reduced Dpp transduction in out competed cells. Our data provide a novel mechanism where the morphogen Dpp regulates the responsiveness to its own survival signal by inversely controlling the expression of a repressor and its corepressor, Brk and dNAB.

**P-193 The DrosophilaHox gene Deformed (Dfd/Hoxb4-d4) modulates cell adhesion and segregation within the eye-antennal imaginal disc.**

**M-A. Tiberghien**1, E. Simon3, G. Lebreton2, C. Faucher1, D. Cribbs1 and C. Benassayag1
1.Centre de Biologie du Développement ,Univ. Paul Sabatier, 118 route de Narbonne, 31062 Toulouse, France - 2.Molecular Biology Institute of Barcelona (IBMB), CSIC, Barcelona Science Park, 08028 Barcelona (Spain) - 3.Centro de Biología Molecular Severo Ochoa, CSIC, Univ. Autónoma de Madrid, Cantoblanco, E-28049 Madrid, Spain

Most of the adult Drosophila head, including the eyes, ocelli, antennae and maxillary palps, derives from the composite eye-antennal imaginal disc. Within this complex rudiment, the antennal disc gives rise to two distinct appendages: the antenna (Ant) and maxillary palp (Mx), both olfactory organs. We found that the Mx territory, detectable within the antennal disc by expression of the Hox gene Deformed (Dfd/ Hoxb4-d4), is separated by lineage from cells of the Ant territory expressing the transcription factor Cut. Further, we have identified an antagonism between Cut and Dfd which may be involved in establishing differential cell properties needed to form or maintain the Mx/Ant boundary. Differential Dfd protein expression leads to a cell-sorting phenotype that appears to reflect modulated cell adhesion properties. We describe here our initial molecular characterisation of Dfd-induced cell sorting seen in cells mis-expressing Dfd. Clones harboring Dfd mis-expressing cells show rapid cell shape remodeling accompanied by clone extrusion from the epithelium, accompanied by considerable disorganization as seen by cell polarity markers. Since the Hox transcription factor Dfd/Hoxb4-d4 is required for adult mx formation, we infer that such Dfddependent cell sorting might play a role in cell sorting and segregation required for normal maxillary organ differentiation. This role for the Hox gene Dfd in Mx cell organization and affinity offers an enticing model for studying cellular Hox functions in organ morphogenesis.

**P-194 The cadherin Fat2 is required for planar cell polarity in the Drosophila ovary.**

**Viktorinová, I**; König, T.; Schlichting, K.; Dahmann, C.
Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307 Dresden, Germany

Planar cell polarity is an important characteristic of many epithelia. In the Drosophila wing, eye, and abdomen, establishment of planar polarity requires the core planar cell polarity genes and two cadherins, Fat and Dachsous. Drosophila Fat2 is a cadherin related to Fat, however, its role during planar cell polarity has not been studied. Here, we have generated mutations in fat2 and show that Fat2 is dispensable for planar cell polarity in the wing, but that it is required for the planar polarity of actin filament orientation at the basal side of ovarian follicle cells. Defects in actin filament orientation correlate with a failure of egg chambers to elongate during oogenesis. Using a functional fosmid-based Fat2-GFP transgene, we show that the distribution of Fat2 protein in follicle cells is planar polarized and that Fat2 localizes where basal actin filaments terminate. Mosaic analysis demonstrates that Fat2 acts non-autonomously in follicle cells, indicating that Fat2 is required for the transmission of polarity information. Our results suggest a principal role for Fatlike cadherins during the establishment of planar cell polarity.

**P-195 Testing whether post-translational modification of tyrosine has a role in the opposing functions of Fushi tarazu in gene regulation.**

**Bath, D**; Blachuta, B; Percival-Smith, A;
Department of Biology, University of Western Ontario, London, Ontario, Canada

Fushi tarazu (FTZ) is a homeodomain-containing, pair-rule transcription factor. FTZ activates expression of Engrailed and FTZ, and represses expression of Wingless and Even-skipped. Although three functional domains of FTZ have been identified (the homeodomain, a PEST degradation signal, and a binding site for the cofactor, FTZ-F1), we are far from understanding the complete functional organization of FTZ. Ectopic expression of FTZ in all cells at late cellular blastoderm results in a pair-rule phenotype, termed “anti-ftz???. We have used the induction of anti-ftz as an indicator of FTZ function. A deletion analysis showed that the N and C termini of FTZ are required for FTZ function, and these regions are functionally redundant. The termini are tyrosine-rich, and deletion of the tyrosines from the termini results in an inactive FTZ protein. However, surprisingly, when a string of tyrosines was attached to an inactive FTZ protein, the fusion protein acts as a repressor of all FTZ-dependent gene expression. These results suggest that tyrosines may be required for both negative and positive regulatory activity. A possible explanation for the opposing function of the tyrosines of FTZ is post-translational modification (PTM) of tyrosines to create two distinct forms of FTZ with opposing functions. In an initial approach to analyze PTM of FTZ, a tagged FTZ fusion protein was immunoprecipitated from extracts of heat-shocked embryos. Using sequence-independent antibodies raised to phosphotyrosine, sulfotyrosine, and ubiquitin, we have yet to identify any PTM of FTZ in a Western analysis. Presently, the tagged FTZ protein is being purified from embryonic extracts for analysis using mass spectrometry to conduct an unbiased test for PTM of FTZ.

**P-196 Identification of Regulators of Hedgehog Secretion by Functional Genomics.**

R. Aikin*, **A. Cervantes***, G. D'Angelo, L. Ruel, S. Lacas-Gervais, S. Schaub, P. Thérond
CNRS, UMR 6543

Hedgehog (Hh) family proteins are secreted molecules that function as organizers in animal development. Recent evidence demonstrates that the Hh signalling pathway is aberrantly activated in several types of cancer (brain, skin, lung, prostate, pancreatic) and is implicated in tumor formation as well as tumor maintenance. In addition to being palmitoylated, Hh is the only metazoan protein known to possess a covalently-linked cholesterol moiety. The importance of these modifications is underscored by the fact that the absence of either modification severely disrupts the global organization of numerous tissues during development. It is currently not known how lipid-modified Hh is secreted and released from Hh-producing cells. To gain insight into the cellular machinery required to secrete lipid-modified Hh, we have performed a genome-wide RNAi screen in Drosophila S2 cells. We identified several genes with known roles in secretion and protein trafficking. Interestingly, we demonstrate that lipid-modified Hh secretion is strongly dependent on COPI but not COPII coat proteins. We also identified several genes with no previously described role in secretion. Using a combination of immunofluorescent and electron microscopy, we demonstrate a role for some of these genes in the regulation Golgi morphology. We also performed in vivo validation of several novel regulators of protein secretion using transgenic RNAi. The current project could create new perspectives for studying how Hh secretion is regulated, as well as provide insight into the secretory pathway taken by lipid-modified proteins.

**P-197 Identification of Spalt and Spalt-related target genes in the Drosophila imaginal wing disc.**

**María, F. Organista;** Jesús, de Celis; Rosa, Barrio and Jose F., de Celis
Centro de Biología Molecular “Severo Ochoa??? and University Autónoma de Madrid Madrid 28049 Spain

The Decapentaplegic (Dpp) signalling pathway has a key role in the patterning of the Drosophila wing. One of the best-characterized Dpp functions is the regulation of the expression of the genes spalt and spalt-related (sal/salr) in the wing imaginal disc, the epithelium that gives rise to the adult wing. The sal and salr genes encode Zn-finger nuclear proteins that contribute to the positioning of the veins and the growth of the wing. The Sal and Salr proteins are though to act as transcription factors, but none of their target genes have been identified so far. We have compared the expression profiles of sal and salr mutant wing discs with control discs, with the aim of identifying candidate Sal/Salr targets. We studied by in situ hybridization the expression pattern of 150 genes which mRNA levels varied significantly comparing wild type with sal/salr mutant wing discs. From this collection we further selected 10 genes showing an expression pattern in the wing blade similar or complementary to those of sal and salr. Finally, we validated for these genes the microarray data using RT-PCR, and studied the changes in their expression patterns in different sal/salr mutant backgrounds. We are now studying whether the Sal and Salr proteins are able to bind the promoter region of some of these candidate targets using ChIP techniques. The identification of direct Sal and Salr target genes could be critical in understanding the control of growth and patterning of the Drosophila wing imaginal disc by Dpp and its downstream genes Sal and Salr.

**P-198 Dynamics of Dpp signalling.**

**Fisun Hamaratoglu**; Markus Affolter
Growth & Development Biozentrum, University of Basel Switzerland

Aim of this project is to monitor Dpp signaling dynamics in vivo. Fusing Dpp responsive elements to cDNA constructs that encode highly unstable versions of GFP or RFP will allow us to detect small and transient fluctuations in Dpp response. Dpp regulates its target genes via various means (activation versus repression; different threshold levels) and therefore every enhancer region provides us with a unique tool. In recent years, enhancers in genes that read out the Dpp morphogen gradient in the wing imaginal disc have been identified and characterized in great detail. Examples of such enhancers include regulatory elements of vestigial, optomotor blind and spalt: all three genes are activated by Dpp signaling (also indirectly via repression of Brinker) but respond to different levels of Dpp. In addition, so-called silencer elements (SEs) have been identified in the regulatory region of brinker. These SEs are short, 16 bp DNA elements that directly read out Dpp signaling levels via interaction with Mad/Med, and repress transcription of neighbouring enhancers via recruiting the large zinc-finger protein Schnurri. Furthermore, we have recently identified activating elements (AEs, similar in sequence to SEs) in enhancer regions of the dad gene. This repertoire of distinct enhancers will serve as invaluable tools in our analysis of Dpp signaling properties in vivo. These experiments aspire to achieve a detailed spatio-temporal analysis of the dynamics of Dpp signaling in growing tissues. This information will be crucial for formulating or excluding certain models of how Dpp functions in making of an organ.

**P-199 Functional analysis of the Drosophila embryonic germ cell transcriptome by RNAi.**

**Ferenc Jankovics**, László Henn, ??gnes Varga, Ildikó Velkeyné Krausz, Margit Ugrainé Szatmári, Péter Vilmos, Miklós Erdélyi
Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary

Drosophila provides a powerful experimental system for the genetic dissection and in vivo analysis of the germ line development. Embryonic germ cell development of Drosophila depends on the maternally provided germ plasm, the most posterior part of the egg cytoplasm. The germ plasm contains all the proteins and localized RNA species necessary to induce germ cell fate. Certain gene products present in the germ plasm or produced by the germ cells might play crucial role in germ cell determination and its subsequent development such as germ cell migration and gonad formation. To identify and functionally characterize genes involved in germ line development we performed a systematic, RNAi-based functional genomic screen combined with fluorescent in vivo video microscopy. First we screened RNA localization databases (BDGP, Fly-FISH) for genes whose transcripts are present or highly enriched in the germ plasm or expressed in the embryonic germ cells. This way, 516 germ line specific genes were found. To test for functional redundancy in the germ cell transcriptome, 390 gene pairs were established based on their homology, similar domain structure or common evolutional history. For the functional analysis, gene specific dsRNAs (alone or in pairs) were injected into the embryos specifically expressing GFP in the germ cells, enabling identification of embryonic germ line defects by live imaging. Altogether ca. 80000 embryos were injected, 12500 movies were generated and analyzed. Phenotypic categories were established involving abnormal germ cell migration, decreased number or absence of germ cells. We have identified 22 genes to be involved in the development of embryonic germ cells. In addition, by simultaneous silencing of genes two genetic interactions were also identified.

**P-200 Caudal, a key developmental regulator, is a DPE-specific transcription factor.**

**Juven-Gershon T.** (a,b) Kadonaga, J.T. (a)
a Section of Molecular Biology, 0347, University of California, San Diego, La Jolla, CA 92093-0347, USA b Current address: The Mina and Everard Goodman

The regulation of gene transcription is critical for the proper development and growth of an organism. The transcription of protein-coding genes initiates at the RNA polymerase II core promoter, which is a diverse module that can be controlled by many different elements such as the TATA box and downstream core promoter element (DPE). To understand the basis for core promoter diversity, we explored potential biological functions of the DPE. We found that nearly all of the Drosophila homeotic (Hox) gene promoters, which lack TATA-box elements, contain functionally important DPE motifs. In addition, the homeotic DPE-dependent core promoters are conserved from Drosophila melanogaster to Drosophila virilis. Furthermore, these observations suggested that at least some of the transcription factors that regulate the Hox gene network might be DPE-specific activators. Following this hypothesis, we discovered that Caudal, a sequencespecific DNA-binding transcription factor and key regulator of the Hox genes, activates transcription with a distinct preference for the DPE relative to the TATA box. The specificity of Caudal activation for the DPE is particularly striking when a BREu core promoter motif is associated with the TATA box. Moreover, Caudal-mediated activation of the Antennapedia P2 enhancer-promoter region as well as the Sex combs reduced enhancer-promoter region was observed to be dependent upon the DPE motifs in their respective core promoters. Taken together, these findings indicate an important role of the DPE in the regulation of the Hox genes. Our findings show that Caudal is a DPE-specific activator and exemplify the role of the core promoter in the establishment of complex regulatory gene networks.

**P-201 A study of epithelial cell delamination in the Drosophila notum.**

**Marinari E** and Baum B
University College of London

Delamination is thought to be a critical early step in epithelial tumour cell invasion and metastasis, and is an important feature of development, where it can help to remove excess cells to balance proliferation to maintain tissue homeostasis. In Drosophila, we have found that over 30% of epithelial cells lying close to the midline delaminate during the normal development of the thorax. Over an extended period, beginning 15 hours after pupariation, these epithelial cells constrict their apices, leave the epithelium basally and start blebbing, before being cleared by macrophages. Interestingly, although these cells derive from the region where the two wing discs fused during thorax closure, the choice of which cell is to leave the epithelium appears to be stochastic. We are using this as a model system in which to study the genetic and environmental triggers underlying epithelial cell delamination. Here we will describe our analysis of this process, its contribution to tissue morphogenesis, and the role of programmed cell death, tissue pressure and Jnk signaling.

**P-202 Identification of nmo-related genes in the ommatidial rotation process by differential expression analysis.**

**Muñoz-Soriano, V**; Chisman, R; Belacortu, Y; Paricio, N.
Department of Genetics, Faculty of Biology, University of Valencia, E-46100, Spain

One of the most important developmental events for the achievement of the final patterning of the Drosophila eye is the ommatidial rotation process. In this process, that involves late larval and early pupal stages, the developing ommatidia rotate 90 degrees in two 45 degrees steps. It is known that some signaling pathways like EGFR and Fz/PCP are involved in this process, however few genes have been proved to specifically affect it. One of these genes is nemo (nmo), a gene coding for a MAP-like protein kinase. nmo loss of function produces an arrest of the ommatidia at 45 degrees, suggesting an essential role of this gene in the second rotation step. Although some attempts have been made to determine whether there is a connection between nmo and the mentioned signaling pathways in the ommatidial rotation the question is still unclear. In such a scenario and to gain further knowledge on the genes affected by nmo loss of function in this process, we performed an analysis of the differential expression of nmo mutant third instard larval eye discs compared to wild type using expression microarrays. From our preliminary results we identified a total of 101 downregulated and 104 upregulated genes in nmo mutant eye discs ($p<0.05$) with respect to control. Interestingly many genes fell into functional categories which could be related to the ommatidial rotation process like those related to the synthesis of extracellular matrix components, cytoskeleton biogenesis and organization, adhesion and signaling. Validation analysis of selected genes is going on, we will present the results of the validation analysis and discuss the information that it gives regarding nmo function and the pathways in which could be involved in this specific developmental process.

**P-203 A genetic screen to identify dominant modifiers of a scabrous overexpression eye phenotype.**

Fabrice C. Durupt, Diego Santos, **Verónica Muñoz-Soriano**, Robin Chisman and Nuria Paricio
Department of Genetics, Faculty of Biology, University of Valencia, E-46100, Spain

Epithelial planar cell polarity (PCP) in the Drosophila eye is generated when immature ommatidial preclusters acquire opposite chirality in the dorsal and ventral halves of the eye imaginal disc and subsequently rotate 90° in two 45º steps towards the equator, an imaginary dorsoventral midline. Although the scabrous (sca) gene is required for the correct spacing of ommatidial clusters in Drosophila eye imaginal discs, it is also involved in the ommatidial rotation process. It was shown that while ommatidial clusters rotate more than 90º in sca mutant eye discs, sca overexpression produces an underrotation defect. Since the Sca protein is synthesized by the morphogenetic furrow and is transported through vesicles to ommatidial row 6-8, where preclusters are in the process of the second 45º rotation, it was proposed that Sca is required to stop the second rotation step. In order to identify genes functionally related to sca during this process we generated a recombinant line (sev>sca) in which Sca is missexpressed in the eye with the sev-GAL4 driver. sev>sca adult flies have rough eyes that in tangential sections show rotation defects, but also a high percentage of symmetric ommatidia. We have screened a total of □1250 RNAi lines from the NIG-Fly collection, and identified either enhancers or suppressors of the external sev>sca rough eye phenotype. These primary interactions have been validated with independent alleles of the genes affected in the RNAi lines. Among the genes identified in the screen, we find components of Notch and JNK signalling, genes involved in cytoskeleton organization or cell polarity, as well as uncharacterized loci.

**P-204 Identification of target genes of cabut, a transcription factor that acts downstream of JNK signailling during Drosophila melanogaster dorsal closure.**

Yaiza Belacortu and **Nuria Paricio**
Department of Genetics, Faculty of Biology, University of Valencia, E-46100, Spain

Dorsal closure (DC) is a morphogenetic movement which the lateral epithermal sheets migrate and fusion over the amnioserosa to form a continuous larval epithelium. There are several genes involved in this process and one of them is cabut (cbt), which encodes a C2H2 zinc finger transcription factor.cbt mutants present an anterior hole and show defects in the elongation of the dorsal-most epidermal cells as well as in the actomyosin cable assembly at the leading edge. We have previously demonstrated that Cbt is required during DC downstream of the JNK pathway, regulating dpp expression in the leading edge cells (Muñoz-Descalzo et al. 2005).To identify genes regulated by Cbt during DC (stage 10-13), we have used Genome 2.0 Affymetrix DNA microarrays. Comparison of the expression profiles of wild type vs cabut mutants embryos have led to the identification of a set of approx. 1000 genes which are either positively or negatively regulated by Cabut with a fold change of at least 1.5. We have identified genes involved in JNK signaling, cytoeskeleton dynamics or ecdysone response. Since these genes can be direct or indirect Cbt targets, we used the microarray data together with binding site and bioinformatics analysis, quantitative real-time PCR, genetics and in vivo expression analysis to identify potential transcriptional targets of Cbt.

**P-205 Omb expression is, and needs to be, graded along the anterior-posterior axis of the wing imaginal disc.**

Shen, J (a, b, c); Dahmann, C (b); **Pflugfelder, GO** (a)
a Institute of Genetics, Johannes Gutenberg University Mainz, 55128 Mainz, Germany b Max Planck Institute of Molecular Cell Biology and Genetics, 01307 Dresden, Germany c present address: Dept. of E

Decapentaplegic (Dpp), one of the best characterized morphogens, is required, among others, for dorso-ventral patterning of the Drosophila embryo and for anterior-posterior (A/P) patterning of the wing imaginal disc. In the larval wing pouch, the Dpp target genes optomotor-blind (omb) and spalt are generally assumed to be expressed in a step function above a certain threshold of Dpp signaling activity. We show that the transcription factor Omb forms, in fact, a symmetrical gradient on both sides of the A/P compartment boundary. Disruptions of the Omb gradient lead to a reorganization of the epithelial cytoskeleton and to a retraction of cells toward the basal membrane suggesting that the Omb gradient is required for correct epithelial morphology. Moreover, by analysing the shape of omb gain- and loss-of-function clones, we find that Omb promotes cell sorting along the A/P axis in a concentration-dependent manner. Graded Omb expression, therefore, appears necessary for normal cell morphogenesis and cell affinity.

**P-206 Notch signalling coordinates tissue growth and wing fate specification in Drosophila.**

**Rafel,N**; Milan,M
ICREA and Institute for Research in Biomedicine (IRB Barcelona) Parc Cientific de Barcelona, Baldiri i Reixac 10-12, 08028 Barcelona, Spain

During the development of a given organ, tissue growth and fate specification are simultaneously controlled by the activity of a discrete number of signalling molecules. We report that these two processes are extraordinarily coordinated in the Drosophila wing primordium, which extensively proliferates during larval development to give rise to the dorsal thoracic body wall and the adult wing. The developmental decision between wing and body wall is defined by the opposing activities of two secreted signalling molecules, Wingless and the EGF receptor ligand Vein. Notch signalling is involved in the determination of a variety of cell fates, including growth and cell survival. We present evidence that growth of the wing primordium mediated by the activity of Notch is required for wing fate specification. Our data indicate that tissue size modulates the activity range of the signalling molecules Wingless and Vein. These results highlight a crucial role of Notch in linking proliferation and fate specification in the developing wing primordium.

**P-207 Testing a genetic model for Sex combs reduced function in Drosophila melanogaster.**

**Sivanantharajah, L** and Percival-Smith, A
Department of Biology, The University of Western Ontario, London, Ontario N6A 5B7, Canada

Sex combs reduced (Scr) is a Drosophila Homeotic selector (Hox) gene required for establishing labial and prothoracic segmental identity. Identification of the antimorphic allele Scr14, a missense allele in the highly conserved octapeptide motif, suggests that SCR may function as a complex during proboscis and sex comb development. In our proposed model for SCR activity, SCR+ exists in a dynamic equilibrium of four forms, three of which are inactive: SCR monomer with octapeptide available for complex formation, SCR monomer with highly conserved, insect-specific C-terminal domain (CTD) preventing complex formation by masking the octapeptide, and SCR dimer. The fourth form is an active complex of two SCR molecules joined indirectly by the octapeptides, mediated by a component(s) of the transcriptional machinery. This equilibrium is disrupted by SCR14 because it forms a locked complex with other SCR molecules, preventing interaction with the transcriptional machinery. We also suggest that Scr6, a missense allele in the CTD, does not interact with Scr14 because it is hyperactive at masking the octapeptide. Three biochemical predictions of this model will be tested. First, to test if the octapeptide mediates complex formation, octapeptides will be purified from bacteria to determine if they form a complex. Second, to test if SCR14 forms a locked complex with SCR+, SCR14 peptides will be expressed in embryos to determine if SCR+ can be co-immunoprecipitated. Third, to test if the octapeptide interacts with the CTD, pull down assays will be used. This study will aid our understanding of how conserved protein domains contribute to HOX function. Since the octapeptide motif is found in most HOX proteins, complex formation may be important for the function of all HOX proteins.

**P-208 An RNAi-based screen to identify molecules required for the formation of the anterior-posterior compartment boundary in the Drosophila wing.**

**Umetsu, D**; Dunst, S; Dahmann, C
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

The subdivision of proliferating tissues into compartments is an evolutionary conserved strategy during development of insects and vertebrates. Boundaries between compartments are lineage restrictions that keep cells of distinct fates sorted during proliferation. As a result, sharp and straight interfaces between compartments are maintained. Compartment boundaries have long been recognized to position signaling centers that control patterning in diverse tissues. Signaling pathways involved in the maintenance of compartment boundaries have been identified, however, the downstream effector molecules remain unknown. In order to identify these downstream effector molecules, we developed a clone-based assay to directly analyze cell sorting at the anteriorposterior compartment boundary in the developing Drosophila wing imaginal disc. We used this assay in a large-scale RNA interference based screen. We primarily focused on genes that encode transmembrane proteins and small GTPase regulators. We have so far screened about 2,500 RNAi lines. Twelve per cent of the RNAi lines showed some defects in the properties of the clones, such as size, shape or coherence of cells. A few RNAi lines affected the segregation of cells at the anterior-posterior compartment boundary. The further analysis of these RNAi lines as well as the continuation of the screen promises to provide novel insights into the molecular mechanisms that implement cell sorting at compartment boundaries.

**P-209 Characterization of adipokine in Drosophila.**

**Arquier, N**; Leopold, P
Institut de Biologie du Développement et Cancer CNRS UMR6543 Parc Valrose 06108 Nice Cedex

In mammals, the energetic status of an organism is controlled by several complex metabolic regulations that involve different tissues such as the gut, the liver, the pancreas, the brain and the adipose tissue. The adipose tissue has two main functions and properties: firstly it is a reservoir for cellular “fuel??? and secondly it is an endocrine organ. It secretes a lot of molecules in the bloodstream that are called adipokines or adipocytokines. Among these molecules are leptin and adiponectin. Adiponectin is mainly involved in the insulin sensitivity of the cells and in the energetic homeostasis through the activation of AMPK. We have identified an adiponectin receptor homologue in Drosophila (dAdipoR) and preliminary results suggest that an adiponectin signalling is active in Drosophila.

**P-210 Nutrient Deprivation Induces Autophagy in Drosophila Ovaries.**

**Julia MI Barth** (1), K Köhler (1), J Szabad (2) and E. Hafen (1)
(1) Institute of Molecular Systems Biology, ETH Zurich, Switzerland (2) Department of Biology, University of Szeged, Hungary

Autophagy, a lysosomal-mediated degradation, promotes cell survival under starvation. In Drosophila, nutrient depletion induces autophagy in the fat body, but it remains unknown whether autophagy also plays a role in other organs responding to nutritional conditions. For example, the size and structure of Drosophila ovaries depend on the nutritional status, since reduced food or limiting growth factor signaling leads to poorly developed oocytes. Interestingly, programmed cell death occurs during oogenesis at different stages; however, mainly apoptotic events have been studied in this process. Here, we show that starvation induces autophagy in different stages and cell types of the developing ovaries in the fruit fly. Using lysotracker, fluorescently labeled autophagy-related (Atg) proteins and dAtg8 antibodies, we show that starvation induces autophagy in follicle cells. This process depends on the Atg machinery since autophagy is blocked in Atg1 mutant clones. Further, we demonstrate that also germ cells at different developmental stages undergo autophagy upon starvation. To confirm that this is Atg dependent, we created mosaic flies mutant for Atg1 solely in the ovaries. The analysis of Atg-deficient ovaries sheds light on the contribution of autophagy to ovarian development. We further show that starvation increases the lipidation of Atg8 and induces the expression of Atg genes in the ovaries. Together, this presents the first study on starvation-induced autophagy during oogenesis, and establishes the ovaries as a new model to study autophagy in Drosophila.

**P-211 Ethanolamine kinase insufficiency causes deregulation of the dSREBP pathway leading to cardiac dysfunction.**

Lim, H.--Y., **Bodmer, R.**
Burnham Institute for Medical Research

How heart function, dyslipidemia and metabolic control mechanisms are connected at the molecular-genetic level is of principal importance in a world with increased incidence of obesity. We found that the easily-shocked (eas) mutant fly exhibits abnormalities in cardiac physiology, including elevated heart rate, thinning of the heart tube and reduced cardiac performance under stress. eas encodes an ethanolamine kinase, suggesting that the cardiac deficits in eas mutants are likely due to a reduction in the level of phosphatidylethanolamine (PE), the principal phospholipid in flies, which is known to negatively regulate the sterol regulatory element binding protein (dSREBP) signaling pathway in Drosophila cell culture. Indeed, we find that cardiac-specific elevation of dSREBP function adversely affects heart function, whereas a reduction or inhibition of dSREBP signaling improves cardiac performance and rescues the cardiac disorders of eas mutants. Finally, a significant rescue of the eas mutant heart defects is also achieved by interfering with cardiac expression of the dSREBP target genes, including ATP Citrate Lyase and Fatty Acid Synthase. This genetic in vivo analysis suggests that dSREBP signaling, when aberrantly elevated in eas mutant hearts, compromises cardiac performance in Drosophila. A similar dysregulation of SREBP targets involved in lipid metabolism in mammalian cardiomyocytes may underlie cardiac disorders associated with obesity.

**P-212 Drosophila melanogaster CYP18A1: a key enzyme of molting hormone (20-hydroxyecdysone) inactivation.**

**Dauphin-Villemant, C**; Blais, C; Lafont, R; Guittard, E; Maria, A; Parvy, JP; Pasricha, S; Lumb, C; Daborn, P
1- Equipe Biogenèse des Signaux hormonaux, Laboratoire Biologie du Développement, UMR7622 CNRS, Université Paris 6, 7 Quai St Bernard, F-75005 Paris, France 2- Centre for Environmental Stress and

An essential aspect of development in multi-cellular organisms is that regulatory processes are tightly regulated to ensure appropriate cell growth and differentiation for each defined life stage. In insects, peaks of the steroid hormone 20-hydroxyecdysone (20E) evoke periodical moults and 20E is essential for coordinating growth, development or reproduction. Both increases and decreases of circulating active hormones are important for the control of hierarchical gene expression cascades. A widespread and prominent route of ecdysteroids inactivation (catabolism) is their conversion into 26-hydroxylated metabolites, and ultimately to the corresponding ecdysonoic acids. Biochemical evidence obtained in several insect species indicates that a cytochrome P450 enzyme (CYP) is involved. However the gene coding for this key enzyme of ecdysteroid metabolism had not been identified in any insect. We demonstrate here that the Drosophila melanogaster 26-hydroxylase is the product of the cytochrome P450 gene cyp18a1. When cyp18a1 is transfected in Drosophila S2 cells, extensive conversion of the active hormone 20-hydroxyecdysone (20E) into 20-hydroxyecdysonoic acid is observed, while no conversion occurs in non-transfected Drosophila S2 cells. In the same cell line, cyp18a1 endogenous expression can be rapidly induced after micromolar treatment by 20E. Further, in flies, cyp18a1 null alleles generated by excision of a P element upstream of cyp18a1, and RNAi knockdown of cyp18a1, both result in a pupal lethality phenotype. Cyp18a1 disrupted larvae show increased 20E levels. Our data show that the inactivation of 20E is important for proper development, and that Cyp18a1 is a key element in this process.

**P-213 In situ regeneration study in the Drosophila imaginal wing disc.**

**Sandra, Díaz-García**; Antonio, Baonza Cuenca
Centro de Biología Molecular “Severo Ochoa” and University Autónoma de Madrid, Madrid 28049, Spain

One of the classic problems in biology is to understand the genetic and molecular mechanisms by which the process of regeneration is regulated during development. Drosophila imaginal discs are a suitable model system for studying this process. A standard approach to study regeneration in Drosophila has been by performing “in vivo” culture of the regenerating structures. One of the problem of this method is that is impossible to reproduce the physiological condition that occur during normal development. We have developed a new method to remove a part of the wing imaginal disc inside the larva. Using this method, it is not necessary to transplant the disc afterwards. Thus, we can study the process of regeneration in its normal developmental context and to study how this process is genetically regulated. Further, the effect of the wound in the final adult structures can be assessed.

**P-214 Long-lived, starvation resistant Drosophila show dramatic changes in gene expression patterns.**

**Doroszuk, A**; Jonker, M; Pul, N; Breit, T; Zwaan, B
Evolutionary Biology, Leiden University, Netherlands; Microarray Department, University of Amsterdam, Netherlands

Although individual lifespan and rate of ageing can vary substantially in populations, the mechanisms behind this natural variability are poorly understood. In our lab, long lived, starvation resistant (SR) Drosophila melanogaster lines were obtained from a naturally polymorphic population after 20 generations of selection for increased SR. We designed a microarray experiment to address two main questions: I) what is the genetic basis of lifespan extension in SR flies? II) What is the influence of diet on age-related gene expression of Control and SR lines? We analysed SR and Control adult females cultured on three different food levels (normal diet, malnutrition and overfeeding). The analysis included two physiological age classes: middle-age and old females. We found, that control flies on normal food showed a substantial change in gene expression at different age classes (5298 differentially expressed genes, DEGs). Functional analysis of these genes identified a “reference signature of ageing??? which was dominated by a decrease of activity of reproduction-related genes with age and upregulation of immunity and stress-related genes. In contrast, SR flies showed a more uniform gene expression pattern across the age classes (168 DEGs) and their signature of ageing involved changes opposite to those observed in the Control line. Moreover, age-related gene expression of Control flies showed pronounced differences across the diets, while it was weakly affected by diet in SR flies. Overall, these results indicate that life-span extension was achieved through adjusting reproductive, metabolic and stress-response strategies at the level of gene expression. The results also demonstrate higher robustness of age-related gene expression in the long-lived genotype.

**P-215 To die or not to die: adenosine phosphorylation versus deamination.**

**Fleischmannova, J**; Simek, P; Zurovec, M.
Institute of Entomology, Biological centre, v.v.i. and Faculty of Science, University of South Bohemia, Branisovska 31, 370 05 Ceske Budejovice, Czech Republic

The role of extracellular adenosine in neurotransmission, ischemia or immune response is well known from mammals. For insects, the importance of tight regulation of the extracellular adenosine is demonstrated by the fact that flies with defects in both adenosine deamination and uptake die at larval stage. Increased level of extracellular adenosine in growth media in vitro has been previously reported to cause growth arrest and cell death for some insect cell lines whereas it is harmless for others. The aim of this study was to uncover the mechanism of adenosine toxicity and tolerance in vitro. In our experiments, we found that adenosine toxicity in “sensitive cells??? is not connected with the receptor, but instead it depends on adenosine transfer into cytoplasm (by nucleoside transporters). Once entered the cell, adenosine is incorporated into adenosine phosphates (AMP/ADP/ATP) and depending on the concentration may cause growth arrest or cell death. In “adenosine tolerant??? cell types, we also observed relatively high levels of adenosine uptake, however, in contrast to the sensitive cells, the ATP level of these cells did not increase significantly and the cells preferred deamination of adenosine to inosine that does not interfere with energy metabolism. Adenosine recycling via salvage pathway enzymes is important for cells to replenish adenosine nucleotide pool; however, it may deleteriously interfere with the cellular metabolism homeostasis at high adenosine levels. In some cell types, adenosine deamination exceeds the adenosine kinase activity to avoid toxic effects of high adenosine concentrations. Our results show that different cell types use different default pathways of adenosine utilization, which may have important role in pathological situations.

**P-216 Loss of lipoyltransferase (lipB) disrupts key metabolic pathways for energy production and leads to shortened lifespan in Drosophila.**

**Kishita, Y**; Tsuda, M; Aigaki, T
Department of Biological Sciences, Tokyo Metropolitan University, Japan

Lipoyltrasferase (LipB) is required for the process of protein lipoylation, which occurs in several enzymes involved in energy production, such as pyruvate dehydrogenase (PDH) and 2oxoglutarate dehydrogenase (OGDH). LipB transfers endogenous octanoyl or lipoyl group from octanoyl(lipoyl)-acyl carrier protein to target proteins. Most of our knowledge on LipB is based on the studies in E. coli, and little is known about the role of lipB in higher organisms. In this study, we have investigated the function of a Drosophila homolog of lipB. Phenotypic characterization was performed using lipB mutants that have piggyback insertions in the locus. We found that lipoylation of PDH and OGDH was dramatically reduced and their enzyme activities were also impaired in these flies. PDH and OGDH play important roles in glycolysis and TCA cycle: PDH connects glycolysis and TCA cycle by converting pyruvate into acetyl-CoA, whereas OGDH converts 2oxoglutarate to succinyl-CoA. HPLC analysis of the metabolites in these pathways revealed that the mutation in lipB caused metabolic disruption of glycolysis and TCA cycle and resulted in a deficit in energy production. Furthermore, the lipB mutants exhibited locomotor defects and shortened lifespan.

**P-217 painless, one trp channel involved in the mecanotransduction.**

**Senatore, S**; Vatrapu, R; Perrin, L ;Semeriva ,M; Lalevee N
IBDML CNRS UMR7216 université de la méditerranée Aix-Marseille 2

In the cardiovascular system, the cells sense diverse mechanical stimuli, which in turn induce adapted responses to modulate cell behaviour. The identity of the sensing receptors and the mechanisms by which the signal is transmitted within the cell are still matter of intense debate. We have shown that the fly cardiovascular system appears to sense and respond to mechanical forces and offers a striking example of mechanosensation. In larvae, the heart is beating with a regular frequency, with however interrupting sessions lasting a few seconds during which the heart stops beating. The interruptions are concomitant with an intense body wall muscles activity, suggesting that tensions arising from muscle activity are at the origin of the heart arrests. This observation suggests that the heart is able to sense mechanical forces and responds to this stress by blocking cardiac muscle fibres contraction. We have shown that Pain, a member of Trp family, is involved in the response of the cardiovascular system to mechanical forces. Firstly, we have confirmed that Painless is specifically expressed in the cardiovascular system. It is one of the downstream transcriptional targets of the genetic program for functional organogenesis. With loss-of-function alleles of pain, I have observed a complete loss of the heart arrests and the same mutant phenotype was reproduced by driving two RNAi lines specifically to the cardiomyocytes, confirming that the function of pain is cell autonomously required. Moreover, expression of the endogenous pain gene was therefore effective by crossing the EP-line with a heart driven Gal4 and was shown to fully rescue the heart mechanosensitive phenotype indicating that the observed cardiac phenotype is effectively due to cardiac inactivation of pain.

**P-218 Aging and phenotypic changes in D. melanogaster flies with altered Mitochondrial functions.**

Rera, M ; Monnier V. ; **Tricoire H**
Unit of Functional and Adaptive Biology (BFA), EAC7059, Université Paris Diderot-Paris 7 /CNRS , Laboratory of Genetics of Stress and of Aging, 4, rue Marie Andree Lagroua Weill Halle, 75013 PARIS, F

One of the main theories of aging is the free radicals theory that links reactive oxygen species (ROS) to cellular damages that lead ultimately to aging phenotypes. Since the main intracellular ROS production site is the mitochondrion, it is believed that this organelle plays a central role in aging. Consistent with this, in several organisms, mutations of mitochondrial components have been shown to modulate lifespan and/or oxidative stress resistance. Surprisingly, while mitochondrial genes inactivation leads to life-shortening diseases in humans, in C. elegans, mild inactivations of some genes encoding mitochondrial proteins (Mit genes), including members of the electron transport chain (ETC), increase longevity. This phenotype is dependent on both the gene inactivation level and on the stage (developmental or adulthood) at which inactivation occurs. We recently investigated whether inactivation of D. melanogaster orthologs of genes encoding components from the electron transport chain (ETC) and mitochondrial ribosomal protein could similarly modulate lifespan and/or oxidative stress resistance. We tested different levels of inactivation during development or adulthood taking advantage of the RU486 inducible GAL4GS (gene switch)/UAS system to ubiquitously express RNAi contructs targeting Mit genes. We observed pleiotropic phenotypes depending on developmental stage of inactivation, sex of individuals and inactivation levels that we will discuss in comparison with data from other organisms.

**P-219 The Effects of Extracellular Adenosine on Energetic metabolism.**

**Zuberova M** and Dolezal T
Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic

Extracellular adenosine is a stress signaling molecule. Many specific responses of the different tissues to extracellular adenosine are known, but many effects are not molecularly well characterized. We are using a Drosophila mutant in the adenosine deaminase (Adgf-a) to study these effects. The adgf-a mutant accumulates extracellular adenosine in the hemolymph which leads to developmental disorders. We have previously shown that a P-element insertion in the gamma subunits of Phosphorylase kinase (Phk-?), the main regulator of glycogen metabolism, rescues the adgf-a mutant. This suggested a connection between the adenosine signaling and the regulation of glycogen metabolism when the accumulation of extracellular adenosine would trigger an activation of the storages in an attempt to provide energy for dealing with stress. The concept of higher energy request is supported by the findings that an increase of sugars in the diet also rescues the adgf-a mutant phenotype while the decrease worsens it. We will present the results from the measure of the glycogen, glucose, trehalose and lipids contents under the different diets in the mutant and wild type larvae. Preliminary data show that the level of the glucose in the hemolymph increases in the adgf-a mutant and this effect is suppressed by blocking adenosine signaling. These results are in agreement with the concept of higher energy request in the mutant supporting the role of extracellular adenosine as a signal regulating energetic metabolism.

**P-220 Small GTPase Ral contribute to the maintenance of the follicular epithelium polarity via JNK signalling cascade.**

**Balakireva,M**; Beraud,C; Camonis,J
Institut Curie - Section Recherche, Inserm U830, Paris, France

The Ral pathway is an essential component of physiological Ras signalling as well as Ras-driven oncogenesis. In mammals, RalA and RalB have distinct function, and in Ras oncogenic transformation RalA is crucial for tumour initiation whereas RalB for transformed cell survival and metastatic progression. Drosophila melanogaster has one Ral gene and the Ral pathway is conserved in human and flies. Reduced Ral expression in Ral mutants has no effect on cell division but leads to post-mitotic cell-specific apoptosis during oogenesis and sensory organs development. Ral counters apoptotic programs by acting as a negative regulator of JNK activity. A pathway from Ral to its effector the exocyst to msn/HGK serves Ral action on JNK. The Ral-dependent restriction of JNK activation and the exocyst/HGK relationship are conserved in mammalian cells. The follicular epithelium of Drosophila ovaries was used to study the role of Ral in cell polarity. Clonal analysis revealed that loss of Ral activity causes loss of aPKC apical localization, loss of Lgl lateral localisation and apical accumulation of ???-catenin. We found that Ral function as a negative regulator of JNK activity was conserved during oogenesis and both aPKC and Lgl Ral-induced phenotypes were affected by JNK signalling cascade. A gain-of-function screen using Ral apoptotic phenotype identified new functional partners of Ral. Two of them, faf, a deubiquitinating enzyme, and Par-1, a protein involved in Bazooka localization, were selected decipher molecular players in Ral signalling in cell polarity. Surprisingly, faf loss-of-function mutants suppressed Ral-dependent loss of aPKC apical localization and Lgl lateral localisation.

**P-221 Chromatin access of transcription and remodelling factors is regulated by cytoskeletal proteins during ecdysone-triggered cell death in Drosophila.**

**Farkaš, R**1; Mentelová, L1,2; Kuchárová-Mahmood, S1; Raška, I3; Mechler, BM4
1Lab. of Genetics, Inst. of Exp. Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia; 2Dep. of Genetics, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia; 3Lab. of

At the onset of Drosophila metamorphosis the steroid hormone ecdysone activates a cell death program that leads larval salivary glands (SGs) to rapidly disintegrate about 14-16 hr after puparium formation. During this process ecdysone acts through the ecdysone receptor (EcR/Usp) heterodimer that regulates primary response genes, including the Broad-Complex (BR-C) critical for SG death. Timing of SG histolysis depends upon the level of p127l(2)gl, a cytoskeletal tumor suppressor that interacts with nonmuscle myosin II heavy chain (nmMHC) encoded by the zipper (zip) gene. Reduced l(2)glp127 expression delays SG histolysis whereas overexpression accelerates this process without affecting larval and pupal development. The p127 and nmMHC regulate chromatin access of BR-C Z, E74 and a series of remodeling factors including SIN3, RPD3 and SMRTER. In wild-type SGs, these factors bind to chromatin but in l(2)gl- they accumulate in the cytoplasm and the cortical nuclear zone and are unable to associate with chromatin. Similar chromatin exclusion can be achieved by overexpression of nmMHC and occurs also in SGs of developmentally delayed zipE(br)/+ larvae. We found genetic interaction between l (2)gl and BR-C, as well as between l(2)gl and zipE(br). Using co-immunoprecipitation we have shown that these nuclear factors can form complex with cytoskeletal proteins, supporting idea that interaction is physical. Thus, p127l(2)gl and nmMHC act jointly to control chromatin access of BR-C Z1, SIN3, RPD3, SMRTER and possibly several other factors, and in this way regulate the early stages of ecdysone cascade leading to SG histolysis. A similar mechanism may occur in l(2)gl tumour cells and can explained the partial genome silencing occurring in these cells.

**P-222 The expression of Drosophila adenosine receptor in response to different stress stimuli.**

**Fenckova M**, Dolezal T
Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic

Adenosine signaling in mammals is very important for several apparently unattached physiological processes. Adenosine can stimulate four different types of adenosine receptors and the type of response is determined by the combination of the type of the receptor stimulated, the type of cells or tissues expressing the receptor and the level of receptor expression. Ensemble of these factors can evoke different responses such as neuromodulation, heart rate, blood clotting, vasoconstriction and even the opposite, vasodilatation. Also the up- and downregulation of immune response and neuroprotection in hypoxia is controlled by adenosine signaling. The importance of adenosine signaling in Drosophila is also emerging however compared to mammals Drosophila possess only one adenosine receptor (AdoR) with very low expression levels under normal conditions. We are interested in effects involving AdoR signailing and therefore we have used different stress conditions to investigate potential changes in the expression of the AdoR gene. Genetic stimulation of immune response, oxidative stress and the accumulation of adenosine itself significantly increase the AdoR expression. On the other hand, heat shock has no effect on the AdoR expression suggesting that adenosine signaling is not involved in this type of response. The AdoR seems to be also affected by an alternative intron splicing site resulting in a putative altered translational product. The level of expression of AdoR mRNA also differs during the development.

**P-223 Characterisation of the regulatory network downstream of the Drosophila Hox protein Deformed.**

**Fuchs, A.L.**; Lohmann, I.
University of Heidelberg, CellNetworks – Cluster of Excellence and Bioquant Center, Im Neuenheimer Feld 267, Heidelberg, Germany aurelia.fuchs@bioquant.uni-heidelberg.de

One of the major discoveries in biology has been that many developmental pathways are conserved even between distantly related species. This was first realised by studying Hox genes, which specify anterior-posterior (A/P) identities in all but the most basal animals, and which were originally discovered in Drosophila through their homeotic mutant phenotypes. Hox genes, which are expressed in specific domains along the A/P body axis, code for transcription factors that assign specific morphologies to individual segments by regulating distinct sets of downstream genes in a segment-specific pattern. Despite this knowledge, the identification of Hox target genes has been hampered over the last decades due to the complexity of the regulatory networks controlled by Hox proteins together with the short and degenerate DNA sites, at which Hox proteins bind. We are focusing on the development of morphological structures controlled by the Hox protein Deformed (Dfd), which is expressed in the maxillary head segment and responsible for correct morphogenesis of the corresponding structures. We center ourselves on one specific structure, the maxillary cirri primordium, which is controlled in a feed-forward loop by Dfd and another homeobox gene, Distal-less (Dll). In order to elucidate the regulatory network downstream of Dfd and Dll, we have performed gene expression profiling experiments and found that many regulated genes are related to olfaction. This is in line with the fact that mutations in each gene alone, Dll or Dfd, are able to cause the loss of cirri, a sensory and feeding structure in the larvae, where olfaction reception is taking place. We provide insight into a Dfd downstream target and its contribution to the Dfd dependent phenotype.

**P-224 Regulation of Hedgehog pathway by the glypican Dally and the hydrolase Notum.**

**Gallet A**, Ayers K, Mteirek R, Therond P.
IBDC, CNRS UMR6543 Université de Nice, Parc Valrose, 06108 Nice Cedex2, France

The Hedgehog (Hh) family of secreted morphogens is vital to direct cell growth and patterning during development in many organisms. Furthermore, ectopic Hh signaling is implicated in developmental disorders and in the cause and maintenance of various cancers. The Hh protein is covalently modified by cholesterol and palmitic acid. These modifications confer high affinity for membranes, and so raise the question as to how Hh moves far from its source of production, to receiving cells where it binds its receptor and turns on signaling. Heparan Sulphate Proteoglycans (HSPGs), proteins which make up part of the extracellular matrix, have been implicated in morphogen movement and signaling in both invertebrates and vertebrates (Lin, 2004). Glypicans are HSPGs linked to the plasma membrane by a GPI anchor, and two homologs exist in Drosophila, Dally and Dally-like (Dlp). We are investigating the role of Dally in Hh signaling; specifically whether Dally is involved in Hh reception and what the role of Dally GPI anchor cleavage is. To better understand Dally GPI cleavage, we are studying the secreted enzyme Notum, a functional homolog of the Phospholipase-C which is thought to cleave GPI anchors in vertebrates (Traister et al., 2007), Through loss of function and overexpression studies we give evidence that both Dally and Notum are involved in Hh reception and signaling. Furthermore, we show that the function of Notum in the Hh pathway is mediated by Dally, as phenotypes caused by ectopic Notum are not displayed when preformed in a Dally mutant background.

**P-225 Rap2L function in Drosophila.**

**Grabbe, C**; Shirinian, M; Palmer, R.H.
Department of Molecular Biology, Umeå University, Building 6L, SE-901 87 Umeå, Sweden.

Among the Ras-like small G proteins, the family of Rap proteins acts as molecular switches in pathways primarily regulating cell-cell junction formation, cell adhesion to extracellular matrix and polarity. For proper regulation of Rap activity, guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs) functions to orchestrate the cycling of Rap proteins between an inactive GDP-bound and an active GTP-bound conformation. Rap proteins are conserved from man to lower vertebrates and include Rap1 and Rap2, which appear to target both shared and unique downstream effectors, with the common theme of being localized at the plasma membrane or different membrane compartments, and involve regulation of the actin cytoskeleton. The Drosophila genome encodes two Rap proteins, Rap1 and Rap2L. Whereas Rap1 is known to be essential for cell migration, multiple morphogenetic events and the establishment of cell-cell adhesion in imaginal disc, there are currently no reports addressing the function of Rap2L. By ISH analysis we have found that Rap2L, which shares 67% amino acid identity with human Rap2 family members, is expressed during all developmental stages, preferentially in the somatic mesoderm and its derivatives. We will present novel information regarding Rap2L protein expression pattern, interaction partners and mutant phenotypes.

**P-226 Groucho/TLE sets a threshold for wingless/Wnt signalling by outcompeting beta-catenin from binding Tcf/Lef1.**

Peleg Hasson1, **Tamar Harel**1, Michal Gilon2, Robert E. Goldstein1, Tama Dinur1, Uri Gat2 and Ze'ev Paroush1.
1Department of Developmental Biology and Cancer Research, IMRIC, The Hebrew University -Hadassah Medical School, PO Box 12272, Jerusalem 91120, Israel; 2Department of Cell and Animal Biology, Silberm

Upon binding of Wingless (Wg)/Wnt ligands to their membranal receptors, beta-catenin is stabilized. It then shuttles from the cytoplasm into the nucleus, where it serves as a coactivator for Tcf/Lef DNA-binding transcription factors, empowering them to induce expression of their targets. In the absence of Wg/Wnt signaling, Tcf/Lef proteins recruit the general transcriptional corepressor Groucho (Gro)/TLE. Possibly, Gro/TLE-mediated repression prevents premature expression of Wg/Wnt-responsive genes in resting cells. Here we confirm and extend the idea that, in the context of Wg/Wnt signaling, Gro/TLE represses gene expression by displacing beta-catenin off Tcf/Lef. We show that the overexpression of Gro in the wing disc leads to repression of Wg-downstream targets such as Distalless (Dll). Notably, Dll is not silenced at the D/V boundary itself, suggesting that Gro cannot block Wg target expression in places where Wg signaling is at its highest. We further demonstrate that the binding of Gro and beta-catenin to dTcf is mutually exclusive. Gro homo-oligomerization is a precondition for Gro-dependent repression in many cases, but a form of the protein that cannot oligomerize is still able to counteract activation mediated by beta-catenin and Lef1. We also show that Gro does not counteract activation of a Wnt-responsive promoter by a Lef1-VP16 chimera. Collectively, our results support the model that Gro utilizes a different repressor mechanism in silencing Wg/Wnt targets than the one it uses when in association with the majority of its partner repressors. We propose that, by competing with beta-catenin for binding Tcf/Lef, Gro/TLE enables cells receiving moderate levels of the Wnt/Wg signal to assess whether they should be turning on target gene expression or not.

**P-227 EGFR signaling induces expression of pathway target genes by downregulating Groucho-dependent repression.**

**Aharon Helman** 1, Einat Cinnamon 1, Sharon Mezuman 1, Tonia Von Ohlen 2 and Ze'ev Paroush 1
1 Department of Developmental Biology and Cancer Research, IMRIC, The Hebrew University -Hadassah Medical School, PO Box 12272, Jerusalem 91120, Israel; and 2 Division of Biology, Kansas State Univer

Groucho is a global co-repressor that is mobilized by a myriad of DNA-binding repressors to silence gene expression during the development and patterning of the Drosophila embryo and adult. Recently we have shown that Groucho is directly phosphorylated by MAPK in response to receptor tyrosine kinase signaling, and that this modification downregulates its repressor function. We now provide evidence indicating that phosphorylation of Groucho by MAPK links EGFR signaling to the expression of pathway target genes. We show that Groucho is phosphorylated in the presumptive neuroectoderm of the embryo in an EGFR pathway-dependent manner. EGFR signaling in this region drives the expression of the pathway target gene intermediate neuroblasts defective (ind), whose protein product is required for the formation of the intermediate neuroblasts column. We find that when we impede Groucho phosphorylation both the expression of ind and the differentiation of intermediate neuroblasts are blocked, phenocopying the defects caused by the loss of EGFR signaling activity. These data suggest that the EGFR pathway promotes ind expression by downregulating - via phosphorylation - the repressor activity of Groucho. Consistent with this idea, we also demonstrate that transcription of ind is restored in ras, gro double mutant embryos. Based on these results we surmise that the EGFR pathway employs phosphorylation of Groucho as a molecular mechanism for relieving transcriptional repression, a prerequisite for turning on the expression of ind and likely other EGFR pathway targets.

**P-228 Molecular mechanisms of EGF signalling dependent regulation of pipe, a gene crucial for dorsoventral axis formation in Drosophila.**

**Meike Knispel**, Martin Technau, and Siegfried Roth
Institute for Developmental Biology, University of Cologne, Germany

During Drosophila oogenesis the restriction of pipe expression to ventral follicle cells is crucial for dorsoventral axis formation. pipe codes for a sulfotransferase sulfating mucin-type glycoproteins which are secreted by ventral follicle cells and act as eggshell cues for the induction of the embryonic dorsoventral axis. A localized oocyte derived EGF signal restricts pipe expression, however the molecular details of how EGF receptor activation turns off pipe transcription in dorsal follicle cells are not known. In order to identify transcription factors acting downstream of the EGF receptor we produced follicle cell clones mutant for several candidate genes known to mediate EGF signalling in other tissues. None of these factors had an influence on pipe expression. To distinguish whether EGF signalling activates a transcriptional repressor or inactivates a transcriptional activator we have delimited a minimal cis-regulatory module in the pipe gene which recapitulates pipe expression and analyzed its structure using a combination of bioinformatics and reporter gene assays. We identified a 31 bp region required for repression of pipe which is bound by an unknown repressor protein. We are currently pursuing biochemical approaches to purify the protein which binds to the 31 bp element.

**P-229 Temporal regulation of Drosophila IAP1 determines the dual functions of caspases in the sensory organ development.**

**Akiko Koto**1, Erina Kuranaga1,2, Masayuki Miura1,2
1 Department of Genetics, Graduate School of Pharmaceutical Sciences, University of Tokyo
2 CREST, JST.

The caspases comprise a family of cysteine proteases that function in various cellular processes, including apoptosis. However, how the balance is struck between the caspases' role in cell death and their nonapoptotic functions is unclear. To address this issue, we monitored the protein turnover of an endogenous caspase inhibitor, Drosophila IAP1 (DIAP1). DIAP1 works as the E3 ubiquitin ligase and suppresses the caspase activation by directly binding to caspases and promoting its degradation. However once DIAP1 degradation is promoted, cell death is executed by activated caspases. DIAP1 degradation is thought as the main trigger to induce cell death. For this study, we developed a fluorescent probe to monitor DIAP1 turnover in the external sensory organ precursor (SOP) lineage of living Drosophila. In the pupal thorax, each SOP cell divides asymmetrically to make four types of cells that compose each sensory organ, which contain the shaft, socket, sheath cell, and the neuron. Our detailed analysis of DIAP1 protein turnover during sensory organ development showed that, depending on the cell-differentiation state, DIAP1 executes two distinct functions, one in the survival of the shaft cell, and one in its morphogenesis; both functions are exerted through the regulation of caspase activity. The precise temporal control of DIAP1 degradation is critical to keep the balance between a cellular viability and the execution of caspase's nonapoptotic functions in the sensory organ development.

**P-230 The Interaction between p127l(2)gl and Armadillo leads to a decreased formation of Arm/Tcf, activation of hid expression, and execution of cell death program in Drosophila salivary glands.**

**Mentelová, L**1,2; Kuchárová-Mahmood, S1; Raška, I3; Mechler, BM4; Farkaš, R1
1Lab. of Genetics, Inst. of Exp. Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia; 2Dep. of Genetics, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia; 3Lab. of

During Drosophila metamorphosis larval tissues, such as the salivary glands, are histolyzed whereas imaginal tissues differentiate into adult structures forming at eclosion a fly-shaped adult. The disintegration of the larval salivary glands is triggered by the steroid hormone ecdysone and takes place 15-16 hr after puparium formation (APF), although vacuolization of the cytoplasm is already noticed 4-5 hr APF, much before the activation of the death genes (12-14 hr APF). Previously we showed that disintegration of the salivary glands requires the presence of the p127 cytoskeletal protein encoded by the l(2)gl tumour suppressor gene and that the timing of histolysis displays a l(2)gl-dose response. We found that in l(2)gl salivary glands the Armadillo (Arm) protein, which is normally associated with the plasma membrane, is released in the cytoplasm where it is diffusely distributed. Immuno-precipitation and the use of the yeast-two hybrid system revealed that p127 and Arm physically interacts together. While ectopic expression of wg, which is known to stabilize intracellular Arm, significantly delays the process of cell death, sgg expression, which encodes the Shaggy/Zeste-white 3 kinase, was found to accelerate this process. RT-PCR analysis showed no expression of hid mRNA in both l(2)g salivary glands or wt glands ectopically expressing arm or wg. However, we found that sgg can induce a level of hid expression similar to that in wild type at ~10 hr APF. The occurrence of Arm/Tcf binding sites within the hid gene indicates that the wg signalling pathway can be involved in the control of cell death gene expression and our data provide the first evidence for a crosstalk between the pathways regulated by ecdysone and wingless.

**P-231 The activity of the RNA-binding protein HOW is regulated by MAPK-dependent signaling.**

**Nir, R**; Volk, T;
Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel

Held Out Wings (HOW) is a Drosophila RNA-binding protein. It controls different developmental processes including differentiation of tendons, mesoderm, muscles, and glia cells by regulating the mRNA levels and splicing profile of key regulatory factors essential for these distinct processes. As HOW protein is expressed in high levels throughout development, regulation by post-translational modifications seems to be required to allow timely alterations of its targets' mRNA levels. We show that HOW undergoes phosphorylation by MAPK in Drosophila SR+ cells. Mutating the Threonine in two possible MAPK consensus sites (phospho Threonine Proline – pTP) into Alanine (phospho mutant) abolished HOW reactivity with anti pTP antibodies, indicating that at least one of these residues is phosphorylated in SR+ cells. Specific inhibition of MAPK activity by a MEK inhibitor reduced significantly the reactivity of the anti pTP antibodies with HOW. Our preliminary results demonstrate that the phosphorylation state of HOW affects its activity in RNA degradation and regulation of splicing. In addition we are attempting to elucidate the role of this mode of regulation on HOW activity in-vivo. Significantly, an antibody raised against a small HOW peptide, containing one MAPK-dependent pTP, reacts with a subset of HOW-positive cells, mainly heart and muscle cells, indicating that HOW undergoes phosphorylation of this site in vivo. Moreover, a transgenic HOW protein, expressed in embryonic heart and muscle cells, reacts with a pTP antibody, while a phospho mutant protein does not, further supporting phosphorylation of HOW in this tissue. In summary our analysis couples HOW activity in regulation of RNA metabolism with MAPKdependent signaling in the developing embryo.

**P-232 A conserved pathway regulating apical membrane morphogenesis in Drosophila photoreceptor.**

**Pinal, N**; Pichaud, F
Laboratory for Molecular Cell Biology,Cell Biology Unit, MRC. University College London

G-protein coupled receptors (GPCRs), such as the sensory visual pigment rhodopsin1 (rh1) are part of a large family of conserved transmembrane receptors. In the fly eye, rh1 is expressed in the outer photoreceptors (R1 to R6) where it is involved in phototransduction. Rh1 is also required in late pupation, for the morphogenesis and maintenance of rhabdomere, which is the apical organelle of the photoreceptors, and that consists of a stack of microvilli. Interestingly, the Rho-GTPases Rac and Cdc42 have been shown to act downstream of rh1 in rhabdomere morphogenesis. Here we demonstrate that rh1 function during this process is conserved through evolution, with vertebrate rhodopsins being able to rescue the rh1 loss of function phenotype in the rhabdomere. Surprisingly, rh1 function in rhabdomere morphogenesis is independent of G?q activity, a key factor required for phototransduction. Furthermore, we show that phosphorylation of the C-terminal domain of rh1 is not required either, demonstrating that both the GPCR-kinases (GRK1 and 2) and ?-arrestin are dispensable for this process. Our work supports the existence of an entirely novel pathway, downstream of rhodopsin, regulating rhabdomere morphogenesis that is independent of the phototransduction cascade.

**P-233 Intracellular control of the Hedgehog pathway.**

**Ranieri N.**;Ruel L. and Therond P.P.
IBDC, Université de Nice-Sophia Antipolis, Parc Valrose, CNRS UMR6543, Nice, France

Hedgehog (Hh) is a secreted protein that controls cell growth and differentiation that is implicated in many diseases and cancer development. Most of the pathway components are conserved between vertebrate and invertebrates. We focused our attention on the two main Drosophila repressors of the pathway: Costal2 (Cos2) and Suppressor of Fused (Sufu). They both undergoes phosphorylation events during pathway activation that are very important step for the continuation of the signalling events, we believe that they are causing inactivation of both proteins to allow activation of the transcription factor and so of the target genes. To better understand the importance and the consequences of these modifications, we developed phospho-antibodies against the phosphorylation sites in both proteins. With these tools we will be able to test the proteins behaviour both in vivo and in vitro assay.

**P-234 Regulation of Drosophila HIFalpha/Sima by oxygen-dependent nuclear export.**

**Romero, NM**; Irisarri, M; Wappner, P.
Instituto Leloir and IIB, FCEyN, Universidad de Buenos Aires; CONICET. Patricias Argentinas 435, Buenos Aires (1405). ARGENTINA

The mammalian Hypoxia Inducible Factor (HIF) is a heterodimeric alpha/beta transcription factor that induces a wide spectrum of genes under conditions of oxygen deprivation. Hypoxia-inducible factor alpha (HIF) proteins are regulated by oxygen levels through several different mechanisms that include protein stability, transcriptional coactivator recruitment, and subcellular localization. It was previously reported that these transcription factors are localized mainly in the cytoplasm in normoxia, and accumulate in the nucleus upon hypoxic exposure. We have characterized the mechanism governing oxygen-dependent subcellular localization of the Drosophila HIF-alpha homologue Sima, and found that this protein shuttles continuously between the nucleus and the cytoplasm. Nuclear import depends on an atypical bipartite Nuclear Localization Signal (NLS) mapping next to the C-terminus of the protein, and nuclear export is mediated by at least tree CRM1-dependent Nuclear Export Signals (NESs). Two of these NESs map to the basic helix-loophelix (bHLH) domain and the other one is localized at the oxygen-dependent degradation domain (ODDD). Site-directed mutagenesis of either NES provoked Sima nuclear retention and increased transcriptional activity. We explored the mechanism that accounts for oxygen-dependent regulation of Sima subcellular localization, and found that both hydroxylation proline 850 in the ODDD and the activity of the E3 ubiquitin ligase subunit VHL are absolutely required for CRM1-dependent nuclear export. We propose that Sima nucleocytoplasmic localization is the result of a dynamic equilibrium between nuclear import and nuclear export, and that the cellular machinery responsible of oxygen-dependent Sima proteolysis also mediates regulation of nuclear export.

**P-235 A novel role for Abd-B during Drosophila Left-Right axis establishment.**

**Coutelis, JB**; Suzanne, M; Noselli, S
IBDC NICE 6 UMR6543/CNRS, University of Nice Sophia Antipolis, Nice, France

Proper animal organogenesis requires correct establishment of the Left-Right body axis. It is apparent in the asymmetric positioning of certain single organs, in the sided dissimilarities of bilateral organs or in the oriented looping of tubular organs. Drosophila melanogaster possesses such Left-Right asymmetric features, for instance, the complex looping of the embryonic gut or the dextral coiling of the spermiduct around the hindgut in male adult flies resulting from the 360degree clockwise rotation of the genital plate. Their orientation depends on the activity of a single gene which codes for the type ID unconventional myosin, Myosin ID (myoID). In myoID null flies, the orientation of the Left-Right axis is fully reversed revealing an underlying sinistral activity only apparent in this mutant context. To identify new members of the Left-Right determination pathway
(s) and to shed light on how MyoID exerts its function, we screened a collection of genomic deficiencies covering individual regions of the second, third or fourth chromosomes crossed to myoID sensitized background. Using this approach we identified several genomic loci genetically interacting with myoID either as suppressor (sinistral pathway) or enhancer (dextral pathway). Here we present our identification of the Hox gene Abdominal-B (Abd-B) as a major upstream regulator of Left-Right determination in Drosophila. This role appears separate from that of Anterior-Posterior specification.

**P-236 Mutation in adenosine receptor decreases the frequency of warts tumorous mosaic clones.**

**Sidorov, R**.; Kucherova, L.; Zurovec, M.
Institute of Entomology, Biology Centre, ASCR, v.v.i.

The mutation in adoR influences cell survival in somatic mosaic clones. When combined with a recessive mutation in a tumor suppressor warts, it causes more than 10-fold decrease of the wts tumor clone frequency in adoR wts/ + + heterozygotes compared to pure wts/+ heterozygotes. The effect was observed for different types of chemically induced (by paraquat or rotenone, causing mutagenesis via oxidative stress and cisplatin, a direct DNA-binding mutagen), as well as spontaneous, mosaicism. The effect of the mutant adoR is cell-autonomous, as there is now significant difference of the tumor frequency in flies with adoR wts / + + (cells surrounding an adoR wts homozygous clone are adoR heterozygous in this case) and adoR wts / adoR + (all cells of the fly are adoR homozygous) genotypes. For them both, the low level of wts tumor clones is characteristic. In contrast, in trans-diheterozygotes, producing twin wts and adoR clones in adoR heterozygous surrounding, the frequency of tumorous wts clones is high, comparable to the level in wts/+ heterozygotes without adoR mutation. Introduction of the adoR[+] expressing transgenic construct to the second chromosome provides a slight rescue of the tumor frequency (nearly 2folds above the level in adoR wts mutants without the transgene).

**P-237 Protein kinase CK2 as a checkpoint in intracellular signalling.**

**Stark, F**; Jauch, E; Raabe, T
Institut für medizinische Strahlenkunde und Zellforschung

The protein kinase CK2 is a heterotetramer consisting of two catalytic ?-subunits and two regulatory ?-subunits, which are ubiquitously distributed in eukaryotic cells. Among its multiple cellular functions, CK2 is known to be involved in cyclic events like circadian rythm and cell proliferation. The fact that its kinase activity is higher in proliferating cells compared to resting and differentiated cells raises the question about its regulation. Till now there is not much known about the exact regulatory mechanisms of CK2. In general, it is clear that the signalling level mediated by CK2 can be influenced by localisation, expression levels, assembly and covalent modification of the kinase as well as by interaction with proteins and other molecules. Our work focuses on the regulation of CK2 mediated by interaction with metabolic intermediates of the polyamine pathway and its effect on cell signalling.

**P-238 Larval translucida (ltl) is a novel feedback regulator of DPP/BMP signaling during Drosophila wing disc development.**

**Milan Szuperak**, Sally Salah, Emily J. Meyer, Aissam Ikmi and Matthew C. Gibson
Stowers Institute for Medical Research

The Bone Morphogenetic Protein (BMP) signaling pathway plays a central role in controlling growth and epithelial morphogenesis throughout the metazoa. In the developing Drosophila wing disc the BMP2/4-like ligand, Decapentaplegic (DPP), forms a morphogen gradient orthogonal to the tissue A/P axis, establishing distinct territories of differential gene expression. At present, the best-studied target genes are transcription factors, while the DPP/BMP effector molecules that ultimately regulate proliferation and morphogenesis remain poorly understood. To identify such genes, we performed a microarray screen, comparing DPP-deprived epithelial cell clones to wild type controls. This approach identified putative DPP target genes exhibiting specific expression patterns in the wing imaginal disc. Here we report the functional analysis of one such gene, larval translucida (ltl), which encodes a novel Leucine Rich Repeat protein with no clear homologues outside the class of insects. The expression pattern of ltl mRNA and protein precisely mirrors the phospho-Mad activity gradient in the wing imaginal disc, suggesting that ltl is a direct and threshold-independent transcriptional target of the DPP/BMP signaling pathway. Knockdown of ltl strongly disrupts larval development and affects wing growth and patterning. Intriguingly, Gal4/UAS-mediated overexpression of ltl specifically inhibits the DPP-dependent process of crossvein formation during wing morphogenesis and also affects wing growth. Together, these findings suggest that ltl encodes a secreted protein that participates in an autoregulatory feedback loop in the DPP/BMP signaling pathway in the developing imaginal wing discs.

**P-239 The Molecular Mechanisms of D-CBL.**

**Wang, P.Y.**; Pai, L.M.
Institute of Basic Medical Sciences, Chang Gung University, Tao-Yuan, Taiwan.

The Cbl family of proteins plays a role in downregulation epidermal growth factor receptor (Egfr) signaling through the internalization-mediated destruction. The Cbl proteins contain two functional domains in the N-terminus, a Ring finger domain with E3 ligase activity and a phosphotyrosine binding (PTB) domain to bind substrates. The proline-rich domain and ubiquitin association (UBA) domain are found at the C-terminus of D-Cbl-L, but that are absent in D-Cbl-S. We previously found that the lack of D-cbl in eggs and wings results in phenotypes characteristic of Egfr hyperactivation. Interestingly, overexpression of D-Cbl-L, but not D-Cbl-S, results in phenotypes characteristic of Egfr hypoactivation in a dosage-dependent manner. This suggests that D-Cbl-L efficiently down-regulates Egfr signaling through the proline-rich domain. Furthermore, we demonstrated that D-Cbl-L promotes the internalization of Egfr-ligand complex, and the E3 ligase activity is required for this activity. In mammals, Grb2 has been demonstrated to play a role in promoting Egfr internalization by recruiting Cbl. Therefore, we tested whether the interaction between Drk, Grb2 homologous, and D-Cbl is critical for the effect of D-Cbl-L on Egfr. Indeed, reduction of Drk function suppressed the effect of D-Cbl-L overexpression. In addition, the dosagedependent effect of D-Cbl-L on Egfr was dramatically reduced while proline rich motif was mutated. Furthermore, we demonstrated this mutation impaired the interaction of D-Cbl-L and Drk. Interestingly, the D-Cbl-L-PRDrk mutant rescued D-cbl null mutant completely, whereas D-Cbl-L rescued partially. Taken together, these results suggest the interaction between D-Cbl-L and Drk is critical for the dosage-dependent effect of D-CblL on Egfr.

**P-240 Hedgehog morphogen gradient is shaped by Sulfatase-1 modified HSPGs during Drosophila wing development.**

**Alexandre Wojcinski**,1, Hiroshi Nakato,2, Cathy Soula,1, and Bruno Glise,1
1) Centre de Biologie du Développement, CNRS, Université Paul Sabatier, UMR-5547, Toulouse, France. - 2) Department of Genetics, Cell Biology and Development, The University of Minnesota, Minneaopolis.

Over the past decade, intensive biochemical and genetic studies have elucidated the central components of the Hedgehog (Hh) signalling pathway. However, several important issues remain to be resolved concerning the mechanisms by which the distribution and movement of Hh is regulated in morphogenetic fields. Heparan sulfate proteoglycans (HSPGs), major components of the extracellular matrix, have clearly been shown to play crucial roles in regulating Hh movement during development. HSPGs consist a core protein to which heparan sulphate (HS) glycosaminoglycan chains are linked. HS chains are characterized by a specific sulfation pattern defined during their biosynthesis that is further modified at the cell surface by extracellular endosulfatase. Our lab has recently shown that such an enzyme, called sulf1, is a modulator of Sonic Hedgehog (Shh) signalling in the developing chick neural tube suggesting that the sulfation state of HS chain may play a role in modulating Hh signalling. In order to investigate this question, we turned to Drosophila melanogaster and analyse the function of sulf1 during wing development. As a first step, we showed that sulf1 is indeed expressed in the wing imaginal disc and its restricted expression pattern corresponds to future wing vein domains in agreement with a function in modulating Hh signalling. Then, we showed that sulf1 loss and/or gain-of-function experiments in the wing disc lead to a misregulation of various Hh target genes indicating that Sulf1 play a role in Hh signalling regulation. Moreover we have established a correlation between these Hh signalling defects and a modification of apical Hh distribution. Our results allow us to propose a model where Sulf1, by modulating sulfation pattern of HSPGs and extracellular distribution of Hh, is necessary to fine-tune Hh signalling during wing development.

**P-241 In vivo analysis of cell death signal during reproductive organ development.**

**Erina Kuranaga**, Masayuki Miura
Department of Genetics, Graduate School of Pharmaceutical Sciences, University of Tokyo, and CREST, JST, Tokyo, Japan

Programmed cell death or apoptosis plays crucial roles during development. While wellknown functions of apoptosis are sculpting and deleting structures in developmental animals, cellular function of apoptosis to coordinate the organ morphogenesis is largely unknown. Apoptosis mechanisms are highly conserved throughout evolution, and caspase activation plays central roles in execution of apoptosis. We previously developed a genetically encoded indicator for caspase activation using FRET system (SCAT3), which enables us to perform a spatiotemporal analysis of caspase activation in living animals. In Drosophila, the orientation defect of adult male terminalia has been observed in mutants of apoptotic pathway including drICE (Drosophila homolog of caspase-3), and dronc (Drosophila homolog of caspase-9). During the course of the maturation of internal genitalia, 360 degrees clockwise rotation of male terminalia is observed. The orientation defect occurs when the rotation is incomplete during development. To address the involvement of cell death in the rotation of male genitalia, we performed imaging analysis of caspase activation using SCAT3 in living animals. During male genitalia rotation, we found caspase activation and cell death occurred in the area nearby the rotating genitalia. Genetic manipulation of cell death indicated the importance of cell death for precise rotation during male terminalia development.

**P-242 The role of mitotic tissues in controlling developmental timing.**

**Andersen, DS**; Colombani, J; Leopold P.
Institute of Developmental Biology & Cancer, University of Nice Sophia-Antipolis, CNRS UMR6543, Centre de Biochimie, Parc Valrose, 06108 Nice cedex 2, France

Previous studies suggest that the larval imaginal discs play a crucial role in coordinating tissue maturation/patterning with developmental timing. Thus, transplantation of young imaginal discs into more developmentally advanced larvae delays pupal molt, allowing the underdeveloped discs to catch up with other larval tissues. In contrast, complete ablation of the imaginal discs has no effect on developmental timing. These results suggest that a signal released from imaginal discs prevents the onset of metamorphosis until patterning/maturation of the discs is complete. Reminiscent of this, wounding of discs delays pupal molt, thereby allowing damaged tissue to regenerate before the onset of metamorphosis. So far, the mechanisms by which immature/wounded discs prevent metamorphosis remains elusive. We are developing a genetic screen for the signals that couple patterning/regeneration of mitotic tissue with the developmental clock. For this purpose we set up two different conditions for which we can induce a developmental delay by manipulating disc growth. Characterization of these two lines will be presented and discussed. These two conditions are being used to screen collections of RNAi lines for candidates that can rescue the developmental delay.