

27th European Drosophila Research Conference

October 20 – 23, 2023

Lyon, France



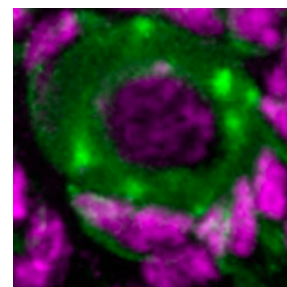
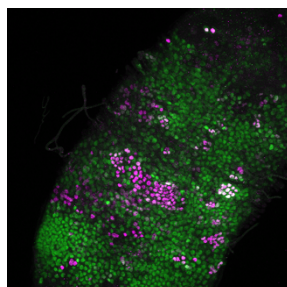
ABSTRACT BOOK (do not print)

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Workshops

Cancer models workshop



Drosophila research has a long history of contributing to our understanding of the mechanisms of cancer initiation and propagation. These studies range from molecular mechanisms of DNA repair to polarity and neoplastic growth. The goal of the workshop will be to bring together scientists interested in these very diverse aspects of modelling cancer using *Drosophila* and to create cross-discipline dialogue spanning distinct cellular contexts from stem cells to epithelial cells.

Organisers: Renata Basto & Allison Bardin (Institut Curie, Paris, France)

13h-13h15: Allison Bardin

“Nucleotide sharing through gap junctions buffers replication stress”.

Department of Genetics and Developmental Biology, Institut Curie, Paris, France.

13:15-13h30: Manon Budzyk

“Gen nuclease is essential for the proliferation of non-programmed polyploid cells”.

Basto lab, Cell Biology and Cancer department, CNRS and Institut Curie, Paris, France.

13h30-13h45: Brian Calvi

“Unscheduled endoreplication impairs the growth and function of cells and tissues”.

Indiana University, Bloomington, USA.

13h45-14h: Wu-Min Den

“Sex dimorphic and systemic regulation of tumor growth by Upd2-JAK/STAT signaling”.

Department of Biochemistry and Molecular Biology, Tulane University School of Medicine, New Orleans, USA.

14h-14h15: Kaustuv Ghosh

“Chromosomal Instability-induced Cell Invasion through Caspase-driven DNA Damage”.

Milan lab, Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Barcelona, Spain.

14h15-14h30: Tatsushi Igaki

“Non-cell autonomous tumor progression by unfolded protein response”

Graduate School of Biostudies, Kyoto University, Kyoto, Japan.

14h30-14h45: Anne-Marie Martinez

“Transient loss of Polycomb components induces an epigenetic cancer fate”.

Institute of Human Genetics, Montpellier, Montpellier, France

14:45-15h: Marta Mira-Osuna

“Contribution of septate junction components to apical and basal extrusion of protumoral cells”

Le Borgne lab, Institute of Genetics & Development of Rennes, Rennes, France.

15h-15h15: Mirka Uhlirova

“Immunosurveillance, understanding the crosstalk between immune cells and epithelial tumors”.

Institute for Genetics Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany.

Sex Workshop



20th October 2023

Organisers:

Elizabeth Rideout (University of British Columbia, Canada)

Jenny Regan (University of Edinburgh, UK)

Bruno Hudry (Institut de Biologie Valrose, France)

Program:

13:00-13:15: Elizabeth RIDEOUT

“Studying sex differences in *Drosophila*”

Department of Cellular and Physiological Sciences, Life Sciences Institute, The University of British Columbia, Vancouver, Canada

13:15-13:30: Brian McCABE

“+/-10%: Sexual Dimorphism in the larval CNS”

Brain Mind Institute, EPFL - Swiss Federal Institute of Technology Lausanne, Lausanne, Switzerland.

13:30-13:45: Deepika VASUDEVAN

“Sex-specific differences in stress response signaling”

Department of Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh Pennsylvania, USA.

13:45-14:00: Galit SHOHAT-OPHIR

“Failure to mate enhances investment in behaviors that may promote mating reward and impairs the ability to cope with stressors via a subpopulation of Neuropeptide F receptor neurons”

The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Israel

14:00-14:15: Carolina REZAVAL

“Is love blind? Mating probability gates threat perception.”

School of Biosciences, University of Birmingham, Birmingham, United Kingdom

14:15-14:30: Pau CARAZO

“Sexual selection and sexual conflict in complex environments”

Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Valencia, Spain.

14:30-14:45: Lesley WEAVER

“The *Drosophila* Estrogen-Related Receptor acts as a central regulator of oogenesis.”

Department of Biology, Indiana University, Bloomington, IN, USA.

14:45-15:00: Benjamin PRUD'HOMME

“How inter-allelic interactions shape sex-specific expression of X-linked genes?”

Aix Marseille Univ, CNRS, IBDM, Marseille, France.

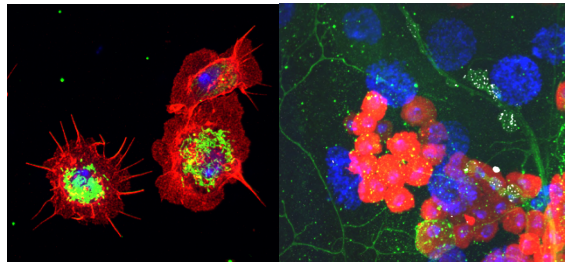
15:00-15:15: Yu XUAN

“Sex, Drugs, and Longevity”

“Immunity beyond Infection” Workshop



20th October 2023



Organisers:

Estee Kurant (Haifa, Israel)
Angela Giangrande (Illkirch, France)
Bruno Lemaitre (Lausanne, Switzerland)
Katrin Kierdorf (Freiburg, Germany)

Program:

13:00-13:15: **Caroline DILLARD**

“Role and regulation of NF- κ B -driven tumorigenesis”

Centre for Cancer Cell Reprogramming, Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, Oslo, Norway

13:15-13:30: **Parvathy RAMESH**

“Immune control of local and systemic manifestations of Colorectal Cancer in *Drosophila*”

Institute of Cancer Sciences–University of Glasgow, Wolfson Wohl Cancer Research Centre, Glasgow, UK

13:30-13:45: **Hila TOLEDANO**

“Spontaneous and massive elimination of germ cell progenitors by phagoptosis”

Department of Human Biology, University of Haifa, Israel

13:45-14:00: **Bayan KHARRAT**

“Dual role for the orthologue of HECA, Headcase, in blood cell progenitor maintenance in the *Drosophila* lymph gland”

Institute of Genetics, Biological Research Centre, Szeged, Hungary

14:00-14:15: **Sara MONTICELLI**

“Early-wave macrophages: novel string-puller of late hematopoiesis”

IGBMC, Illkirch, France

14:15-14:30: **Fabian HERSPERGER**

“Crosstalk between hemocytes and fat body regulates energy mobilization during oxidative stress”

Institute of Neuropathology, University of Freiburg, Germany

14:30-14:45: **Naden KHATEB**

“Masking phosphatidylserine in adult *Drosophila* brain prevents developmental neuronal phagoptosis and rescues neurodegeneration”

Department of Human Biology, University of Haifa, Israel

14:45-15:00: **Francesca DI CARA**

“Metabolic alteration of the gut-brain axis triggers IMD-mediated immune signaling in aging brains leading to neurodegeneration”

Dalhousie University, Halifax, Canada

15:00-15:15: **Martina MONTANARI**

“Larval microbiota primes the *Drosophila* adult gustatory response”

Institut de Biologie du Développement de Marseille, Aix-Marseille Université, Marseille, France

The Gut Workshop-EDRC 2023

Organizers: Irene Miguel-Aliaga, Imperial College London / MRC London Institute of Medical Sciences, London, UK; Julia Cordero, Institute of Cancer Sciences, University of Glasgow and CRUK Beatson Institute, Glasgow, UK.



Since its conception in 2011 'The Gut Workshop' has become one of the flagships of the EDRC. After the long COVID-imposed hiatus, we are excited to re-launch it at EDRC 2023 in Lyon. Groundbreaking work has been done in the past three years on the role of the digestive tract as a fundamental barrier epithelium and coordinator of multiorgan physiology. The goal of this workshop is to bring together the most exciting ongoing research on local and systemic functions regulated by the intestine. Our exciting program includes research that spans cellular, tissue level and organismal scale. Topics will encompass intestinal stem cell biology, including their interaction with the

microenvironment, gut/microbiome interactions and interorgan signalling between the intestine and metabolic, immune, and neuronal systems.

Talk length: 12 min talk + 3 min questions.

Speakers' list and talk titles:

Lucy O'Brien: *'Cutting loose: Mechanosensitive cell extrusions shrink the gut following food withdrawal'*

Golnar Kolahgar: *'Mechanotransduction in the stem cell niche'*

Ditte Andersen: *'Pvf1-PvR-mediated crosstalk between the trachea and the gut stimulates intestinal stem cell migration and divisions during gut regeneration'*

Sa Kan Yoo: *'Ereboris, cell death during homeostatic turnover of gut enterocytes'*

Chrysoula Pitsouli: *'The sterol transporter Npc2c controls intestinal stem cell mitosis and host-microbiome interactions in Drosophila'*

Fumiaki Obata: *'Gut Microbiome for metabolic homeostasis and inflammaging'*

Edan Foley: *'Immune Regulation of Intestinal Stem Cell Survival'*

Ryusuke Niwa: *'A high-protein diet-responsive enteroendocrine hormone regulates feeding behavior and metabolic optimization in Drosophila'*

Katerina Siudeja: *'Somatic genome instability and endogenous retroelement activity'*

Ageing Workshop



20th October 2023

Organisers:

Helena Cochemé (MRC LMS, London)

Gilles Storelli (CECAD, Cologne)



Program:

15:30-15:45: Sara AL ISSA

“Characterization of a cluster of three snoRNAs, including *jouvence*, involved in lifespan, neurodegeneration, and metabolism, in *Drosophila*”

Institute of Neurosciences Paris-Saclay (NeuroPSI), CNRS/University of Paris-Saclay, Saclay, France.

15:45-16:00: Geetanjali CHAWLA

“miR-375 regulates dietary restriction dependent enhancement of lifespan in *Drosophila*”

Department of Life Sciences, Shiv Nadar Institute of Eminence, Greater Noida, Uttar Pradesh, India.

16:00-16:15: Gaia FABRIS

“PWP1 affects aging by mediating intestinal stem cell homeostasis in a nutrient dependent manner”

Faculty of Biological and Environmental Sciences and Institute of Biotechnology, University of Helsinki, Finland

16:15-16:30: Claudia LENNICKE

“Redox regulation of autophagy extends lifespan in *Drosophila*”

MRC London Institute of Medical Sciences and Institute of Clinical Sciences, Imperial College London, UK

16:30-16:45: Polina REICHERT

“Mitochondrial redox protein quality control as a key determinant in ageing”

School of Molecular Biosciences, University of Glasgow, UK

16:45-17:00: Michael RERA

“Studying ageing as a two phases process: into Smurfness”

Centre de Recherche Interdisciplinaire, U1284, Paris, and Université Paris-Saclay, AgroParisTech, INRAE, UMR PNCA, Palaiseau, France.

17:00-17:15: Mathilde SOLYGA

“Regulation and function of ribonucleoprotein granules in the aging *Drosophila* brain”

Institute of Biology Valrose, Cote d’Azur University, Nice, France.

17:15-17:30: Wei SONG

“Renal NF- κ B activation impairs uric acid homeostasis to shorten lifespan in the context of tumors”

Department of Hepatobiliary and Pancreatic Surgery, Frontier Science Center for Immunology and Metabolism, Medical Research Institute, Zhongnan Hospital of Wuhan University, and TaiKang Center for Life and Medical Sciences, Wuhan University, Wuhan, China

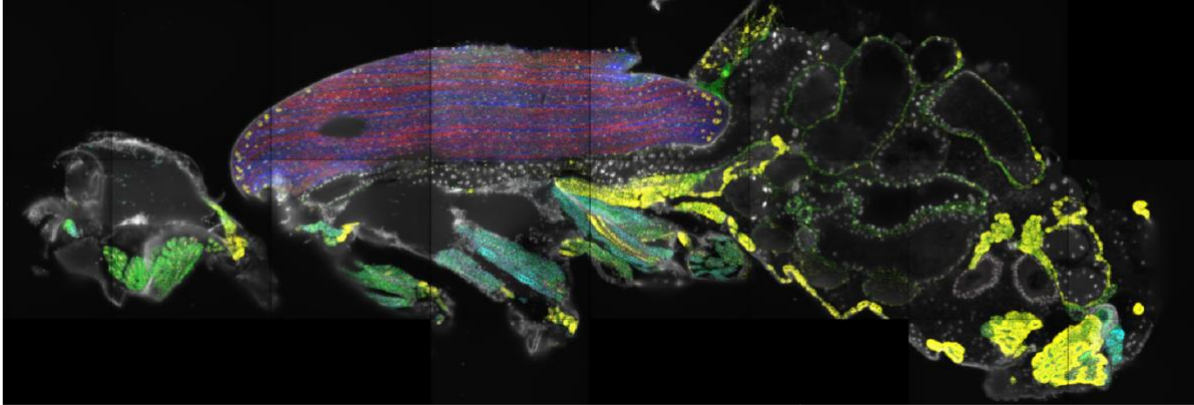
17:30-17:45: Alessio VAGNONI

“*In vivo* characterisation of mitochondrial contact sites in *Drosophila* neurons: implications for ageing and neurodegeneration”

Institute of Psychiatry, Psychology and Neuroscience, King's College London, UK, and Multidisciplinary Institute of Ageing, University of Coimbra, Portugal.

@EDRC2023
#EDRC2023_Ageing

Imaging and quantitative image analysis workshop



Organisers:

Frank Schnorrer (IBDM, Marseille)
Pavel Tomancak (MPI CBG, Dresden)

20th October 2023

Program:

15:30-15:45: Pavel Tomancak

"Mastodon: the real deal for developmental lineage analysis"
MPI CBG, Dresden, Germany.

15:45-16:00: Romain Levayer

"Automatic detection of cellular events in large movies through machine learning"
Institute Pasteur, Paris, France.

16:00-16:15: Katja Röper

"Imaging the mechanisms and mechanics of tube morphogenesis"
LMB, Cambridge, United Kingdom.

16:15-16:30: Stephan Preibisch

"Reconstructing the complete fruit fly central nervous system connectome from isotropic FIB-SEM data"; *Janelia Research Campus, Ashburn, United States.*

16:30-16:45: break – buffer.

16:45-17:00: Virginia Pimmitt

"The central dogma goes live in the early embryo"
Mounia Lagha lab, IGMM, Montpellier, France.

17:00-17:15: Matteo Rauzi

"Composite morphogenesis: how can a tissue fold and extend at the same time"
Institute of Biology Valrose, Nice, France.

17:15-17:30: Markus Affolter

"Engineer the fly genome for protein visualization and manipulation"
Biozentrum, Basel, Switzerland.

17:30-17:45: Frank Schnorrer

"Nanobodies, DNA-PAINT super-resolution imaging and spatial transcriptomics in adult fly tissue"
IBDM, Marseille, France.



Plenary talks

Ultra Long-Range Enhancer-Promoter Interactions in the *Drosophila* Brain

Mike Levine

Lewis-Sigler Institute, Princeton University, USA

Transcriptional enhancers located near (<10 kb) their target genes immediately convey regulatory information to the adjacent promoter. However, distal enhancers (>20 kb) often employ “tethering elements” to interact with their targets. These elements are a few hundred base pairs in length and contain repeated sequences such as GAGA. All of the enhancers that regulate stripes of Hox gene expression map 25-50 kb from their target promoters. These interactions employ tethering elements to accelerate gene activation. For example, a stripe of *Scr* expression is regulated by a distal enhancer located ~35 kb upstream of the promoter. It maps near a tethering element that interacts with another tether at the *Scr* promoter. Disrupting this interaction leads to a delay in the onset of *Scr* expression and weak homeotic transformations.

There are ~400 tethering elements active in the early *Drosophila* embryo, forming ~200 regulatory loops. About half facilitate enhancer-promoter interactions, while the other half mediate promoter-promoter interactions. Such a loop is seen within the *knirps* locus, which contains both the *knirps* transcription unit and a duplicated gene, *knirps*-related. Tether-tether interactions help coordinate the transcription of the two genes via shared enhancers.

A high-resolution Micro-C map identifies hundreds of tether-tether regulatory loops in the *Drosophila* brain. These loops are often associated with genes involved in neuronal adhesion and the formation of specific synapses, such as *Dprs* and *Dips*. ~50-100 of these brain-specific tethering elements are engaged in ultra long-range interactions, linking distant TADs over distances of 1-25 Mb across chromosome arms (meta-loops). There are ~25 meta-loops in the *Drosophila* brain, separated by an average of 5-6 Mb. An in-depth analysis of one of these meta-loops provides evidence for the most distant enhancer-promoter interaction documented to date, ~6.2 Mb. Different models and mechanisms for such ultra long-range interactions will be discussed.

Transposable elements: drivers of gene expression and gene structure novelty relevant for adaptive evolution

Josefa González

Institute of Evolutionary Biology, CSIC, UPF. Barcelona, Spain

Transposable elements are mobile DNA sequences that represent a substantial fraction of eukaryotic genomes: 20% in *Drosophila melanogaster*. Transposable elements contain regulatory sequences that can affect the expression of nearby genes. They are also enriched for repressive histone marks that can spread into flanking sequences, although in this case the effect on gene expression is less clear. Instances in which gene expression changes driven by transposable element insertions are adaptive have also been described. However, because of their repetitive nature, transposable elements are difficult to annotate in genomes, which has precluded the systematic genome-wide characterization of their effect on gene expression, structure and evolution. We have generated reference genomes, transcriptomes, and epigenomes for several natural *D. melanogaster* strains collected across Europe by the European Drosophila Population Genomics Consortium. We have shown that transposable elements are important contributors to adaptive evolution with stress response, behavior and development being the most influenced traits. We also found that transposable elements do affect gene expression through epigenetic changes and that these effects are not restricted to gene silencing. Moreover, we showed that 19% of body-part specific transcripts are gene-TE chimeras that on average contribute 43% to total gene expression, with some of them adding new protein domains. Overall, our results provide evidence for the genome-wide impact of transposable elements in the rewiring of gene expression and the generation of novel transcripts with some of these changes playing a role in the adaptive capacity of the species.

How to rapidly switch cell types: Alternative processing of nascent transcripts controls a dramatic shift in the proteins expressed in proliferating vs. differentiating cells

Margaret Fuller

Department of Developmental Biology, Stanford University School of Medicine, USA

Many differentiated cell types are regenerated from adult stem cells, a process essential for tissue maintenance and repair. *Drosophila* germ cells have long provided a powerful model to investigate the mechanisms that regulate self-renewal, proliferation and differentiation in adult stem cell lineages. In all adult stem cell lineages, relatively undifferentiated precursor cells must switch from mitotic proliferation to onset of the terminal differentiation program specific for that lineage. Failure to properly switch can lead to scarring, tissue dysmorphia, or cancer. How do cells make such an abrupt switch from proliferation to differentiation in one cell cycle? To answer this question, we are studying the switch from proliferating spermatogonia to differentiating spermatocytes in the *Drosophila* male germ line. Clearly a robust new transcription program turns on in early to mid-stage spermatocytes, driven by opening of chromatin at spermatocyte specific promoters by action of the cell type specific tMAC complex. However, our analysis has revealed that cell type specific alternate processing of nascent transcripts also dramatically changes the proteins expressed in proliferating spermatogonia vs. spermatocytes. 3'end-seq revealed that over 500 genes expressed in both spermatogonia and spermatocytes utilize an alternative site for the final endonuclease cut that generates the 3' end of nascent transcripts, a phenomenon known as alternative polyadenylation (APA), producing mRNA isoforms a long 3'UTR in spermatogonia but a short 3'UTR in spermatocytes. Analysis by polysome profiling and/or antibody staining indicated that for at least half of these genes the cell type specific switch from long to short 3'UTR led to changes in translation state, switching from protein ON in spermatogonia to OFF in spermatocytes, or vice versa. Thus a single molecular event, developmentally regulated change in the site at which to make the 3-end cut, can drastically change the proteins expressed in the two cell states, with ON to OFF vs OFF to ON presumably specified by sequences in the 3'UTR extensions expressed in spermatogonia. Our current results suggest that abundance of specific cleavage and polyadenylation (CPA) machinery components expressed at different stages regulates the choice of 3' cleavage site. In addition to this ON/OFF mechanism, analysis of stage specific full length transcripts from PacBio long reads and splice junctions from short read RNA-seq revealed that over 600 genes express different predicted protein isoforms in spermatogonia vs spermatocytes due either to spermatocyte specific alternative promoters, alternative splicing, or in a few cases alternative 3'end cuts upstream of the normal stop codon. Through these mechanisms, cell type specific alternative processing of nascent mRNAs rapidly switches both the suite of proteins expressed and an extensive slate of cell type specific protein isoforms turned on or off at consecutive steps during differentiation in the male germ line adult stem cell lineage. Our results indicate that in addition to looking for cell type specific transcription factors that might drive new developmental programs, we should probe the role of developmentally regulated alternative processing of nascent mRNAs in rapid cell stage changes.

Impacts of nutrition histories in larval stages on growth, organogenesis, and lifespan

Tadashi Uemura

Graduate School of Biostudies and Center for Living Systems Information Science (CeLiSIS), Kyoto University,
Kyoto, Japan

Postembryonic development is characterized by massive and rapid growth of juveniles. This developmental stage, in early life, is heavily influenced by the quality and quantity of nutrients consumed by the juveniles. To study how juveniles adapt to various nutritional environments, we have been characterizing growth and organogenesis in *Drosophila melanogaster* larvae under various diets, and in addition have been performing comparative analyses of *Drosophila* species with distinct feeding habits in nature. On the other hand, the impact of the nutritional environment in the early life - designated as nutrition history- is not restricted to that stage, but that it also exerts long-term health effects later in life, even to adult stages. However, the underlying cellular and molecular mechanisms of these effects are only just emerging.

Here, to dissect the far-reaching effects of the nutrition history, we imposed various dietary interventions during larval stages of *D. melanogaster*, and subsequently quantified lifespan of adults maintained on the standard food. As a larval diet, we used budding yeast, *Saccharomyces cerevisiae*, which is one of the major ingredients of laboratory media for *D. melanogaster*. Taking advantage of a single-gene knockout collection of *S. cerevisiae* as a source of diverse nutrition histories, we fed larvae with individual yeast strains from this collection and isolated those causing larval growth retardation and/or a decreased pupariation rate. One of the isolated yeast strains, *nat3D*, led to a shorter lifespan in adults compared to a control yeast diet. To understand how this *nat3D* diet in larval stages shortens adult lifespan, we performed a series of multi-omics analyses of the yeast diets, the yeast-fed larvae, and adults with the yeast diet-history. Our results strongly suggest the possibility that the function of a histone acetyltransferase, Gcn5, in larvae was reduced by the *nat3D* diet. We are addressing whether the reduced Gcn5 function in larval stages is the cause of the shorter lifespan in adults, investigating the larval tissue or cell type that contributes to the early death of adults, and identifying key nutrients in *nat3D* that reduce Gcn5 function in larvae.

References:

- Watanabe et al. Interspecies comparative analyses reveal distinct carbohydrate-responsive systems among *Drosophila* species. *Cell Rep.* 28:2594-2607 (2019).
- Kanaoka et al. Inter-organ Wingless/Ror/Akt signaling regulates nutrient-dependent hyperarborization of somatosensory neurons. *eLife* 12:e79461 (2023).
- Tsuyama et al. Dynamic de novo adipose tissue development during metamorphosis in *Drosophila melanogaster*. *Development* 150:dev200815 (2023).

Exploring the impact of physical forces on morphogenetic mechanisms at the tissue-scale

Bénédicte Sanson

Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK

Early embryos undergo rapid morphogenetic changes, starting with gastrulation. Morphogenetic deformations are primarily caused by genetically programmed cell behaviours, such as polarised cell intercalation, apical cell constriction or cell division. But morphogenesis is also influenced by physical inputs at the tissue or embryo-scale. Because multiple morphogenetic deformations can occur at the same time, tissues can be pulled or compressed, which in turn impacts cell behaviours. We have been investigating the contribution of physical stresses in epithelial morphogenesis, using the *Drosophila* early embryo as a model. With automated image segmentation and tracking to quantitate cell and tissue behaviours [1], we have shown for example that axis extension in *Drosophila* embryos is driven by an extrinsic force that acts in parallel to actomyosin-dependent polarised cell intercalations [2]. We showed subsequently that the source of this force is endoderm invagination, highlighting the importance of mechanical interactions between tissues [3]. Recently, we have been investigating whether mesoderm invagination, which pulls the ectoderm perpendicular to the direction of axis extension, has an impact via mechanical constraints or mechano-transduction feedbacks [4]. We have also been investigating how genetic programming, manifesting as actomyosin planar polarisation, combines with local and global tissue stresses to orient cell divisions in the plane of the epithelium [5,6]. We will summarise our latest findings using these tissue-scale approaches.

- [1] Blanchard et al. (2009) Nat Methods doi:10.1038/nmeth.1327
- [2] Butler et al. (2009) Nat Cell Biol doi:10.1038/ncb1894
- [3] Lye et al. (2015) PLoS Biol doi:10.1371/journal.pbio.1002292
- [4] Lye et al. (2023) bioRxiv doi.org/10.1101/2023.07.18.549479
- [5] Scarpa et al. (2018) Dev. Cell doi:10.1016/j.devcel.2018.10.029
- [6] Blanchard et al. (2023) bioRxiv doi.org/10.1101/2023.07.12.548728

Waking up “Sleeping” Neural Stem Cells

Hongyan Wang

Duke-NUS Medical School, Singapore

The ability of stem cells to switch between quiescent and proliferative states is crucial for maintaining tissue homeostasis and regeneration. In *Drosophila*, quiescent neural stem cells (qNSCs) extend a primary protrusion, which is removed prior to NSC reactivation. Recently, we showed that in qNSCs, microtubules are predominantly acentrosomal and oriented plus-end-out, which is similar to that in neuronal axons. Here, we have unravelled that qNSC protrusions can be regenerated upon injury, similar to immature neurons in the central nervous system. This regeneration relies on the Golgi apparatus which acts as the major acentrosomal microtubule-organizing centre in qNSCs. A Golgi-resident GTPase Arf1 and its guanine-nucleotide exchange factor Sec71 promote NSC reactivation and regeneration via a novel role in regulating microtubule growth. Arf1 physically associates with its new effector Mini Spindles (Msps)/XMAP215, a microtubule polymerase. Our findings have established *Drosophila* qNSCs as a new regeneration model and a novel Arf1/Sec71-Msps pathway in the regulation of microtubule growth, NSC reactivation and regeneration.

The co-evolution between transposable elements and the piRNA pathway

Julius Brennecke

Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Vienna BioCenter, Vienna, Austria

In the ancient genetic conflict between transposable elements (TEs) and their hosts, TEs strive to proliferate within the host genome, while hosts develop defense strategies to suppress TE activity. With progressive upgrades in their defense mechanisms, host organisms began to tolerate increasing amounts of TEs in their genomes, with profound evolutionary consequences. However, while the influence of TEs on the evolution of host genomes is becoming increasingly understood, delineating the driving forces behind the diversification of TEs is much more challenging. Using *Drosophila* as a model system, I will first present our findings on the core evolutionary principles of insect endogenous retroviruses, a monophyletic group of LTR retrotransposons that acquired the trait of cell-to-cell infectivity. I will then focus on how the host piRNA pathway, a small RNA-based genome defense system, continuously co-evolves and exploits TE vulnerabilities to counter the persistent threat of mobile genome invaders.

Energetic leaders opening unlocked doors; how immune cells infiltrate tissues

Daria Siekhaus

Department of Molecular, Cellular, and Developmental Biology, University of California, Los Angeles, USA

Vertebrate immune cells can effectively regulate organ homeostasis and clear infections if they are able to enter into tissues, during development and later in life. To identify what enables this cell biological feat, we study embryonic *Drosophila* macrophages. We find that BMP signaling specifies a macrophage subgroup that leads tissue infiltration. Leaders are powered by a previously unknown program that boosts mitochondrial energy production through shifts in metabolic enzyme levels and the translation of key mitochondrial components. Leaders infiltrate where surrounding cells have reduced their focal adhesions during cell division, a process we have found is also conserved in vertebrates. Our results raise the tantalizing possibility that insights gained in the fly could be relevant to the immuno-oncological treatment of tumors, the suppression of autoimmunity and the amelioration of other disease pathologies.

Visual processing strategies in dynamically changing environments

Marion Silies

Johannes Gutenberg University Mainz, Germany

A major challenge for the brain is to keep stable neural representations of stimulus features while facing a wide range of sensory inputs in natural scenes. Visual systems must stably compute contrast and motion cues, while actively navigating the environment. However, self-motion of the animal for example leads to rapid changes in background illumination, and to global motion cues occurring across the animal's eye. How does the visual system deal with such changes caused by animal behavior? Here, I will discuss how *Drosophila* stably processes visual information under constantly changing conditions. First, I will talk about changes in luminance that occur slowly throughout the day, but also happen at millisecond time scales when our eyes saccade across a natural scene, or when the visual input changes rapidly due to self-motion. We identified a mechanism that allows flies to handle diverse changes in illumination. This post-photoreceptor luminance gain control adjusts contrast signals when background luminance rapidly changes quickly, but also at slow timescales. We have elucidated the circuit mechanisms that achieve luminance-invariant visual behaviors, and are currently investigating the biophysical mechanisms that combine different types of signals to achieve stable contrast computation. Stable contrast signals are then used to compute higher-order visual features, one of which is the direction of motion. In the second part of my talk, I will show how motion computation is tuned to the behavior of the animal already in local direction-selective cells, which encode patterns of optic flow at the population level. Together, our work argues that visual processing strategies have evolved to handle the demands imposed by the animal's own behavior.

Imaginal discs mark their physical boundary

Aurelio Teleman

German Cancer Research Center (DKFZ), 69120, Heidelberg, Germany

The wing disc at the end of 3rd instar is composed of 50,000 cells that are differentially specified but mostly proliferative and roughly equipotent. One aspect of a cell's identity besides its expression profile is its metabolic state. We asked whether cells in the wing disc all have the same metabolic state, or whether this differs in a region-specific way. We find that indeed cells on the rim of the wing disc have a different metabolic state compared to cells on the inside, and that this leads to differences in their epigenetic state and hence gene expression. Interestingly, this difference is due to physical proximity to the edge of the disc, indicating that this metabolic and epigenetic state marks the physical boundary of the disc.

Impact of cell extrusion on tissue dynamics, in developmental and tumoral contexts

Magali Suzanne

Centre de Biologie Intégrative (CBI), Université de Toulouse, Toulouse, France

How mechanical forces drive morphogenesis is a fundamental question in the field of biomechanics. We found a few years ago, combining imaging, genetics, biophysical and modeling approaches, that apoptotic cells, far from being eliminated passively, exert a force before dying and thus actively participate in tissue remodeling. This force is transient, generated in the depth of the epithelium by an actomyosin structure, and constitute a mechanical signal involved in tissue folding (Monier et al, 2015). We characterized the cellular mechanism responsible for the generation of this apoptotic apico-basal forces and discovered that, during the initial force-producing stage, these cells reorganize their actomyosin cytoskeleton to create a contractile tether connecting the apical surface to a basally localized nucleus. This work identifies an alternative cellular organization supporting mechanical force production through the anchoring of the actomyosin cytoskeleton to the nucleus and highlights a critical non-genomic role of the nucleus during morphogenesis (Ambrosini et al, 2019). To test how this applies to vertebrate morphogenesis, we used the formation of the neural tube, an initially flat epithelium that bends and fuses, to determine how apoptosis contributes to morphogenesis in vertebrates. Our results strongly suggest that apoptotic forces, cumulatively, contribute actively to neural tube bending, revealing an important similarity between apoptotic cell dynamics and role in *Drosophila* and chicken, and suggesting that apoptotic morphogenetic force is evolutionarily conserved (Roellig et al, 2022).

We turned to delaminating cells to decipher how they extrude compared to apoptotic cells. We found that apico-basal forces are also produced by cells undergoing EMT before their delamination, similarly to what was observed in apoptotic cells. This apico-basal force produces a mechanical signal, which constitutes an important driving force and actively influences the surrounding tissue, as shown by mesoderm invagination failure when this force is prevented (Gracia et al, 2019). This finding was surprising, revealing unexpected similarities between EMT and apoptosis as driver of morphogenesis, although they lead to very different outcomes (cell survival and cell death, respectively). This work leads us to further investigate the cellular mechanism of delaminating cell extrusion. We further addressed the impact of apoptosis and tumor cell mechanics in tumor progression and identified them as unexpected factors that could influence tumor development, and more specifically the hyperplasia/dysplasia transition, a critical step in tumor aggressiveness (Montemurro et al, in revision).

Altogether, these works allow us to better understand how cell extrusion impact the surrounding tissue without altering epithelial integrity.

Apoptosis, cellular senescence and tumorigenesis in imaginal cells

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Programmed cell death (apoptosis) is a homeostasis program designed to remove cells that are unwanted or are damaged by physiological insults. To assess the functional role of apoptosis, we have studied the consequences of subjecting *Drosophila* imaginal cells unable to execute apoptosis to stress or genetic perturbations that normally cause massive cell death. We find that many of those cells acquire permanent activity of the JNK pathway, which drives them into senescent status, characterized by arrest of cell division, cellular hypertrophy, Senescent Associated β -gal activity (SA- β -gal), production of reactive oxygen species (ROS), Senescent Associated Secretory Phenotype (SASP) and migratory behaviour. We have identified two classes of senescent cells in the wing disc: 1) those that localize to the appendage part of the disc, express the *upd*, *wg* and *dpp* signalling genes and generate tumour overgrowths, and 2) those located in the thoracic region, which fail to express *wg* and *dpp* and do not induce tumour overgrowths. Whether a senescent cell becomes tumorigenic or non-tumorigenic depends on its original identity prior to the transformation. We also find that the growth pathways JAK/STAT, Dpp and Wg are down regulated in the senescent cells.

ORAL Presentations

by topics

Cell biology

A cell surface code mediates tissue-intrinsic defense against aberrant cells in epithelia

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Tissue-intrinsic error-correction mechanisms allow epithelial cells to detect aberrant neighboring cells and cause their removal from the tissue. The molecular mechanisms which grant cells the ability to compare their internal states is unknown. Here we demonstrate that comparison of cell identity, created by cell-fate-specifying transcription factors and patterning pathways, is conveyed through a specific set of cell surface molecules. We demonstrate that *Drosophila* imaginal discs express a range of cell surface molecules previously implicated in neuronal axon guidance processes, such as members of the Robo, Teneurin, Ephrin, Toll-like or atypical Cadherin families. Expression of these molecules is regulated by intrinsic fate-patterning pathways of the disc but also by aberrant expression of oncogenic RasV12. Importantly, mosaic clones deregulating individual cell surface molecules are sufficient to induce all hallmarks of ‘interface surveillance’, a tissue-intrinsic error-correction mechanism previously shown to be induced by cells with aberrant activation of fate-patterning pathways. Specifically, cells with deregulated expression of Robo2 and Robo3 induce actomyosin enrichment, bilateral JNK signaling and apoptosis at mosaic clone interfaces in imaginal discs. Moreover, deregulation of Robo2 levels, which is normally expressed in a complex endogenous pattern, induces these interface surveillance hallmarks in a Robo2-pattern-specific manner. Taken together, our work indicates that these cell surface molecules mediate cell fate recognition in epithelial tissues and thereby contribute to the maintenance of epithelial health by initiating detection and removal of aberrant cells during development and adult tissue homeostasis.

Keywords: epithelia, axon guidance receptors, patterning, interface surveillance

^{*}Speaker

An intruder-targeting system eliminates paternal mitochondria after fertilization in *Drosophila*

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An intruder-targeting system eliminates paternal mitochondria after fertilization in *Drosophila*
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Maternal inheritance of mitochondria occurs in almost all organisms, spanning from fungi and plants to humans. Upon fertilization, a single sperm fully penetrates the egg, resulting in a short period where mitochondria from both gametes populate the early embryo. Within a few cell cycles, however, paternal mitochondria (**PM**) are eliminated, and propagating maternal mitochondria take over the mitochondrial network. Several reports illustrate PM elimination as an outcome of passive dilution by the vast maternal mitochondrial pool. Yet, recent studies, performed on different organisms, suggest active elimination of PM by egg-derived mechanisms. In particular, our group previously demonstrated that egg-derived multi-vesicular bodies (**MVBs**) associate with PM immediately after fertilization to promote PM degradation. Nevertheless, the mechanisms by which MVBs mediate paternal mitochondrial destruction remain unknown¹.

Here, I will present our recent findings aiming to identify the mechanisms by which egg-derived MVBs target and destroy PM in *Drosophila*. We identify a cell intruder-targeting pathway, called LC3-associated phagocytosis (LAP), which is a common endocytic, autophagic and phagocytic pathway, as the main executor of PM elimination. Our model indicates that MVBs loaded with LAP-specific components engage with PM to mediate its elimination. LAP-specific phosphatidylinositol 3-phosphate (PI3P) kinase complex is recruited to PM for PI3P production. The presence of PI3P, together with generation of ROS, promote Atg8 (LC3) conjugation to PM, facilitating sequestration of PM to lysosomes. Finally, I will also present initial evidence

^{*}Speaker

for possible conservation of some of these mechanisms during corresponding processes in mammalian eggs.

1. Politi, Y. *et al.* Paternal mitochondrial destruction after fertilization is mediated by a common endocytic and autophagic pathway in *Drosophila*. *Dev. Cell* **29**, 305–320 (2014).

Keywords: Fertilization, Mitochondria, Mitochondrial inheritance, LC3, associated phagocytosis (LAP)

Assembly of apical integrin-based focal adhesion contacts sustain adherens junctions remodeling during epithelial cytokinesis

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In proliferative epithelial tissue, cell divisions entail cell contact remodelling which weakens the mechanical tissue integrity. Our previous work contributed to reveal that formation of the new adhesive interface is coordinated with cytokinesis, and that the strength of adhesion with neighbors impacts both the kinetics of adherens junctions (AJs) remodeling and the geometry of the new AJs. Nonetheless, how cell adhesion and cytoskeletal forces are maintained when E-Cadherin complexes are partially disengaged between mitotic and neighboring cells at the cleavage furrow remains poorly understood. While the nonmuscle type II myosin (Myo II) is recruited in the neighboring cells and act as a force generator to set the length of the new interface, how Myo II is recruited and activated there is not fully understood. In addition, it takes several minutes to disassemble AJ with the neighbors and form AJs between daughters, a time during which mechanical integrity is partly impaired.

Here, we report that focal adhesion contact (FAC) containing the beta-Integrin/Myospheroid, the alpha-PS3- integrin/Scab, Talin/Rhea and Vinculin assembles transiently in the neighboring cells, within the plane of adherens junctions. FAC assembles at the time of the reduction of E-Cadherin signal during furrow invagination, at both edges of the presumptive novel adhesive interface. FAC then propagate along the presumptive adhesive interface until formation of AJ and then disappear. Integrin-based adhesion is known to act as a mechanotransducer able to regulate actomyosin contractility. Interestingly, concomitantly to assembly of FAC, we report that the p114RhoGEF/Cyst is also be transiently recruited in the neighbours where it regulates the recruitment of MyoII at the edges of the forming adhesive interface. Loss of Cyst impact the formation of the novel adhesive interface. Whether FAC assembly and p114RhoGEFs recruitment are interdependent is under investigation. We propose a model according which local pulling forces exerted by furrow ingression triggers FAC assembly in the neighbors to modulate force transmission, actomyosin contractility and contribute to ensure the mechanical integrity at the place were AJ disassembly with neighbours and AJ assembly between daughters is taking place.

Keywords: cytokinesis, cadherin, integrin, myosin, mechanical forces

*Speaker

Going for a swim: What drives the ‘swimming’ cell migration of *Drosophila* adipocytes?

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Cell migration plays a fundamental role in health and disease. It enables immune cell recruitment, wound healing and cancer invasion but also many other cellular processes. Cells can use various migration modes which can either be dependent or independent on cell-matrix adhesion. The most extreme type of adhesion-independent migration is the swimming mode, which allows cells to move through liquids without close contact to any substratum. The mechanism underlying swimming migration is still ill-defined and has so far only been studied *ex vivo*. We have previously shown that adipocyte-like fat body cells (FBCs) in *Drosophila* pupae do not float passively in the hemolymph, as previously believed, but actively migrate. This allows them to move towards epithelial wounds using swimming migration to promote wound healing and combat infection.

Here we use pupal FBCs as an experimental *in vivo* model to study this swimming mode of cell migration further. Our data suggest that during migration, the nucleus of FBCs together with the perinuclear microtubule-organising centre, is positioned at the front of the cell. This correlates with higher speed and directional persistence.

Their unusual cellular organization and unconventional swimming migration mode, raises the question of how these giant cells generate internal forces and transmit them to their environment to migrate. We find that migrating FBCs generate internal forces by producing cortical actin waves which propagate towards the cell rear resulting in actomyosin based contraction. By using a newly developed high-throughput screening method, we discover that FBC migration is controlled by small Rho GTPases and several of their downstream effectors. Altogether our data provide valuable insights into the elusive *in vivo* mechanism underlying swimming cell migration.

Keywords: cell migration, fat body, GTPases, wound healing

^{*}Speaker

Identification of α -tubulin detyrosinases in human and *Drosophila* reveals an unexpected enzymatic diversity.

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Tubulin post-translational modifications (PTM) represent an important mechanism involved in the regulation of microtubule functions. Detyrosination is a PTM which consists in the removal of the C-terminal tyrosine residue from α -tubulin, generating $\alpha 1$ -tubulin which can be further processed to $\alpha 2$ -tubulin. In 2017 the vasohibins VASH1 and VASH2 were identified as the first class of enzymes catalyzing tubulin detyrosination. Using biochemical approaches in human cells, we discovered that two closely related proteins, KIAA0895L and KIAA0895, have Tubulin Metallo-CarboxyPeptidase activity and thus we termed them TMCP1 and TMCP2 respectively. We show that TMCP1 is primarily a detyrosinase but it can also generate $\alpha 2$ modification. In contrast, TMCP2 mainly generates $\alpha 2$ -tubulin and interestingly also processes β I-tubulin leading to the formation of previously uncharacterized $\beta 3$ modification associated with centrioles and primary cilia. Thus, TMCPs represent a new family of tubulin modifying enzymes. Strikingly, neither VASHs nor TMCPs have homolog in *Drosophila* despite the presence of α -tubulin detyrosination, suggesting the existence of additional class of enzymes catalyzing this modification. In *Drosophila*, α -tubulin detyrosination is found almost exclusively in testes where it is present on the meiotic spindles and is highly abundant on sperm axonemes. Using a RNAi-based genetic screen, we have identified a previously uncharacterized carboxypeptidase, renamed TCP (Tubulin CarboxyPeptidase), which is responsible for this modification in flies. CRISPR-mediated deletion of the *TCP* gene completely abolishes α -tubulin detyrosination. *TCP(KO)* males are viable and show almost normal fertility but produce gender-biased progeny. As a complementary approach to analyze the role of tubulin detyrosination *in vivo*, we engineered *Drosophila* carrying point mutations in the major α -tubulin gene *α Tub84B* to generate fully detyrosinated ($\alpha 1$) and non-cleavable tubulin mutants. Strikingly, whereas absence of detyrosination is rather well tolerated, excess of detyrosination results in male sterility. Thus, a balanced level of detyrosination is necessary for proper *Drosophila* spermatogenesis. Altogether our results reveal an unanticipated diversity in the enzymes catalyzing α -tubulin detyrosination.

Keywords: microtubules, post, translational modifications, detyrosination, spermatogenesis

*Speaker

Integration of cortical and lamellar actin networks are required for the migration of *Drosophila* macrophages

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The actin cortex is a thin network of actin filaments and actin-binding proteins reversible attached to the plasma membrane of many eukaryotic cells and plays a fundamental role in processes such as cell division and cell migration. It is structurally distinct from other organisations of the actin network, such as the lamellipodia, which contains flattened, crosslinked fibres that are in intimate contact with the cell substrate. One open question is how cells generate and transit between these distinct actin geometries.

Here we show that embryonic *Drosophila* macrophages (hemocytes) during their developmental dispersal simultaneously contain both, a planar polarised lamellipodial network and an actin cortex around their cell body. Surrounding the soma of hemocytes is a distinct actin network consisting of cortical proteins, such as Moesin, and cortical regulators, such as PIP2. Live imaging reveals that Moesin is binding the actin network within the lamellipodia and is rapidly advected through the retrograde actin flow to the rear of the lamella where it concentrates within the actin cortex, suggesting that the actin flow has a role in regulating the cortex organisation. Additionally, we generated a new actin probe in flies that reports on actin filaments in proximity to the plasma membrane (MPAct), which confirms the presence of an actin cortex by highlighting that the actin filaments surrounding the cell body, in contrast to the lamellipodia, are distinctly associated with the lipid bilayer.

Intriguingly, loss of Moesin or constitutive activation of Moesin leads to a reduction in hemocyte speed and aberrant embryonic dispersal. Moreover, inhibition of Moesin and the subsequent loss of cortex organisation also affects the dynamics of the actin retrograde flow within the lamellipodial network, pointing to the existence of a bidirectional cortex-lamellipodia crosstalk. While migrating cells are often thought to have either a cortical or a lamellar actin network, these data support the idea that these two distinct actin geometries can coexist, and we are now further investigating how they are coordinated during hemocyte migration.

Keywords: Cell migration, Actin, Moesin, PIP2, ERM, Actin cortex, Macrophages, Hemocytes, Actin network, Lamellipodia

^{*}Speaker

Microtubules coordinate mitochondria with myofibril morphogenesis during flight muscle development

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Muscle morphogenesis is a complex multi-step process that results in highly specialised cells containing contractile myofibrils and energy producing mitochondria. Myofibrils are long chains of a succession of stereotyped nanomachines called sarcomeres that contract by pulling on actin filaments by central myosin filaments at the expense of ATP. Recent studies have elucidated how contractile sarcomeres are assembled during myofibrillogenesis. However, how muscle cells regulate and specialise their mitochondria to support the high energy demands of sarcomere function remains largely unknown. The *Drosophila* indirect flight muscles is a well-characterised myofibrillogenesis model, also useful for studying mitochondrial dynamics. Recently, we have shown that mitochondria remodel their morphology and relocate extensively during flight muscle development (Avellaneda et al. 2021): initially, mitochondria are excluded from the large developing myofibrils bundles, however, quite rapidly, they intercalate in between each myofibril and insulate them from one another. This close contact guarantees an optimal supply of ATP for all sarcomeres. How this rapid morphogenesis happens is still unknown. Here, we provide a mechanism for rapid and stereotyped mitochondria intercalation between myofibrils during flight muscle development. Using high-speed *in vivo* imaging of developing flight muscles, we show that mitochondrial intercalation is surprisingly fast taking less than two hours, suggesting an active mechanism. By tracking mitochondrial populations with a photoconvertible fluorophore Dendra2, we show that mitochondrial transport dynamics increase dramatically after intercalation, largely along the long axis of the muscle cell. Interestingly, anti-parallel microtubules are oriented along the same axis, in proximity to the assembled myofibrils and the moving mitochondria suggesting a role for microtubule-based transport. Indeed, light-induced microtubule severing directly affects myofibril orientation and assembly, whereas knock-down of the *kinesin heavy chain* motor protein specifically blocks mitochondrial transport resulting in mitochondria agglomeration outside of the myofibril bundles. I will present data showing the dynamics of mitochondrial re-localization during muscle development as well as a key role for microtubules in coordinating mitochondria with myofibril morphogenesis.

Keywords: mitochondria, drosophila, muscle development, microtubule

*Speaker

Snazarus acts at the crossroads of endocytic and secretory transport in nephrocytes

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The endomembrane system of eukaryotic cells represent an intricate network the components of which are connected with each other either via vesicular trafficking routes or permanent physical contacts. This complex, dynamic system plays a pivotal role in cell physiology and its proper function requires the concerted action of a multitude of proteins. Members of the Sorting nexin (Snx) protein family play important roles in numerous locations of the endolysosomal system. All Snx proteins contain the lipid-binding PX-domain that enables their membrane association where they utilize other protein domains to take part in versatile molecular events. However, exact cellular functions of many Snx proteins are currently unknown. We use various fruitfly tissues to study the roles of the less characterized Snx proteins in the endolysosomal system. The subfamily of transmembrane domain-containing sorting nexins have four members in mammals, while only one such protein, Snazarus (Snz) is encoded in the fruitfly genome. In fruitfly fat cells, Snz localizes to contact sites between the ER, plasma membrane (PM) and lipid droplets (LDs) and is involved in lipid trafficking. We report a surprising novel function of Snz in larval garland nephrocytes, the highly endocytic, podocyte-like cells of *Drosophila*. Garland nephrocytes are spherical cells that continuously take up material from the hemolymph and display a highly organized endosomal system. Loss of *snz* leads to prominent morphological and functional changes in the endomembrane system of nephrocytes. These include upregulation of endocytosis, redistribution of recycling endosomes and alterations of the nephrocyte diaphragm, the molecular filter highly similar to the mammalian podocyte slit diaphragm. We show that the effects of *snz* loss are linked to its role in Rab11-dependent recycling and are independent of ER-PM-LD contact sites in this cell type. We propose a model in which Snz negatively regulates exocytosis downstream of Rab11, thus contributing to the maintenance of membrane balance in the endosomal system of nephrocytes.

Keywords: nephrocyte, Snz, Rab11, endocytosis, recycling

*Speaker

The ER protein Creld regulates dopaminergic neuron activity via mitochondrial hydrogen peroxide production

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Organelle communication of endoplasmic reticulum (ER), mitochondria and peroxisomes integrates multiple metabolic and signaling functions. Dynamic contacts between ER and mitochondria enable the exchange of calcium and phospholipids. Disturbed contact formation impairs mitochondrial dynamics and is a molecular hallmark of Parkinson's disease (PD). PD is also characterized by impaired mitochondrial respiratory complex I activity and dopaminergic neurodegeneration. The ER protein Cysteine-rich with EGF-like domain (Creld) is a poorly characterized risk gene for PD. Here we found that Creld regulates mitochondrial dynamics and function. Loss of Creld leads to mitochondrial hyperfusion and reduced ROS signaling in *Drosophila melanogaster*, *Xenopus tropicalis* and human cells. We used electron and super-resolution microscopy to analyze mitochondria-ER contact sites (MERCs): Creld mutants show enhanced, but less functional ER-mitochondria contacts. Lipidomics analysis of subcellular fractions revealed that phospholipid transfer at MERCs is reduced. This impairs mitochondrial respiratory complex I activity. Using optogenetics we show that the resulting low hydrogen peroxide levels are linked to disturbed neuronal activity and lead to impaired locomotion, but not neurodegeneration, in Creld mutants. MERCs might recruit a third organelle, the peroxisome. Peroxisomes are important regulators of hydrogen peroxide homeostasis. Creld interacts with the peroxisome biogenesis factor Pex19 on the protein level, and loss of Creld blocks peroxisome function in dopaminergic neurons. We conclude that Creld regulates ER-mitochondria-peroxisome communication and thereby hydrogen peroxide formation, which is required for normal neuron function (Paradis et al., 2022). Paradis M, (...) Bülow MH. The ER protein Creld regulates ER-mitochondria contact dynamics and respiratory complex 1 activity. Sci Adv. 2022 Jul 22;8(29):eabo0155. doi: 10.1126/sciadv.abo0155.

Keywords: Organelles, Parkinson's disease, ROS, dopaminergic neurons

*Speaker

The emerging role of small GTPase Rac1 on basal myosin oscillation

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Pulsatile actomyosin contractility, occurring at apical or basal domain of epithelial cells, can play multiple important roles in tissue morphogenesis. Rho1 signaling has been well studied to govern myosin activity as well as pulsed formation and patterning in many systems. However, we have limited information on upstream signals that control the cortical F-actin networks to support pulsatile myosin contractility and the synergy between F-actin and myosin signals.

Recently, we unexpectedly identified the importance of Rac1 and its binding protein Sra1 for the basal actin networks to control basal myosin oscillation and stress fiber strength during stages 9-10 of *Drosophila* oogenesis. Mechanistically, Sra1 and Rac1 downstream Wave complex are distributed at both basal junctions and medial-basal regions, while Rac1 downstream effector cofilin is located mainly at medial-basal regions. While we did not detect significant oscillatory signals of Wave complex, genetic inhibition of Scar, Abi or Arp2/3 by RNAi in follicle cells can strongly reduce basal actomyosin intensity and oscillation, indicating the strength control of basal F-actin network by the Wave complex. Differently, Cofilin signals strongly oscillates together with basal myosin signals; genetic activation or inhibition of cofilin in follicle cells unraveled that cofilin activity can significantly contribute to the dynamic range of basal actomyosin oscillation, thereby implying an oscillatory control. Simultaneous modification experiments confirmed that Wave complex and cofilin synergize to control actomyosin pulse formation.

Furthermore, basal stress fibers are gradually consolidated from stage9 to stage10, and this fiber consolidation seems to be associated with the remodeling of focal adhesions and stress fibers by these pulsatile actomyosin networks. Optogenetic activation or inhibition of Rac1 not only confirmed that spatiotemporal activation of Rac1 signals is indispensable for basal myosin oscillation, but also revealed that Rac1 can spatiotemporally modify stress fibers and their linked focal adhesions for gradual consolidation process of basal stress fiber networks.

Altogether, my studies highlight, for the first time, that Rac1 signaling cascade and its spatiotemporal activity function as an important upstream factor to govern basal F-actin networks and pulsed myosin contractility for tissue morphogenesis.

Keywords: Rac1, Basal F, actin and myosin oscillation, Optogenetic

*Speaker

Methods

CRISPorting: Static and dynamic monitoring of endogenous gene expression using sgRNA

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Alterations in gene expression underlie most physiological and pathological processes, including cellular development, growth regulation and oncogenesis. Hence, the ability to trace endogenous gene activities would represent a powerful tool for studying biological phenomena. We are developing, validating, and implementing a novel CRISPR-based technology for monitoring gene expression *in vivo* called CRISPorting (CRISPR-based reporting of gene activity). The principle of this method is to integrate a tRNA-flanked sgRNA into a non-coding region of a gene without interfering with normal gene function. When the gene is expressed, the generated sgRNA can either associate with Cas9 to activate a static reporter or engage with dCas9-VPR to activate a dynamic reporter. Activation of the static reporter irreversibly marks the expressing cells and its decedents (lineage tracing). We are testing this application by monitoring tumor progression in different tissues. In contrast, dynamic reporting is reversible and allows us to monitor transient gene expression changes in gene activity. We are benchmarking dynamic reporting by reproducing the patterns for well characterized genes and will use it to explore the function and activity of uncharacterized genes. Finally, we explore the use of CRISPorting for monitoring the activity of signalling pathways during development and disease by targeting key target genes.

Keywords: CRISPR, gene expression, monitoring endogenous gene activity, lineage tracing, gene expression changes, tRNA, flanked sgRNA, static and dynamic reporter

*Speaker

FlyBase 2023: new features and tools to accelerate your research.

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We will present an overview of features and tools recently added to FlyBase (www.flybase.org) with the aim of promoting their utility to researchers. Several new features support exploration of scRNA-seq datasets, including graphical displays of cell type expression data from the Fly Cell Atlas. Chemicals used in *Drosophila* research can now be browsed in dedicated report pages and searched via a new aspect of our Vocabularies tool. Additions to functional data/analysis include the provision of stacked Gene Ontology (GO) Summary Ribbons, an option to export gene lists to the PANGEA enrichment tool at the DRSC, and updates to our signaling pathway pages. Other improvements include new orthology data from DIOPT and OrthoDB, a better representation of split-GAL4 lines, and a new 'gene toolkit' feature that highlights the most useful alleles/transgenes for each gene. Finally, we have made a number of additions to our community and outreach resources, including a 'New to Flies' portal, a refreshed set of FAQs, and a newly generated directory of worldwide fly labs.

Keywords: FlyBase

*Speaker

Optogenetics for All: NinaB Cleavage of Beta-Carotene as a Source of All-Trans Retinal

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Optogenetics has become a valuable tool for investigating memory, learning, and various behaviors in *Drosophila* and many other species. It also has become an exciting science educational tool that has been successfully implemented in elementary and secondary schools. There are many different variants of opsins: ion channels (channelrhodopsin), pumps (halorhodopsins), or coupled G-Proteins (visual opsins). They also vary in wavelength of light absorption ranging from blue to red light. All opsins share a similar structure of 7 transmembrane domains and bind a retinal molecule (chromophore) within the 7th transmembrane domain. The retinal chromophore absorbs a photon of light and rapidly isomerize inducing a structural change in the protein. *Drosophila* are unable to synthesize retinal and it must be derived from carotenoids in their diet. In the majority of channelrhodopsin variants, the amounts of carotenoids obtained from standard cornmeal fly food is insufficient to supply both the visual system and the transgenic channelrhodopsins. Fly food is usually supplemented for optogenetic experiments in the range of 0.2 -0.4 uM of *all-trans*-retinal which is costly. NinaB, a *beta*, *beta*-carotene-15,15'-oxygenase, cleaves carotenoids into two retinal molecules. Confocal imaging of *ninaB-GAL4/UAS-GFP*, identified glial cells as the putative site of *ninaB* expression. Since *ninaB* mutants are unable to form Rhodopsin due to the lack of retinal, I tested if glial expression of wild type *ninaB* could restore the endogenous levels of Rhodopsin. Western blot analysis of mutant *ninaB* flies with *repo-ninaB* showed rescue Rhodopsin protein. We are investigating the efficacy of NinaB cleaved *beta*-carotene as the source of *all-trans* retinal in optogenetic experiments. The cost of *beta*-carotene is approximately 20-fold less than that of *all-trans* retinal. Current experiments are comparing the cost-effective *beta*-carotene supplementation versus *all-trans* retinal in optogenetic behaviors and electrophysiology (e.g., Moonwalker behavior, EJP responses at the larval neuromuscular junctions, and learning within the mushroom body).

Keywords: optogenetics, beta, carotene, retinal, behavior, ninaB

*Speaker

Reconstructing spatiotemporal gene expression and enhancer activity in *Drosophila* embryos using scRNA-Seq and optimal transport

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Enhancers are DNA sequences that tightly regulate the spatiotemporal expression of genes. The *in vivo* activity of enhancers is routinely characterized by imaging the expression pattern of a reporter gene under the control of a given enhancer. However, these experiments are tedious and not easily scalable genome-wide. Recent advances in single-cell technologies offer new ways to investigate such biological questions with cellular resolution. This project aims to build an atlas of spatial and temporal enhancer activity during *Drosophila* embryogenesis by combining a single-cell protocol for high throughput enhancer activity characterization with a mathematical model for virtual tissue reconstruction.

To do so, we developed a massively parallel enhancer-reporter assay, which measures the activity of multiple enhancers *in vivo* in one experiment using single-cell sequencing. In the downstream bioinformatics analysis, we can retrieve for each cell its cell type of origin and the identity of the enhancer it contains. By combining this information we can infer the tissue-specific activity of each enhancer under study.

But staying at tissue resolution can be limiting if the enhancers’ activity is not strictly restricted to a cell type, in the case of stripe patterns for example. To overcome this limitation, we are using novoSpaRc, a tool which exploits an optimal transport model to reconstruct a virtual embryo at cellular resolution from single-cell data. This software maps single cells on a virtual tissue based on the hypothesis that cells having a similar transcriptomic profile will be closer in physical space. NovoSpaRc was used to reconstruct basic 2D shapes of different organisms and organs, especially a stage 6 *Drosophila* embryo.

However, the complexity of *Drosophila* development with the growth of internal organs can not be summarised in 2D. Therefore, we adapted novoSpaRc to reconstruct an embryo undergoing gastrulation in 3D, and obtained patterns of gene expression and enhancer activity that recapitulated faithfully previously available *in situ* images. This preliminary analysis comforts

^{*}Speaker

us in the strength of using optimal transport to jump from transcriptomic to spatial level. When complete, this atlas will benefit the *Drosophila* community for further studies on enhancer redundancy or enhancer-promoter interactions in time and space.

Keywords: embryogenesis, enhancer, single, cell RNA sequencing, spatial reconstruction

Towards a whole-larvae atlas of chromatin accessibility to map the developmental enhancer space in *Drosophila*

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Single-cell epigenomics provides a powerful means of cataloguing known cell types, discovering novel ones, and deciphering their underlying regulatory principles. By only sequencing adult tissue over a limited time window, crucial mechanisms in cell identity formation are not captured. We present a first version of a comprehensive atlas spanning the larval development of the fruit fly, *Drosophila melanogaster*. To sequence the whole larva across different developmental stages, we performed a protocol optimization for single-cell ATAC (scATAC) using the in-house microfluidic system ‘HyDrop’ to enable upscaling of data collection of closely set time windows. We tested protocols on fresh and frozen tissue for different developmental stages. We have established an adapted protocol omitting fluorescence-activated cell sorting for whole-larvae sequencing to increase the throughput in high-quality scATAC sequencing. For wandering 3rd instar larvae, we combined whole-larvae sequencing with dissected tissues, namely the brain, wing disc, and eye disc. Next to scATAC, we performed scaleATAC and multi-ome, both at single-cell level. We compared the data quality from scATAC, single-cell scale ATAC and multi-ome using the newly developed PUMATAC pipeline for preprocessing and QC, followed by topic modelling using cisTopic. For the 3rd instar larval stage, this resulted in more than 90K cells so far with 52 clusters of differentially accessible chromatin. We annotate these clusters using our whole-larvae multi-ome and existing tissue-specific single-cell RNA-sequencing data. We are currently expanding this atlas to cover closely set time windows of larval development. We further are training enhancer-driven Gene Regulatory Networks (eGRNs) and use motif enrichment to investigate key transcription factor combinations and pathways driving the formation of cell type identity. Our atlas provides a valuable resource for understanding enhancers and regulatory control mechanisms that drive cellular identity and underlie the development of complex organisms.

Keywords: Development, Gene Regulatory Network, Chromatin Accessibility, single, cell ATAC sequencing, Enhancer, Topic Modelling, Epigenomics

*Speaker

Disease models

A *Drosophila* model for Dent's disease unveils a role for chloride/proton exchanger Clc-c in regulating the cortical actin cytoskeleton

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Dent's disease is an inherited disease characterized by the loss of protein uptake receptors cubilin and megalin as well as the impairment of the endocytic pathway in the proximal tubule of the kidney. The predominant cause for Dent's disease is mutations in the human *CLCN5* gene which encodes the kidney-specific chloride-proton exchanger: Clc-5. Despite extensive research, dialysis and kidney transplantation remain the predominant treatment options for Dent's disease. *Drosophila* nephrocytes share many functional characteristics of proximal tubular cells and podocytes in humans, making them an attractive model for *in vivo* studies of renal diseases. In this project, we have identified Clc-c as the functional orthologue of human Clc-5. Utilizing Clc-c-deficient *Drosophila* nephrocytes as a model for Dent's disease, we were able to recapitulate loss of cubilin and endocytic activity phenotypes characteristic of Dent's disease. We also identify an accumulation of actin at the cortex of Clc-c deficient nephrocytes and show that its reduction by overexpression of twinstar (*tsr*) or knockdown of its negative regulator LIMK1 rescues not only the actin accumulation but also the expression of cubilin and albumin uptake. Additionally, loss of Clc-c caused a distinct mislocalization of slit diaphragm proteins and strong perturbations of the endolysosomal pathway, such as the clustering of Rab7, Lamp1 and cholesterol in large perinuclear compartments. These phenotypes could not be rescued by regulation of Twinstar, suggesting that they occur through an actin-independent mechanism. Together, we have uncovered a role of cortical actin in the pathogenicity of Dent's disease, paving an avenue for potential therapies targeting actin polymerization in the kidney. Additionally, we have also identified a broader range of phenotypes associated with loss of Clc-c which upon further study may elucidate additional mechanisms underlying Dent's disease.

Keywords: Kidney, Dent's disease, endocytosis, protein uptake, cytoskeleton, actin, nephrocyte

*Speaker

A multi-model approach investigating patient-derived gene candidates underlying Atrial Fibrillation disease manifestation.

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Atrial fibrillation (AF), the most common heart rhythm disorder, is reaching epidemic proportions in the aging population. In both human and model systems, it is not understood how aging, genetic predispositions, and external environmental factors synergize to promote arrhythmia, nor which gene regulatory networks initiate and maintain AF. Over 200 genetic variants have been associated with increased AF susceptibility, suggesting that the underlying cause is multifactorial, involving networks of interacting genes. Resolving complex interactions modulating cardiac function in AF is difficult in mammalian systems, but approachable using *Drosophila*. We utilize a multi-platform approach encompassing the genetically tractable *Drosophila* cardiac-aging model and hiPSC-atrial-like cardiomyocyte (ACM) model. High-speed imaging of ACMs and fly hearts permit quantification of cardiac parameters such as action potential duration and arrhythmicity in ACMs, and contraction intervals and arrhythmicity in flies. In collaboration with an arrhythmia center in France, we screened 75+ human gene candidates in our fly model via cardiac-specific RNAi knockdown of the orthologous fly genes. Genetic variants in human AF patients were identified via screening tissue samples against a biomarker panel of 250 candidate genes. Additional candidate genes were selected from AF meta-GWAS analyses. Preliminary research has shown single genetic insults rarely produce robust arrhythmicity in our models, but we see robust arrhythmia when testing interactions between genes or incorporating aging and pharmacological stressors (isoproterenol in ACMs, octopamine in flies). Testing candidate genes in conjunction with a Shaker heterozygous mutant (atrial-specific K⁺ channel KCNA5 homolog), we were able to tease out certain interactions that altered contraction intervals, and either induced arrhythmia at young ages or reduced arrhythmia at older ages. For example, KD of *Dorsocross*, *Piezo*, or *Ryandine Receptor* in a *Sh* heterozygote background produces robust arrhythmicity, even at younger ages, whereas knockdown of *Sarcolamban* in a *Sh* mutant background mitigates age-dependent cardiac alterations. Networking the corroborated hits with first degree neighbors identified novel candidate genes (e.g. Fasciclin, Cubitus interruptus) and pathways (Hedgehog) underlying AF susceptibility. Characterizing identified human AF genetic variants across multiple platforms will not only improve our genetic and molecular understanding of AF pathogenesis but also guide novel experimental and therapeutic strategies to treat AF.

^{*}Speaker

Keywords: cardiomyopathy, arrhythmia, atrial fibrillation, aging, atrial like cardiomyocytes

Chromosomal Instability-induced Cell Invasion through Caspase-driven DNA Damage

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Genomic Instability is a known hallmark of almost every solid tumor. Epithelial cancers, better known as carcinomas, like that of the prostate, lung, and breast amongst others are frequently correlated to numerous genomic instabilities. One such instability is that of Chromosomal Instability (CIN), characterized by an enhanced rate of change of chromosome number and structure, and is persistently found amongst the metastases of carcinomas. CIN helps in driving the gain and loss of proto-oncogenes and tumor suppressors respectively and additionally contributes towards rapid tumor evolution and increases the probability for a tumorigenic mass to metastasize. Metastasis arises due to a cascade of multiple events starting with the invasive behavior of cells, which is characterized by the movement of cancerous cells into a mosaic of non-cancerous tissue. The molecular insights of invasive behavior have recently gained attraction and are being actively pursued. In this study, we present a *Drosophila* model of invasion developed by inducing CIN in the wing epithelium. A tissue with, otherwise, strictly maintained compartment boundaries is breached upon induction of CIN and causes these cells to invade the surrounding non-cancerous tissue. The tumorigenic mass presents tissue hyperplasia, epithelial structure disruption, and active cellular dynamics of invasive cells in the tissue microenvironment. c-Jun N-terminal kinase (JNK) has previously been described as a major regulator of most of these characteristics, however, the sheer lack of an invasive model occluded the unearthing of the molecular dynamic governing the phenomena of invasion. In this study, we show how CIN leads to DNA Damage as a consequence of JNK-mediated transcriptional activation of proapoptotic genes and subsequently, caspases. We furthered our study by showing how this activation is sustained by an autocrine feed-forward loop mediated by ligand-dependent JAK-STAT activation. A cytokine homologous to interleukin-6, Upd3, controls this cascade of events and maintains caspases at a sub-lethal level, promoting tissue invasiveness by enhancing CIN-induced DNA damage. This body of work unveils effector caspases and JAK/STAT pathway as potential therapeutic targets towards effectively impeding CIN-induced metastasis.

Keywords: Chromosomal Instability, Cancer, Metastasis, Invasion, JNK, Upd3, JAK, STAT, Caspases, DNA Damage

*Speaker

Deregulations of miR-1 and its target Multiplexin promote dilated cardiomyopathy associated with myotonic dystrophy type 1

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Myotonic dystrophy type 1 (DM1) is the most common muscular dystrophy in adults. DM1 is autosomal dominant disease caused by an expansion of CTG repeats in 3'-untranslated region of the *dystrophin myotonic protein kinase* (*DMPK*) gene. Healthy individuals have 5 to 37 CTG. Whereas DM1 patients carries from > 50 to ≈4,000 CTGexp. The mutated *Dmpk* transcripts affects the functions of RNA-binding factors with adverse effects on alternative splicing, processing, and stability of a large set of muscular and cardiac transcripts. Among these effects, inefficient processing and down-regulation of muscle- and heart-specific miRNA, *miR-1*, have been reported in DM1 patients, but the impact of reduced *miR-1* on DM1 pathogenesis has been unknown. Here, we use Drosophila DM1 models to explore the role of *miR-1* in cardiac dysfunction in DM1. We show that *miR-1* down-regulation in the heart leads to dilated cardiomyopathy (DCM), a DM1-associated phenotype. We combined *in silico* screening for *miR-1* targets with transcriptional profiling of DM1 cardiac cells to identify *miR-1* target genes with potential roles in DCM. We identify *Multiplexin* (*Mp*) as a new cardiac *miR-1* target involved in DM1. *Mp* encodes a collagen protein involved in cardiac tube formation in Drosophila. *Mp* and its human ortholog *Col15A1* are both highly enriched in cardiac cells of DCM-developing DM1 flies and in heart samples from DM1 patients with DCM, respectively. When overexpressed in the heart, *Mp* induces DCM, whereas its attenuation rescues the DCM phenotype of aged DM1 flies. Reduced levels of miR-1 and consecutive up-regulation of its target *Mp/Col15A1* might be critical in DM1-associated DCM.

Keywords: drosophila. dilated cardiomyopathy. miR, 1, multiplexin, collagen15. myotonic dystro-

*Speaker

phy type 1

EyaHOST: A modular dual binary-expression system for investigating tumor-host interactions in vivo

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The cancer field has attained a deep understanding of cell-intrinsic pathways driving and supporting tumor growth. We know comparably less about how healthy tissue interacts locally or remotely through signalling networks or metabolism to ultimately influence the fate of cancer cells. The exploration of the molecular underpinnings of tumour-host interactions remains constrained in part due to lack of effective tools to address these interactions. In this work, we have generated a modular fruit fly model for genetic manipulation of host tissue independently of tumour generation. Through a dual binary expression system approach (QF-QUAS and GAL4-UAS), we have made avatars for the study of any gene in genetically normal epithelial stromal microenvironment, hemocytes, fat body, muscle, or whole organism, in a *RasV12*, *ScribIR* Eye-antennal disc tumour context. In this model, we replicate previous findings that an autophagy stress response is induced by *RasV12*, *ScribIR* tumors and is required in the host for tumour growth. Furthermore, we have conducted pilot genetic screens which have identified novel players that are potentially involved in tumour-host crosstalk to support tumor growth. We believe this genetic model will serve as a powerful platform for large scale screening of genes important in host cells to influence tumour growth, and ultimately highlight important processes for future therapy design.

Keywords: Tumour, host, Cancer, tumour, Cachexia, Cell competition, Supercompetition, Microenvironment

^{*}Speaker

Investigating pathogenic human CDK19 variant functions in *Drosophila* reveals insight into normal and disease mechanisms of Cdk8/19 family proteins

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Drosophila cyclin-dependent kinase 8 (Cdk8), the orthologue of vertebrate CDK8 and CDK19, is a threonine/serine kinase that is best known to function with the Mediator Complex to regulate gene expression. Patient mutations affecting *CDK19* are associated with a complex syndrome in which clinical features include epilepsy and neurodevelopmental delays. Using flies to investigate Cdk8 family function, we found that depletion of *Cdk8* severely reduces lifespan and causes bang sensitivity and impaired mobility. We also found that Cdk8 can modulate mitochondrial morphology under physiological conditions. Depletion of *Cdk8* leads to an elongated mitochondrial phenotypes and mitochondrial dysfunction, whereas expression of Cdk8 leads to a fragmented phenotype, like what is seen with modulation of Drp1, a protein required for mitochondrial fission. Of note, these defects were rescued by expression of either wildtype or cytoplasmically-targeted human CDK19, implicating a non-nuclear function of CDK19/Cdk8. De novo patient mutations in CDK19 enhance mitochondrial fusion in neurons and muscles due to the depletion of *Cdk8*, demonstrating that they cause dominant negative effects. We found that Cdk8 promotes phosphorylation of Drp1 and that it is required for proper fission of mitochondria. Cdk8 and Drp1 associate in the cytoplasm of fly larval and adult muscles and S2 cells, demonstrating a non-nuclear role of Cdk8 family members. Preliminary data from patient-derived fibroblasts reveals similar elongated mitochondrial phenotypes, consistent with our findings in flies. To test whether mitochondrial dysfunction underlies the cellular phenotypes, we raised Cdk8-depleted flies on a diet containing the clinically approved antioxidant NACA and observed rescue of climbing defects and mitochondrial fusion phenotypes, establishing elevated ROS as one of the key hallmarks of Cdk8 dysfunction. We found a compelling further link between Cdk8 and familial Parkinsonism, which is due to defective mitochondrial quality control. Elevated Cdk8 could rescue mitochondrial and climbing defects in a fly model of Parkinsonism by promotion of Drp1 activity. These findings implicate Cdk8 family members in rare neurodevelopmental syndromes and also point to a potential therapeutic direction for Parkinsonism.

^{*}Speaker

Keywords: Disease model, Cdk8/Cdk19, mitochondrial dynamics, Drp1, Parkinsonism

Lamp1, lipid transport, and Parkinson

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Aging-associated diseases are an increasing socio-economic burden despite efforts to improve healthspan. Pathologies that cause degeneration of the nervous system are particularly devastating, and in many cases are associated with lysosomal malfunction and a decline in proteostasis. Prime examples are Parkinson and Alzheimer's disease that are characterized by changes in the pH of the endolysosomal system causing accumulation of insoluble protein aggregates that lead to neuronal decay. In addition to their role in proteostasis, Lysosomes are also important regulatory hubs that integrate nutritional signals and participate in lipid metabolism. Therefore, many lysosomal storage and neurodegenerative diseases are associated with alterations in lysosomal pH and accumulation of lipids, particularly cholesterol in the lysosome. *Drosophila* Lamp1 is a bona fide homolog of the mammalian LAMP1/2 proteins that have partially redundant roles in autophagy and cholesterol assimilation and are required for lysosomal integrity. Consistently, we find that *Drosophila* Lamp1 localizes to late endosomes and lysosomes. In contrast to *Lamp1/2* in mice, Lamp1 is not required for development, autophagy, or viability, although mutant males have a reduced mean lifespan. However, *Lamp1* mutant larvae have elevated levels of sterols and diacylglycerols, indicating functions of Lamp1 in lipid assimilation and transport. Most intriguingly, *Lamp1* deficiency results in an increase in acidic organelles in the endolysosomal system, thus identifying a novel role for Lamp1 the regulation of the lysosomal pH. The functions of Lamp1 in endolysosomal acidification and lipid metabolism are reminiscent of the functions of Glucocerebrosidase 1 (GBA1), the major, age-dependent risk factor for Parkinson, suggesting a connection of Lamp1 to neurological disorders. Indeed, we find that mutation of *Lamp1* in adult flies enhances the progressive locomotor defects induced by the expression of mutant α -synucleinA30P in brain dopaminergic neurons. We thus have identified a novel role for Lamp1 in protecting flies from neurotoxicity in an established Parkinson model, which we will further elucidate on.

Keywords: Neurodegenerative disease, lysosome storage disease, lysosome, autophagy, stress, lipid transport

*Speaker

Sickness sleep: Gut hyperplasia disrupts normal sleep and affects brain circadian outputs in *Drosophila*

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Gastrointestinal disorders including inflammatory bowel syndrome (IBS) and colorectal cancer (CRC) are increasing worldwide. While a copious amount of work has been devoted to unravelling the mechanism and effects of these pathologies in the tissue they inhabit, less is known about the repercussions of gastrointestinal disease in distant organs and tissues. The brain and central nervous system (CNS) are intimately related to the gastrointestinal tract through the gut-brain axis. Disruption of intestinal homeostasis can generate profound changes in behaviours, such as sleep and locomotor activity. The mechanism mediating such phenomenology remains largely unknown. Here, we have successfully modelled behavioural disruptions as a consequence of gut hyperplasia in *Drosophila melanogaster*. Our results suggest that intestinal stem cell (ISC) hyperproliferation caused by oncogenic stress or acute damage of the adult intestine, disrupts the amount and quality of sleep in *Drosophila* as well as various behavioural and central circadian outputs. Through genetic analysis of gut derived peptides and their cognate receptors in the brain, we are beginning to unveil gut/brain signaling mechanisms that may explain how critical illness of peripheric tissues impacts CNS controlled behaviours.

Keywords: Gastrointestinal disorders, Intestinal stem cells, sleep, gut brain axis

*Speaker

Spastin elevating approaches and their validation in *Drosophila melanogaster* models of Hereditary Spastic Paraplegia type 4 (SPG4-HSP)

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Hereditary spastic paraplegias (HSPs) are a group of motor neuron disorders, characterized by a progressive spasticity and weakness at the lower limbs. Although mutations in more than 85 genes were found to be associated with HSP, the most frequent affect SPG4 gene and display autosomal dominant inheritance. SPG4 encodes spastin, a microtubule severing ATPase which controls microtubule network dynamics affecting cell division, intracellular membrane trafficking and, axonal transport. There is no cure to alleviate motor neuron degeneration in HSP. Since SPG4 haploinsufficiency is thought to be responsible of disease, restoring spastin physiological levels in SPG4-HSP patients might represent a promising therapeutic approach. Thus, the aim of our work is to identify the molecular machinery involved in the regulation of spastin protein stability to find a way to slow down its turnover. Recently in our laboratory, it has been shown that the kinase HIPK2 phosphorylates spastin at serine S268 and this prevents its polyubiquitylation and proteasomal-mediated degradation. Further, we observed in human cell line (preliminary results) that a Cullin 4-Ring-ubiquitin-Ligase complex may be involved in spastin degradation. Here, we have established a *Drosophila melanogaster* model of SPG4 haploinsufficiency, by RNAi-mediated downregulation of spastin in fly. By exploiting *Drosophila* genetics, we have studied the effects of spastin downregulation in different fly tissues and in different types of neurons. Accordingly, we show that the *in vivo* inhibition of Cullin 4-Ring-ubiquitin-Ligase complex, which is highly conserved in *Drosophila*, significantly affects spastin-mediated phenotypes. We have demonstrated that Cullin 4 silencing considerably rescues alteration of neuromuscular junction morphology and locomotor defects of spastin-deficient flies. Finally, we are now assessing the possibility of using this *Drosophila* model of HSP to perform drug screening to identify drugs able to prevent spastin degradation.

Keywords: HSP, spastin, disease model, cullin 4, neuromuscular junction, locomotor defects.

*Speaker

Unmasking the competitive loser status of tumours with ribosomal mutations.

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Ribosomes are the most abundant protein complexes in cells and single-copy mutations in most ribosomal protein genes (RPGs) have a dominant effect, which causes numerous cellular pathologies including insufficient protein synthesis, nucleolar stress, p53 activation, the accumulation of toxic protein aggregates and proteotoxic stress and chronic activation of stress signalling pathways. Furthermore, when epithelial cells with single-copy loss in a RPG are present in epithelial tissues in a mosaic fashion with wild-type cells, they behave as loser and are eliminated by wild-type cells through non-cell autonomous induction of apoptosis. This process is known as Minute cell competition, as it was discovered in *Drosophila* where RPG mutations are called Minute. Minute cell competition is thought to act as a quality control mechanism to clear tissues from aberrant cells. Surprisingly, recent bioinformatics analysis shows that heterozygous RPG deletions can be found in a staggering 43% of all cancers sequenced across 24 different tumour types. This is likely an inevitable consequence of cancer genome instability. Given the profound cellular malfunctions associated with single-copy RPG loss, it is surprising that RPG loss is so frequent in tumours. How do so many cancers then thrive despite being ribosome mutant? Genome sequencing data shows that despite being present in 43% of tumours, single-copy RPG mutations are still under-represented in human cancers and are often co-deleted with tumour suppressors, suggesting that RPG loss is detrimental and is compensated for by other mutations in tumours. Identifying these compensatory mutations could offer an opportunity to expose those vulnerabilities therapeutically. To this end, we undertook a human cancer bioinformatics analysis on 15 different tumour types and identified a list of genes and pathways associated with single-copy RPG loss common to multiple tumour types. We are currently screening these genes and pathways in *Drosophila*, a prime model for the study of cell competition, to discover cancer-associated mutations modifying Minute cell competition. With this approach our goal is to identify cancer-specific genes that mask the loser status of cancer cells and thus could constitute cancer vulnerabilities that could be exploited therapeutically. Both the bioinformatics work on human cancers and progress on the ongoing screen will be presented at the meeting.

Keywords: Cell competition, Cancer genetics and genomics, Minute

*Speaker

Cell stress, growth, proliferation &
death

A cross talk between p53 and the cell cycle regulates apoptotic induction and tumor formation in *Drosophila*

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A precise coordination between cell proliferation and apoptosis is essential to maintain tissue homeostasis and to prevent tumor formation after DNA damage. Cells respond to this threat by arresting the cell cycle and activating the DNA repair mechanisms or by inducing apoptosis. These "life" *vs* "death" cell fate decisions are often regulated by the tumor suppressor gene *p53*. However, how the proliferating status of the cell can influence p53 cell fate decision making, is mostly unexplored. In this work, we address this important question by studying the apoptotic response after DNA damage in both experimentally arrested and endocycle-induced cells of the wing imaginal disc of *Drosophila*. The endocycle is of special interest as it is a modified cell cycle that alternates G and S phases without entering mitosis through the downregulation of Cdk1 activity. Remarkably, we show that irradiation-induced apoptosis is suppressed in these cell cycle arrested and endocycle-induced cells. Specifically, we found that the ability of p53 to bind and to transcriptionally activate the expression of the pro-apoptotic genes is regulated by the G2/M promoting factor Cdk1. Moreover, we describe the physical interaction between Cdk1 and p53 and its influence on apoptotic induction.

In addition, we study the tumorigenic potential of p53 when its apoptotic role is inhibited. We find that cells with p53 that have inhibited its apoptotic potential acquire a persistent activity of the JNK pathway, which drives them into a senescent-like status and induce the non-autonomous overgrowth of the surrounding tissue.

These results lead us to propose a model in which cell cycle progression and p53 pro-apoptotic activity are molecularly connected to coordinate the appropriate response after DNA damage.

Keywords: DNA damage, apoptosis, cell cycle, p53, JNK, Cdk1

*Speaker

An autophagy-related phagocytosis pathway in glia promotes axon debris clearance and fly survival after nervous system injury

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Glial engulfment of dead neurons and neurites after trauma, during development and in neurodegenerative diseases plays a crucial role in nervous system maintenance. Axon debris generated after traumatic injury is cleared by phagocytic glia via Draper receptor signalling in *Drosophila*. However, mechanisms governing the efficiency of axon debris degradation have remained largely unexplored. We find that LC3-associated phagocytosis (LAP), an engulfment pathway assisted by certain components of the macroautophagy machinery, promotes clearance of degenerating axons by glial cells both in the periphery (wing nerve) and the brain. A LAP-specific subset of autophagy-related (Atg) genes functions in glia to aid debris elimination, encoding members of the Atg8a (LC3) conjugation system and the Vps34 lipid kinase complex subunits UVRAG and Rubicon but not Atg14 or the Atg1 kinase complex. We find that Rubicon and Atg8a lipid conjugation on debris-containing phagosomes is essential for efficient clearance of engulfed axon fragments, and Rubicon overexpression in glia accelerates axon debris elimination. Finally, LAP promotes survival of animals following traumatic brain injury. Our results reveal a critical role of LAP in glia in the clearance of neuronal debris *in vivo*, with important implications for the recovery of the injured nervous system.

Keywords: autophagy, glia, neuron injury, phagocytosis

^{*}Speaker

Caspases promote tissue regeneration by compensatory proliferation

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Apoptosis is a major form of programmed cell death (PCD) that eliminates unnecessary and potentially dangerous cells in all metazoan organisms, thus ensuring tissue homeostasis and proper development. Apoptosis is executed by the activity of a unique family of cysteine proteases called caspases which are activated in a proteolytic cascade, such that the initiator caspases (e.g., caspases 8 and 9) cleave and activate the executioner/effector caspases (e.g., caspases 3 and 7), which in turn carry out the mass proteolysis that leads to apoptosis. However, in addition to their established role in apoptosis, an ever-growing list of studies is suggesting the essential role of caspases in ensuring nonlethal cellular functions during normal development, tissue repair, and regeneration. Since most caspase-dependent non-lethal cellular processes (CDPs) can only be investigated *in vivo*, in specific cell types, tissues, and organs, progress in identifying and characterizing new CDPs has been relatively slow. However, the recent technical advancements and the latest findings are assigning an unanticipated biological significance to these nonapoptotic functions, providing new insights about the efficiency and risks of current therapies for diseases, such as cancer and neurodegenerative diseases, as well as offering new directions for therapeutic interventions. Compensatory proliferation (CP) is a phenomenon of tissue regeneration after lethal stress or developmental cell death. That tissue regeneration occurs in *Drosophila* imaginal discs following massive radiation stress has been known for decades. Several mechanisms have been suggested to explain this phenomenon; most notably is the phenomenon of ‘apoptosis-induced proliferation’ (AiP), which is mediated by the initiator caspase-9-like Dronc, and through which dying apoptotic cells signal to their neighboring cells to proliferate and replace them. However, since these mechanisms have mainly been investigated using non-physiological conditions (i.e., by generating ‘undead cells’ that are induced to undergo apoptosis but kept alive through inhibition of the effector caspases), the physiological mechanisms behind CP in the imaginal discs, as well as the extent or possible involvement of AiP mechanisms in CP, remained to be discovered. Here, I will present our new findings about the physiological mechanisms underlying CP and the extent of resemblance with known AiP paradigms.

Keywords: Compensatory proliferation, cell death, caspases, apoptosis, induced proliferation

^{*}Speaker

Deciphering the role of Histidine during early colorectal cancer

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Intestinal stem cells (ISCs) play a critical role in maintaining tissue homeostasis by regulating intestinal turnover. They have the ability to self-renew or differentiate into specialized cells in response to environmental cues such as nutrient availability, ensuring the appropriate balance of ISCs versus differentiated cells. Notably, the host laboratory has recently discovered a mechanism that couples nutrient-dependent control of ISC fate regulation. However, the dysregulation of stemness and differentiation programs is a hallmark of colorectal cancer (CRC), and the mechanisms by which nutrient signaling influences these programs during early CRC are not well understood. As distinct tumorigenic genotypes display different fate profiles, I hypothesise that nutrient-dependent control of ISC fate differentially modulates phenotypes of CRC associated gene variants.

During a screening for individual nutrients that affect the phenotype of oncogenic ISCs, I have observed that Histidine inhibits Apc,Ras tumorigenic phenotype. Upon further analysis, I discovered that Histidine plays a significant role in regulating the ISC differentiation program. These preliminary findings suggest that Histidine metabolism is involved in maintaining the balance of ISC programs, potentially providing protection against early CRC.

Keywords: intestinal stem cells, Apc, Ras, midgut

*Speaker

Revealing the mechanism behind the RpS12 dependent regulation of Xrp1 in Minute cells and its implications in Cell Competition.

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Cell competition phenomenon, where cells are eliminated via a non-autonomous mechanism in genetic mosaics but not in a homogeneous environment, was first documented in *Drosophila* between wild-type cells and cells with heterozygous dominant mutations in ribosomal protein (*Rp*) genes (known as *Minute* genes). In *Drosophila* 66 of the 79 *Rp* genes belong to the Minute class. In cells with *Minute* mutations, RpS12 protein (encoded by a non-Minute *Rp* gene), via an uncharacterized mechanism activates the Xrp1 transcription factor. Xrp1 is responsible for the cellular responses in Minute cells, including reduced cell competitiveness, slow growth, reduced translation, and even for the developmental delay that Minute flies present. Interestingly, the RpS12-Xrp1 pathway is responsible for the competitive elimination of some aneuploid cells in mosaics, since the loss of chromosomes can lead to *Rp* gene haploinsufficiency. In addition, Xrp1 and cell competition have been found to result from multiple other genetic insults, other than *Rp* mutations, highlighting the significance of unraveling the mechanisms of Xrp1 activation. Here, I will present unpublished work from my research group that reveals the mechanism of Xrp1 regulation by RpS12 in Minute cells. Our findings contribute to the elucidation of the mechanisms of the Xrp1 pathway in cell competition, and additionally to the understanding of the underlying causes responsible for the Minute phenotype.

Keywords: cell competition, ribosomal proteins, Xrp1 pathway, RpS12, Minutes

*Speaker

Role of c-Jun N-terminal kinases (JNK) signalling in balancing cell removal and renewal in a stressed epithelium

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The development of functional tissues relies on the fine balance between cell death and cell division. Little is known about how tissue size and cell numbers are maintained in stress situations, when additional proliferation is needed to compensate for cell loss. The c-Jun N-terminal kinases (JNK) signalling pathway is a stress pathway that has been proposed to trigger not only cell death, but also cell survival and proliferation. To decipher the regulatory events that control these diverse responses, we have set out to characterise the spatial-temporal dynamics of cell fate (proliferation, cell death, cell cycle arrest and migration) upon JNK activation. We optogenetically activate signalling in a subset of cells (*salmon* expression domain) by expressing either a constitutively active form or the wild-type version of the JNK Kinase *hemipterous* (*hep*). Then, we reconstruct the cell-autonomous and non-autonomous response to stress signalling. The response is very fast: we observe cell death and cell cycle arrest within two hours of Hep activation. However, the response lasts for an extended time, with a population expressing low JNK levels being observed 24 hours after the light pulse. We reconstructed the pattern of gene expression in time and space, using both biased and unbiased approaches. First, we used the Hybridization Chain Reaction to visualise the expression of genes known to be regulated by JNK signalling, such as Wg, Dpp, JAK-STAT and ERK. Finally, we performed single-cell and bulk RNA sequencing to identify the genes that could potentially account for the varied effect of JNK signalling activation. We are in the process of deciphering how this pathway orchestrates the replacement of lost cells, thus ensuring the maintenance of a functional epithelium. We hope that our results will guide further studies of more complex tissues of direct biomedical relevance.

Keywords: Stress signalling, Cell death, Proliferation, Tissue Homeostasis

*Speaker

Stress-induced organismal death is genetically programmed by the Zeste-Phae1 axis

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All organisms are exposed to various stressors, such as heat and cold. These stressors sometimes induce organismal death depending on their intensities. Although stress-induced organismal death is observed in many living organisms including humans, the underlying molecular mechanisms remain unclear. Here, we focused on a chymotrypsin-like serine protease *Phaedra1* (*Phae1*) as a death mediator of the fruit fly *Drosophila melanogaster*. RNA-seq and subsequent quantitative PCR analyses revealed that *Phae1* expression was upregulated by lethal heat stress (40 °C) but not non-lethal heat stress (38 °C or lower). While *Phae1* expression was induced after lethal heat stress in various tissues, the most significant induction of *Phae1* was observed in the central nervous system (CNS). Neuron-specific knockdown of *Phae1* significantly increased survival rate and reduced neuronal caspase activity after lethal heat stress. These results suggest that the transcriptional upregulation of *Phae1* in the CNS is essential for stress-induced organismal death. Additionally, we showed that a 10-bp region in the *Phae1* promoter/enhancer was responsible for stress-induced *Phae1* expression. The 10-bp sequence was predicted to contain a consensus binding site of a transcription factor *zeste* (*z*). Consistent with the prediction, Z protein physically interacts with *Phae1* promoter region. Loss of *z* function significantly reduced *Phae1* protein levels in the CNS, resulting in an increased survival rate after lethal heat stress. Taken together, these results suggested that *z* is critical in stress-induced organismal death through *Phae1*. Our study demonstrates that the Z-Phae1 axis genetically programs stress-induced organismal death.

Keywords: stress, organismal death, protease

*Speaker

The contribution of compensatory growth to cell competition and tissue invasion

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Tissue regeneration is widespread among animal species. Following cell death, regeneration requires compensatory proliferation of the surviving cells to replace lost cells. I study the coordination between proliferation and cell death during epithelial development and in cell competition. I use the wing imaginal disc in order to address the spatio-temporal regulation of regeneration following cell death with single cell resolution. The localization of regeneration is important because it can impact tissue shape, and thus the function of the adult tissue. In order to study the response of the tissue to localized cell death, I transiently express the pro-apoptotic gene *reaper* in clones. Using a quantitative approach, I show that cell proliferation is not locally increased around dying clones, but rather globally upregulated on the level of the whole tissue. This burst of proliferation is transient and resolves within ten hours after the end of death induction. Interestingly, adult wing size is not affected, indicating that compensatory proliferation ensures the robustness of development after cell death. To further study the link between compensatory proliferation and developmental robustness, I transiently induce cell death in the anterior compartment of the wing disc, using the *cubitus interruptus* driver. I show that after an initial loss of tissue proportions between the anterior and posterior compartments (the anterior compartment drastically decreasing in size), there is a partial recovery of proportions after an increase in proliferation at larval stage. Interestingly, tissue proportions are not fully recovered in the adult wing, while total wing size is rescued, indicating an uncoupling between tissue size and shape regulation. I am currently investigating the nature of the pro-proliferative signal (mechanics/secreted factors) giving rise to compensatory proliferation. Furthermore, since cell death occurs in cell competition, I wish to determine the contribution of compensatory proliferation to winner cell expansion and tissue invasion. Cell competition is the context-dependent elimination of unfit cells (so-called loser cells), that are progressively replaced by winner cells. Using a cell competition scenario where Discs large is depleted in loser clones, I show that winner clones grow more in presence of loser Dlg-depleted clones. Similar to clone apoptosis, I do not observe a local increase in proliferation of winner clones in proximity to loser clones. I am currently investigating whether winner and loser cells have a different sensitivity to compensatory signals, which could be a novel mechanism for tissue invasion by winner cells.

Keywords: regeneration, apoptosis, compensatory proliferation

*Speaker

The new glial phagocytic receptor Santa-maria acts with SIMU in recognition and engulfment of apoptotic neurons

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Development of the central nervous system (CNS) involves elimination of superfluous neurons through apoptosis and subsequent phagocytosis by glial cells. Phagocytic glia must distinguish between living and dying neurons with high level of specificity, which is achieved through the function of phagocytic receptors that recognize ‘eat me’ signals exposed on apoptotic cells. In mammals, phagocytic receptors are highly redundant, making it difficult to study their function *in vivo*. In *Drosophila*, two glial transmembrane phagocytic receptors, Six-Microns-Under (SIMU) and Draper (Drpr), have been previously shown to mediate glial phagocytosis of apoptotic neurons during embryogenesis. Importantly, in the double mutant *simu;draper* embryos phagocytic glial cells still engulf apoptotic neurons suggesting involvement of additional receptors. Here, we discovered a new role for the transmembrane receptor Santa-maria, a *Drosophila* CD36 homologue, which was shown previously to act in retinoid formation in neurons and glia of adult brains. We found that Santa-maria is specifically expressed in embryonic phagocytic glia and plays a major role in removal of apoptotic neurons during CNS development. Our data demonstrate that Santa-maria, like SIMU, binds apoptotic cells *in vivo* and *in vitro*, and is involved in the recognition and engulfment steps of glial phagocytosis. We show that Santa-maria and SIMU physically interact, thus, acting together as a complex to execute the phagocytosis process. Moreover, we discovered that in the absence of all three glial phagocytic receptors SIMU, Drpr and Santa-maria, phagocytosis of apoptotic neurons is abrogated causing lethality that illuminates the vital role of apoptotic cell clearance in the developing CNS. Interestingly, we revealed that Croquemort (Crq), Santa-maria’s closest homologue expressed in embryonic macrophages but not in glia, could replace Santa-maria’s function in glial cells supporting the notion that different complexes of phagocytic receptors may act in specific phagocytic cell populations and biological compartments.

Keywords: cells death, apoptosis, phagocytosis, glia, CNS, Santa, maria, SIMU, Draper, embryogenesis, development

^{*}Speaker

Turandot proteins promote resilience to stress by preventing antimicrobial peptides-dependent tracheal cell death

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Turandot (*Tot*) genes encode a family of eight small secreted proteins found in the hemolymph of *Drosophila melanogaster*. These genes are found highly expressed in response to a variety of stresses, as diverse as heat exposure, bacterial and viral infection, neurodegeneration or metal poisoning. *Tots* are transcriptional targets of several stress pathways, including the IMD, JAK/STAT and p38 MAPK pathways. For this reason, *Tots* have been extensively used as readouts of the stress response. Despite this large interest, Turandot genes function in the stress response remains unknown. We have generated a *Drosophila* fly line that lacks 6 *Tots*. These *Tot* mutant flies are susceptible to several challenges, including, starvation, bacterial infection and osmotic stress. Turandot proteins secreted from the fat body bind to the tracheal system. Tracheas from *Tot*-deficient flies undergo apoptosis, leading to a reduced number of terminal tracheal cells and tissue hypoxia. In this context, increasing atmospheric oxygen levels or blocking trachea apoptosis restores *Tot*-deficient flies' resilience to stress, showing that oxygen delivery is limiting in these mutants. Tracheas susceptibility to stress is not restricted to *Tot*-deficient flies. Indeed, tracheal apoptosis also happens, to a lesser extent, in wild-type animals exposed to osmotic stress or immune challenge. This process is mediated by antimicrobial peptides (AMP) that target and kill tracheas in a phosphatidylserine-dependent manner. Importantly, deleting several AMPs in *Tot*-deficient flies restores their tracheation and rescues their susceptibility to stress. *In vitro*, TotA inhibits the pore-forming activity of several AMPs and prevents subsequent membrane lysis. Collectively, our data demonstrate that the tracheal system is a critical tissue in the stress response. The phospholipid composition of tracheal membranes makes them prone to autoimmune targeting by AMPs. *Tots* cytoprotect tracheas by preventing AMP-induced cell death. Therefore, Turandot proteins foster resilience by mitigating collateral damages caused by AMPs on host tissues during the stress and immune responses.

Keywords: stress response, cytoprotection, antimicrobial peptides, immunity, tracheas

*Speaker

Population genetics & evolution

Assimilation of an acquired character by plasticity induced genetic and epigenetic inheritance

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Since Waddington’s demonstration of the inheritance of an acquired trait by genetic assimilation, there has been an intense debate about the role of phenotypic plasticity in evolution. Plasticity is proposed to induce the establishment of heritable epigenetic alleles that contribute to evolution by genetic assimilation. We recapitulate Waddington’s assimilation of an acquired character experiment using a set of inbred and outbred populations of flies to address the role of phenotypic plasticity and epigenetic inheritance in genetic assimilation. The population of flies used for the experiment exhibit spontaneous ectopic veins at low penetrance levels, giving us a unique opportunity to test the effect of plasticity in the assimilation of ectopic veins by selection upon a heat shock induction or without it (“assimilated” and “non-assimilated”, respectively). Interestingly, the ectopic veins were assimilated at high penetrance levels after a few generations of selection for both the assimilated and non-assimilated in inbred and outbred flies. We combine transcriptome, epigenome, and genomic sequencing analysis to uncover the underlying genetic and epigenetic changes responsible for the evolution of ectopic veins in these flies. The assimilated and non-assimilated inbred flies share the fixation of genetic alleles that provoke the deregulation of the *Cad96Ca* gene which we show to have a previously uncharacterized function in wing vein development. Furthermore, the flies selected under heat-shock treatment showed the deregulation of additional genes that generate a stronger ectopic veins phenotype. We could not identify mutations associated with these genes in the assimilated flies that may explain their deregulation, suggesting a putative causal role of epialleles associated with these genes. In the outbred populations, flies have evolved by differential selection of alleles already present in the original populations that induce population-specific regulatory changes in different wing developmental genes. We propose that phenotypic plasticity plays a crucial role in evolution through genetic assimilation, which may induce the establishment of heritable epialleles as well as expose otherwise naturally cryptic alleles.

Keywords: adaptation, genetic assimilation, phenotypic plasticity, epigenetic inheritance, Waddington

*Speaker

Dynamics of multitrophic interactions in a specialist's ecological niche

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All organisms belong to ecological communities in which different species cohabit and establish multitrophic interactions such as mutualism, competition and predation. To understand the niche adaptation of a species, we require knowledge of all of these interactions. *Drosophila sechellia* is a powerful model to study the evolutionary and mechanistic basis of niche adaptation. This drosophilid is closely-related phylogenetically, anatomically and genetically to the cosmopolitan generalists *D. melanogaster* and *D. simulans*. However, *D. sechellia* is endemic to the Seychelles islands and is a specialist, feeding and reproducing exclusively on *Morinda citrifolia* "noni" fruit, which, at some ripening stages, is toxic for other drosophilids and insects. Our research work addresses the following questions: how does specialization on a toxic fruit impact interactions of *D. sechellia* with other species? Are there traits associated with these interactions that have been lost or gained in *D. sechellia*, in comparison to the generalist *D. melanogaster* and *D. simulans*? What is the selective advantage of specialization on noni? To answer these questions, we have analyzed the behavioral responses of *D. sechellia* towards selected species including the noni host itself, competitor drosophilids and parasitoid wasps. Using ecologically-relevant behavioral paradigms we have found that *D. sechellia* prefers a narrow time window of the noni host that is highly toxic for its competitor *D. simulans* and its predator *Leptopilina boulardi*. Using *D. sechellia* olfactory receptor mutant animals, together with an analysis of volatiles of different ripening stages of noni, we have identified potential olfactory pathways underlying *D. sechellia*'s preference for this ripening stage. Moreover, our results indicate that by laying eggs on ripe noni fruits, *D. sechellia* avoids competing with the more fecund *D. simulans*, which prefers the less toxic overripe fruits. Together our data suggest that the evolution of *D. sechellia* exploitation of a very narrow time window of its host is a powerful way for this species to increase its fitness in an ever-changing ecological community.

Keywords: *Drosophila sechellia*, behavioral ecology, interspecific interactions, niche adaptation

^{*}Speaker

Engrailed anterior compartment expression and the evolution of pre-adaptive novelty

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The re-use, or co-option, of developmental genes in new organs forms the base of many evolutionary novelties. Although there is abundant research on gene co-option, few studies have considered the functional consequences of full gene network co-option. One of the best-characterised co-option cases is the recruitment of the posterior spiracle gene-network to the posterior lobe of the *Drosophila melanogaster* male genitalia. We have found that the posterior spiracle gene network has also been co-opted to the testis mesoderm where it is required for sperm liberation (spermiation), providing a unique example of sequentially repeated developmental co-options to different germ layers. We will present evidence showing that during embryogenesis, *engrailed* (*en*) is activated in the anterior compartment of the A8 segment (A8a). This expression novelty appeared in the Drosophilids before the evolution of the male genital posterior lobe, and is associated to a previous posterior spiracle gene network co-option event to the cells holding the sperm heads, the Head Cyst Cells (HCC). We have discovered that the *cis* and *trans* regulatory elements responsible for *engrailed* expression in the anterior compartment A8 cells are also activated in the testis HCC. CRISPR deletion of the enhancer responsible for anterior A8 *engrailed* expression shows that A8a compartment activation is not required for the spiracle's development, but that this regulatory element is required in the adult testis. Homozygous flies lacking the posterior spiracle *en* enhancer are completely sterile due to sperm degeneration. Our work presents an example where gene network co-option resulted in the generation of a *bona fide* pre-adaptive developmental expression novelty: the activation of the En transcription factor in the anterior compartment of the embryonic A8 segment where, despite having no specific function, has opened the possibility of this important developmental factor acquiring one in the future. We suggest that the expression of *en* in the anterior compartment of the A8 segment, was likely caused by the regulatory "interlocking" of the co-opted networks. We propose gene network interlocking occurs as the result of the use of the same gene network in several organs, so that any change to the network because of its functionality in one organ, will be mirrored in all organs even if it has no selective advantage in some of them.

Keywords: cooption, gene network, organogenesis, preadaptation

^{*}Speaker

Functional impact of SNP alleles in *Eip75B* on life-history evolution

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Pinpointing genetic loci that control natural variation in life-history traits, and correlated responses therein, is critical to understand and predict evolutionary adaptation and health. Rapid technological advances in genome sequencing technologies in the past decade have provided unprecedented possibilities to identify single nucleotide polymorphisms (SNPs) associated with these complex and highly polygenic traits. However, the presence of hitchhiking loci that are genetically linked to causal sites makes it difficult to identify the exact SNPs with functional impact among the huge majority of neutral variants.

SNPs within the nuclear hormone receptor and PPAR γ -homolog *Ecdysone-induced protein 75B* (*Eip75B*) have been linked to the evolution of ageing and life history in the fruit fly. Using RNAi knockdown, we have demonstrated that reduced expression of this gene indeed affects lifespan, egg laying rate and egg volume, but it is unknown how representative these tests are for the functional effects of natural allelic variants. We, therefore, aimed to functionally validate a naturally-occurring SNP variant located within a cis-regulatory domain of *Eip75B* that has been associated with the experimental evolution of longevity and fecundity. For this, we used (1) a Mendelian randomization approach, in which we screened wildtype lines with alternative SNP alleles, and (2) we performed allelic replacement with the precision of a single nucleotide using CRISPR/Cas9. These experiments revealed that this SNP has a significant effect on fecundity and egg-to-adult viability, but not on longevity or other life-history traits. This indicates that, although life-history traits are highly polygenic, individual SNPs can have remarkably large phenotypic and pleiotropic effects. Moreover, our results illustrate the importance of performing functional tests on candidate nucleotide variants to understand the genetic architecture of phenotypic variation in populations.

To facilitate the selection the SNPs for further functional validation, we are currently developing a Combined Annotation-Dependent Depletion (CADD) for *D. melanogaster*, which is a unique analytical framework that combines a wide range of sequence annotations into a single score for functional impact. This tool will help us prioritize the most promising candidate SNPs for further functional validation.

Keywords: life history, SNP, CRISPR/Cas9, evolution, fecundity, longevity

*Speaker

Genetic and morphological basis of brain miniaturization in *Drosophila melanogaster*.

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Inter-species comparisons of the brain in vertebrates often reveal a positive relationship between relative brain size and cognition. However, studies in invertebrates invite us to question this commonly accepted association. Indeed, insect's brains are another extreme example of evolution, this time following a miniaturization trend, rather than growth, while still maintaining cognitive ability rivalling that of many mammals. Brain miniaturization, just like growth, relies extensively on allometric, spatial, and energetic constraints which need to be overcome to maintain functional integrity. Yet, we know very little about the genetic and morphological underpinning of the brain's miniaturization. Here, we use *Drosophila* and its natural genetic diversity to explore the genetic basis of the natural structural and spatial variation of the miniature brain. We developed a high-throughput and sensitive imaging method to quantify brain morphology of diverse fly lines from the *Drosophila* Genetic Reference Panel (DGRP) reared in tightly controlled environmental conditions. We used a micro-computed tomography scanner to image intact fly heads at a 3 micron/voxel resolution and at a population scale. We then train a convolutional neural network to virtually dissect the brain from the head. Unlike standard methods which require brain dissection or slicing, ours preserves the structure of the brain in its native state and allows for true isotropic measurement. This allows us to explore fine-resolution variation of brain morphology arising from natural genetic variation. By taking advantage of the tissue-density dependence of the micro-CT, we define a structural entropy quantity measuring the degree of structural and spatial organisation of the neuropils at a near-cellular level. Using this method, we found more than three-fold difference in absolute and relative brain volume between DGRP lines as well as extensive, genetically regulated variation of structural organization of the brain. The mapping of those structural variations onto the variation in DNA sequence across DGRP lines will enable us to identify the genetic mechanisms influencing brain structure and morphology and decipher the molecular pathways associated with those traits. Furthermore, as predicted by the miniaturization hypothesis, we have found a positive relationship between brain volume and structural entropy at multiple organizational levels. The extensive natural genetic and structural variation of the brain we unravelled compels us to consider more than a single connectome when studying the brain, as well as more than the sequence of individual neurons, but also their spatial organization with respect to the rest of the brain, when studying neuronal circuits.

^{*}Speaker

Keywords: Miniaturization, GWAS, Brain morphology, Neuropils, Evolution

Larvae of both sexes make conserved hyper-long hydrocarbons required for the desiccation barrier at eclosion

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An epicutaneous lipid barrier that protects against water loss is found in all terrestrial organisms. In adult *D. melanogaster*, it is well established that sexually dimorphic cuticular hydrocarbons (CHCs) act not only as pheromones but also as a barrier to dehydration. Here, we show that adult flies at eclosion are more desiccation tolerant than older flies. This surprising early stress resistance correlates with a cuticular blend of hyper long CHCs (27-37 carbons) that is identical in males and females but gets replaced within three days of eclosion with shorter sexually dimorphic CHCs. Hyper long CHCs are synthesized in larval but not adult oenocytes and subsequently stored in the larval fat body. During pupal stages, hyper-long CHCs are mobilized from the larval fat body to the pharate adult cuticle. We identify a larval-specific oenocyte fatty acid elongase (eloHL) that is uniquely required for the synthesis of hyper-long CHCs. Larval-specific knockdown of eloHL, or the pan-CHC biosynthetic enzyme Cyp4g1, is sufficient to ablate the desiccation barrier at adult eclosion. An evolutionary survey of elongases and hydrocarbons across the *Drosophila* genus suggests that sexually monomorphic hyper-long CHCs are more invariant than their shorter sexually dimorphic counterparts. Together, these findings provide evidence that larval as well as adult oenocytes make CHCs, albeit of different chain lengths. They also suggest that sexual selection favours shorter semi-volatile CHC pheromones at the cost of a suboptimal desiccation barrier.

Keywords: Oenocyte, Cuticular hydrocarbons, reproductive trade off

*Speaker

Regulatory evolution tuning pigmentation intensity quantitatively in *Drosophila* wings

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Elucidation of the molecular mechanisms underlying the morphological diversification is a central issue in the field of evolutionary developmental biology. However, it is not well-understood how variation in these mechanisms modulating quantitative phenotypic differences during evolution. Here, we investigate the developmental process tuning quantitative changes in wing pigmentation intensity among closely related fly species. Preliminary work demonstrated that different expression level of *yellow*, one of the genes involved in melanin synthesis, is highly correlated with the wing pigmentation variation ranging from light grey to pitch black among *Drosophila* species. Quantitative changes in *yellow* activity levels could be a consequence of the changes in its upstream regulatory regions. To understand the origin of the variable regulation, we mapped regulatory changes between two sister species with different shades of grey and found that the changes are broadly distributed along its cis-regulatory sequence. Meanwhile, a key enhancer region is highlighted, acting as a knob ensuring diverged wing pigmentation morphology. Our results specify that this single node is sufficient to modulate trait properties quantitatively in a gene regulatory network.

Keywords: transcriptional regulation, regulatory evolution, enhancer

*Speaker

Role of sugar perception in oviposition host shift of the pest *Drosophila suzukii*

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Behavior evolution can promote the emergence of agricultural pests by changing ecological niche. The neuronal modifications underlying this type of evolutionary shift are still poorly understood. The pest *Drosophila suzukii* has shifted its oviposition (egg-laying) niche from fermented fruits to ripe, non-fermented fruits, causing significant damage to fruit crops worldwide. We investigate the chemosensory changes underlying this evolutionary transition and ask whether fruit sugars, which are depleted during fermentation, are important gustatory cues that direct *D. suzukii* oviposition to sweet, ripe fruits. We show that *D. suzukii* has expanded its range of oviposition responses to lower sugar concentrations than the model *D. melanogaster*, which prefers to lay eggs on fermented fruits. The increased response of *D. suzukii* to sugar correlates with an increase in the value of sugar relative to a fermented strawberry substrate in oviposition decisions. In addition, we show by genetic manipulation of sugar-Gustatory Receptor Neurons (GRNs) that sugar perception is required in *D. suzukii* for preference of a ripe substrate over a fermented substrate. Thus, sugar is a major determinant of *D. suzukii*'s choices on complex substrates. Interestingly, calcium imaging experiments in the brain's primary gustatory center (subesophageal zone) show that the dynamic range of sugar-GRNs responses is compressed in *D. suzukii* compared to *D. melanogaster*, suggesting differential processing, encoding and decoding of sugar concentration information. Taken together, our data suggest that evolutionary changes in sugar valuation participate in driving *D. suzukii*'s oviposition preference for sweet, ripe fruits.

Keywords: Behavior, evolution, *Drosophila suzukii*, oviposition, sensory neurons, gustation, sugar perception

*Speaker

Size matters: investigating the genetic and developmental bases of the rapid diversification of male *Drosophila* genital morphology

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External male genital morphology has diverged rapidly among many closely related animal species driven by sexual selection. For example, *Drosophila simulans* and *D. mauritiana*, which last shared a common ancestor only about 240,000 years ago, exhibit striking differences in the size, shape, and bristle composition of male external genital structures, such as the claspers and posterior lobes. It has been shown that the genetic basis of differences in the morphology of the claspers and posterior lobes between these two species is polygenic, however only a few of the causative genes have been identified and the regulation of the development of these genital structures and how this differs between *D. simulans* and *D. mauritiana* is poorly understood. To characterise the genetic and developmental bases of differences in genital structures we have combined mapping and functional genetics to show that *tartan* (*trn*) and *Sox21b* contribute to differences in clasper and posterior lobe morphology, respectively, between *D. simulans* and *D. mauritiana*. Moreover, genetically identical males reciprocally hemizygous for either *trn* or *Sox21b*, and therefore carrying only a working allele from *D. simulans* or *D. mauritiana*, differ in their copulatory behaviour. Analysis of the regulation and interactions of *trn* and *Sox21b* has also allowed us to further characterise the gene regulatory networks for clasper and posterior lobe development respectively. Taken together, our findings provide new insights into the architecture and evolution of the gene regulatory networks as well as the developmental and evolutionary mechanisms underlying rapid phenotypic evolution.

Keywords: phenotypic evolution, gene regulation, tartan, Sox21b, development

*Speaker

The evolution of a complex morphology at a single-cell resolution

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The evolution of animal body form results from genome divergence. How genome divergence is translated into the morphogenetic events that generate new phenotypes is one of the most challenging questions in modern evolutionary biology. We address this question by studying the evolution of male genitalia in *Drosophilids*. Among males of the *D. melanogaster* subgroup of species, many genital structures display striking differences in size and shape. For example, the posterior lobe, a novel anatomical structure that emerged in this clade, diverged extensively from a "hook-shape" in *D. melanogaster* to an elaborated "clam-shell shape" in its sibling species *D. simulans*. Genetic mapping studies revealed that these interspecies differences in genitalia morphology are affected by multiple loci, but the evolved genes and how they coordinately function to generate diverse structures remains unknown. Here, we use single-cell RNA-seq to generate gene expression atlases of genital discs of *D. melanogaster* and *D. simulans* at three developmental time points. By combining unsupervised cell-clustering with published gene expression pattern data, we obtained transcriptomes for each of the anatomical substructures of the genital disc across development. This approach allowed us to elucidate the network components operating in particular genital structures and to compare their expression between the two species. Despite the dramatic change in morphology, we find that the molecular signature of each genital structure is highly conserved. Nonetheless, we were able to identify dozens of genes with species-specific expression in each genital structure. For example, we find that targets of the Toll pathway are expressed specifically in the posterior lobe of *D. melanogaster* and restrict its growth. These data will allow us to identify new genes that are involved in genitalia development and evolution, and the regulatory networks that contributed to the morphological differences between these species.

Keywords: Morphological evolution, scRNAseq, Gene Regulatory Networks, Genital disc, Evo Devo

*Speaker

Neural development, circuits & behaviour

A Sense of Need: How Single Amino Acid Deprivations Remodel Sensory Systems and Behavior

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In order to overcome dietary imbalances, all animals including humans reorganize their behavior to regain nutrient homeostasis. The process of turning a physiological "need" for a specific nutrient to a psychological "want" driving specific food search strategies remains poorly understood. In our work we use single essential amino acid (eAA) deprivations to induce a specific need for protein in *Drosophila*, resulting in increased intake of protein rich food, namely, yeast. To identify the molecular mechanisms by which a lack in eAAs changes protein appetite we used BRB-seq to systematically characterize transcriptional changes happening upon deprivation of each single eAA compared to fully fed condition. Focusing on the head we found that deprivation of any eAA leads to a significant change in transcription, with more transcripts being down-regulated rather than upregulated. Importantly, a significant number of regulated transcripts (203) are shared across all deprivation conditions, indicating common mechanisms of response to deprivation in parallel to the unique effects induced by the lack of each eAA. Not unexpectedly, many of the modulated genes are related to metabolism and protein synthesis. Surprisingly chemosensory receptor genes were also found amongst the consistently diet-modulated genes. This indicates that sensory input can be modulated by eAA deprivation already at the level of receptor expression. Genetic and neuronal manipulations of the affected chemosensory channels indicate that the transcriptional remodeling of the chemosensory systems supports specific changes in behavioral strategies underlying nutrient homeostasis. These results support the concept that dietary challenges alter sensory processing and expand these ideas to include transcriptional remodeling of sensory systems. Our work uncovers the common and unique transcriptional changes induced by all eAA deprivations supporting discovery of novel factors and mechanisms underlying the organism's behavioral and physiological ability to manage dietary challenges.

Keywords: Behavior, diet, nutrient, deprivation, amino, acids, chemosensory, receptors

*Speaker

A novel post-developmental role of the Hox genes underlies normal adult behaviour

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The molecular mechanisms underlying the stability of mature neurons and neural circuits are poorly understood. Here we explore this problem and discover that the *Hox* genes – which encode a family of evolutionarily-conserved developmental regulators, key for axial patterning – are a component of the genetic program that maintains normal neural function in adult *Drosophila*. Using a conditional expression approach we first show that post-developmental downregulation of the *Hox* gene *Ultrabithorax* (*Ubx*) in adult neurons leads to flight anomalies. To investigate the mechanisms underlying this novel role of the *Hox* system we apply a range of genetic tools to modulate *Hox* inputs within specific sub-populations of neurons and observe that *Ubx* expression within a subset of dopaminergic neurons located within the animal’s ventral nerve cord (VNC) is essential for normal flight. Cell circuitry analyses using the GFP Reconstitution Across Synaptic Partners (GRASP) technique, in combination with optogenetic manipulations, allow us to link VNC’s dopaminergic neurons to the flight apparatus. Furthermore, functional imaging experiments show that *Ubx* is necessary for normal dopaminergic activity: in the absence of normal *Ubx* levels, neurons show reduced neural activity patterns, whilst an artificial increase in TH neurons’ activity generated by conditionally expressing the voltage-gated bacterial Na⁺ channel (NaChBac) rescues flight deficits resulting from *Ubx* downregulation, confirming a neurophysiological role. Finally, using neuron-specific transcriptomics we identify two previously uncharacterized ion channel-encoding genes as potential mediators of the *Ubx* physiological roles in adult neurons. Our study thus reveals a novel role of the *Hox* system in controlling adult behaviour and neural function. Based on the broad evolutionary conservation of the Hox system across distantly related animal phyla, we predict that the Hox genes might play neurophysiological roles in adult forms of other species, including humans.

Keywords: Hox genes, Ubx, flight, neuron, dopamine, transcription, adult

^{*}Speaker

A temporal sequence of probabilistic processes determines axon target choice in the developing CNS

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Developmental variation in brain-wiring pattern contributes to behavioural individuality. However, **how and when individualized wiring diagrams emerges and becomes stable during development remains largely unknown.**

In the *Drosophila* visual system, the Dorsal Cluster Neuron (DCN/LC14) wiring diagram varies between the left and right hemispheres and among isogenic flies leading to individualized object response behaviour. This variation relies on a differential innervation of proximal (lobula) versus distal (medulla) optic lobe neuropils by DCN axons, L-DCN and M-DCN respectively. While we previously showed that within a DCN lineage, sub-fates are established post-mitotically, it is unclear **when, where and how during development DCN neurons become irreversibly committed.**

Using live-imaging techniques to explore axon targeting dynamics, combined with mathematical modelling and experimental validation, we discovered that **targeting choice is an algorithmic multi-step growth process with variable outcomes.** DCNs progressively lose their potential to target the medulla through two successive stochastic developmental decision steps distinct in space and time. First, using optogenetic to temporally activate the Notch pathway, we found that Notch acts within a specific temporal window to maintain the majority of axons in the lobula (Notch-ON) through lateral inhibition, while Notch-OFF status permits the formation of extending transient amplifying axonal structures that are required but not sufficient for distal targeting. *Notch* loss of function increases the number of M-DCN but still produces L-DCNs. These Notch-OFF neurons do not adopt a definite M-DCN fate, but rather have a probabilistic potential to innervate distal targets.

^{*}Speaker

To further assess the mechanism behind medulla targeting choice and thus the acquisition of M-DCN fate, we combined cytoskeleton live-imaging together with temporal pharmacological treatments. We found that a subset of these Notch-OFF axons stabilizes by microtubule probabilistic growth, while those without tubulin retract. This results in the stochastic selection of a different number of distal targeting axons in each hemibrain.

Microtubule destabilization suffices to inhibit this targeting. We observed a similar axonal amplification-stabilization process in the developing chick spinal cord, suggesting a conserved mechanism. Finally, early microtubule patterns predict the adult brain-wiring of an individual in a target-independent manner prior to synapse formation. Thus, we show here **that a temporal succession of genetically encoded stochastic processes explains the emergence of individual wiring variation.**

Keywords: Axon targeting, Fate choice, Axon dynamics, Cell competition, Structural variation, Individuality

Creating true and false memories from forgotten information in *Drosophila*

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Recovering forgotten memories is a double-edged sword. While regaining access to forgotten information can be beneficial, the process carries the risk of generating false memories. The neuronal circuits that guide either true memory recovery or false memory implantation are not understood. We find in fruit flies that a reminder can recover forgotten aversive memories. This recovery requires a silent memory to recruit gated reinforcement provided by a specific pair of dopamine neurons. Manipulating the reminder procedure with suggestive cues implants a false aversive memory. The experience within the contextual boundaries of the learning event defines the extent to which cues misguide recovery. Compared to true memory recovery, false memory implantation recruits a distinct dopamine pathway. Understanding the functional separation for true and false memory recovery will help inform strategies to prevent the acquisition of misleading information.

Keywords: memory, forgetting, recovery, false memory, dopamine, retrieval

^{*}Speaker

Glia sub-types differentially regulate food-odor tracking and feeding behavior in hungry flies.

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Glia sub-types differentially regulate food-odor tracking and feeding behavior in hungry flies.

Jean-François De Backer & Ilona C. Grunwald Kadow

Like most animals, hungry flies have to seek for food supply. As foraging, feeding and digestion are also energy-costly activities, these behaviors must be repressed when the animal reaches satiation. In the central nervous system, glial cells seem to be good candidates to play that role. They are known to provide metabolic support to neurons but also to modulate their activity and to have a direct impact on various types of behavior. Here, we hypothesize that glial cells could sense the metabolic state of the animal and then promote and/or repress the decision of engaging food-seeking and feeding behavior. We are addressing this hypothesis in food-deprived *Drosophila* using single-fly food-odor tracking and feeding behavior assays. We observed that the expression of the adenosine receptor (AdoR) in glial cells is necessary in both cases. Interestingly, the role of AdoR seems to be different according to the cell types composing *Drosophila* glia population. While adenosine is often presented as a stress-signaling molecule it is also the product of ATP dephosphorylation and could potentially be used by glia to sense energy level and/or consumption of neurons locally or systemically. In addition, we found a correlation between metabolic state and internal calcium concentration depending on the glia sub-types. We therefore induced calcium influx in these cells using optogenetics and again observed glia type-dependent inhibition or enhancement of food-odor tracking and feeding behavior in starved flies. Together, our data suggest that different populations of glial cells sense the metabolic state of neurons and of the fly and differentially tune behavior toward food-seeking or feeding.

Keywords: Glia, Feeding, Foraging, Hunger, Internal State, Metabolism, Adenosine Receptor, Neural Circuits, Behavior

*Speaker

How the Brain Controls Motor Circuits in the Adult Nerve Cord

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In most animals, a relatively small set of descending neurons (DNs) connect higher brain centers in the animal’s head to neural ganglia in the animal’s body that comprise the premotor networks controlling a variety of complex behaviors. To understand how brain signals generate behavior, it is critical to understand how these descending pathways are organized onto the motor neurons contained in the body ganglia. In the fly, *Drosophila melanogaster*, these body ganglia form a ventral nerve cord (VNC), analogous to the mammalian spinal cord. In a companion project (Takemura et al. unpublished) we introduced a new connectome of the *Drosophila* Male Adult Nerve Cord (MANC), including cell type and developmental lineage annotation (Marin et al. unpublished), which provides unprecedented access to the complete VNC connectivity at synaptic resolution. Here we provide a first look at the organization of the VNC networks connecting descending neurons to motor neurons based on this new connectomics information. We proofread and curated all the DNs and MNs, ensuring their accuracy and reliability. We then systematically matched DN terminals and MN dendrites to published light microscopy data, allowing us to link their morphology in the VNC with their structure in the brain or to their muscle targets. We report both broad organization patterns of the entire network, as well as fine-scale analysis of specific circuits of interest. We discover that the VNC is a very shallow network with relatively short distances between DNs and MNs, although direct connections between them are less common than might have been expected. We identify broad sets of DNs that control specific subsets of MNs. Notably, we unraveled leg circuits primarily responsible for walking, wing circuits that potentially contribute to flight steering and power generation, and lower tectulum circuits involved in escape responses. Our analysis generates hypotheses for future functional experiments and together with the MANC connectome empowers others to investigate these circuits and others of the *Drosophila* Ventral Nerve Cord, in richer mechanistic detail.

Keywords: Descending neurons, Motor output, Male Adult Nerve Cord, EM, Circuits, Connectivity

*Speaker

Identification of new regulators controlling *Drosophila* brain laterality

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Left-right (LR) asymmetry of the brain is widespread throughout the animal kingdom and is thought to play an essential role in cognitive functions. To try better understanding brain laterality, our team developed *Drosophila* as a new model system. "H-neurons" represent a unique, small group of bilateral neurons projecting asymmetrically into the asymmetrical bodies (ABs), a pair of neuropils that are part of the central complex region of the brain. H-neurons project unilaterally into the right AB in 95% of wild-type flies (ASYM phenotype), while the remaining 5% are symmetrical (SYM phenotype), with "H-neurons" projecting in left and right ABs. We recently identified the NetB pathway as being central to establish "H-neurons" asymmetry. In NetB mutants, all flies become symmetrical. Time course analysis during development reveals that projection of the H-neurons is initially symmetrical, eventually resolving into an asymmetrical circuit during pupal stage, and that the NetB pathway is required just prior symmetry breaking. One key finding is that the NetrinB ligand (*NetB*) has a unilateral activity specifically on the right side to control neural projections into the right AB, while the Unc-5 receptor is required bilaterally. To identify the mechanisms controlling the lateralized activity of NetB, we performed genetic screening and characterized new potential transcriptional and post-transcriptional NetB regulators. Results will be presented showing the role of a single transcription factor whose knock-down leads to randomization of H-neuron projections, including inversion of the brain with H-neurons projecting in the left AB. The mapping of neurons involved in this original phenotype will be presented as well as the interaction with the NetB pathway. This work sheds new light on the gene network and mechanisms involved in *Drosophila* brain symmetry breaking and neuronal lateralization.

Keywords: Left Right Brain Asymmetry, NetrinB, regulators, RNAi screen

*Speaker

Mitochondria transfer from neuronal soma to synapses is required to initiate long-term memory formation

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Neurons have differential and fluctuating energy needs across distinct cellular compartments, shaped by brain electrochemical activity associated with cognition. *In vitro* studies show that mitochondria transport from soma to axons is key to maintaining neuronal energy homeostasis. Nevertheless, whether the spatial distribution of neuronal mitochondria is dynamically adjusted *in vivo* in an experience-dependent manner remains unknown. In *Drosophila*, associative long-term memory (LTM) formation is initiated by an acute upregulation of mitochondrial pyruvate flux in the axonal compartment of neurons in the mushroom body (MB). Through behavior experiments and super-resolution analysis of mitochondria morphology, we show that LTM induction, contrary to shorter-lived memories, causes and necessitates anterograde mitochondria trafficking from MB neurons soma. Accordingly, impairing mitochondrial transport abolished the LTM-triggering increased pyruvate consumption specifically in the MB axonal compartment. Our results thus promote reorganization of the mitochondrial network in neurons as an integral step in elaborating high-order cognitive processes.

Keywords: mitochondria motility, long, term memory, brain energy metabolism, mushroom body, 3D, STED microscopy

*Speaker

Sensory neuron population expansions enable persistent odour-evoked behaviour in an ecological specialist

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How nervous system adaptations confer species-specific behaviors is largely unknown. *Drosophila sechellia* is a powerful model for comparative neurobiology as it is closely related to *D. melanogaster* but adapted to a distinct ecological niche, the noni fruit of *Morinda citrifolia*. Noni emits volatiles that attract *D. sechellia* but not *D. melanogaster*, and this requires Or22a (which has enhanced sensitivity to noni odors) and Or85c/b. Intriguingly, the number of olfactory sensory neurons (OSNs) expressing these receptors has increased three-fold in *D. sechellia*. However, the functional significance of such OSN population expansions is unknown. Using (neuro)genetic tools in *D. sechellia*, we have compared anatomical, behavioral and physiological properties of the olfactory circuitry of *D. sechellia* and *D. melanogaster*. While Or22a and Or85c/b OSN population expansions are unique in *D. sechellia*, the number of downstream projection neurons (PNs) for these pathways are conserved between species. We developed a tethered fly assay enabling closed-loop stimulation of OSNs. As expected, noni odor induced persistent attractive behavior in *D. sechellia* but not *D. melanogaster*. Moreover, odor-independent, optogenetic activation of Or22a OSNs induced more persistent attractive behavior in *D. sechellia* than in *D. melanogaster*. By contrast, activation of a subset of OSNs in *D. sechellia* (~40%; similar to the *D. melanogaster* population size) was insufficient to induce behavioral attraction. Using calcium imaging, we observed that, despite the greater sensory pooling, Or22a and Or85c/b PN sensitivity is not enhanced in *D. sechellia* beyond that conferred by olfactory receptor sensitization. However, these PNs exhibit more persistent responses to pulsed or long-lasting odor stimuli. Such persistent responses were not observed in olfactory pathways where there is no OSN expansion. Weak blockage of cholinergic neurotransmission rendered these responses transient. Computational modelling supports the notion that OSN number increase is sufficient to render the decaying PN responses more persistent via non-linear elevation of PN activity. These observations suggest that OSN number increases enhance host fruit location in *D. sechellia* by sustaining PN responses, synergizing with sensitivity increases conferred by receptor tuning.

Keywords: olfaction, sensory neurons, projection neurons, evolution, species comparison, calcium imaging, tethered fly, optogenetics

^{*}Speaker

Temperature directs nervous system development during a critical period, involving mitochondrial reactive oxygen species and HIF 1 alpha

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How can robust network function emerge from neuronal ensembles that ultimately are subject to variability by each of its constituent cells? As developing networks become active, they undergo plastic tuning phases, termed "critical periods"; "critical" because disturbances during these phases lead to lasting changes in function. The underlying molecular and cellular mechanisms remain poorly understood. In the *Drosophila* larval locomotor network, central neurons and muscles have defined critical periods, during which their functional properties are specified. We find that critical period adjustments occur at the single cell level: targeted manipulations of single motoneuron or muscle cells during, but not outside, their critical periods lead to predictable, lasting cell-specific changes. For example, transient critical period experiences of 32°C heat stress cause significant differences in synaptic terminal growth, synaptic transmission, plasticity, and post-synaptic receptor composition at the neuromuscular junction. As primary signals inducing change, we have identified reactive oxygen species (ROS) produced by complex I of the mitochondrial electron transport chain. Reverse electron transport (RET) is a complex I phenomenon known to be induced by elevated temperature. We show that ROS by RET during the critical period are necessary and sufficient to generate lasting impact, both at the level of neuromuscular junctions and central neurons. We identified the *Drosophila* homologue of hypoxia inducible factor, *sima*, as an effector downstream of ROS. Overall, our data suggest a model, where during the critical period, warm temperatures trigger mitochondrial ROS signalling, which cause the stabilisation and thus nuclear import of *Sima*, leading to changes in gene expression and thus how neurons and muscles develop.

Keywords: development, nervous system, reactive oxygen species, HIF 1 alpha, temperature, mitochondria, reverse electron transport, critical period

^{*}Speaker

Morphogenesis & organogenesis

A genetically guided mechanical wave propagates to drive the formation of an epithelial furrow

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Epithelial furrowing is a morphogenetic process that is pivotal during embryo gastrulation, neurulation or the shaping of the animal body. A furrow often results from a fold that propagates along a line. How fold formation and propagation is initiated, driven and controlled is still poorly understood. To shed new light on this fundamental morphogenetic process, we study the formation of the cephalic furrow: a fold that runs along the dorsal-ventral axis of the embryo during early *Drosophila* gastrulation and which developmental role is still unknown. Here, we provide evidence of its function and show that the cephalic furrow is initiated by two groups of cells located on the left and right lateral sides of the embryo. These cellular clusters work as a pacemaker triggering a bi-directional morphogenetic wave powered by actomyosin contractions and sustained by *de novo* medial apex-to-apex cell adhesion. The position of the pacemakers is under the cross-control of the embryo anterior-posterior and dorsal-ventral gene patterning systems. Thus, cephalic furrow initiation and propagation are driven by a mechanical trigger wave that travels along a genetic guide.

Keywords: Epithelial furrowing, multi, view light sheet microscopy, mechanical forces, two, photon optogenetics, adherens junctions, trigger wave, gene patterning

*Speaker

Biochemical and mechanical control of a morphogenetic wave of contractility

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Morphogenesis is the process that shapes cells and tissues during development. In epithelia, biochemical cues and self-organization orchestrate spatio-temporal dynamics of actomyosin contractility driving cell movements and tissue deformations. Here, we study the interplay between biochemical signalling and mechanics during a contractility wave driving morphogenesis of the posterior endoderm in *Drosophila* embryos. In the posterior-most region called the endoderm primordium, genetic patterning induces apical non-muscle Myosin-II (MyoII) contractility and tissue invagination via expression/secretion of the ligand Fog and G-protein coupled receptor (GPCR) induced Rho signalling. Next, a wave of MyoII activation travels anteriorly inducing further invagination and tissue movement. The MyoII wave is regulated by a mechanochemical 3D cycle of deformations involving cell adhesion to the overlying membrane, integrin-mediated activation of MyoII and subsequent cell detachment. We asked whether Fog-GPCR signalling is also involved in wave propagation and how it cross-talks with integrins and tissue mechanics. Using mosaic mutants and tissue-specific expression of a membrane-tethered Fog, we found that Fog-induced GPCR signalling is required for MyoII activation and cell invagination during wave propagation. We show that Fog production in the endoderm primordium alone is sufficient to rescue MyoII wave propagation in Fog-depleted embryos and that Fog extracellular diffusion is required for this. Modulating Fog production in the primordium or its degradation in the neighbouring tissue modulates the amplitude and the range of an exponential gradient of MyoII activity during wave propagation, suggesting that Fog might act as a morphogen to activate MyoII. Strikingly, however, an endogenous Fog tagged with YFP is distributed uniformly in the extracellular space, indicating that other factors are involved in the shaping of the MyoII activity gradient. Interestingly, we find that integrins are epistatic to Fog signalling to regulate the amplitude and range of the MyoII gradient. Altogether, we propose that integrin-dependent Fog-GPCR signalling regulates a mechanochemical propagation of a self-organized morphogenetic wave of MyoII.

Keywords: morphogenesis, contractility, diffusion, ligand, receptor signalling

^{*}Speaker

Cellular and molecular mechanisms regulating FGFR/Btl and TNFR/Wgn receptors during morphogenesis

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Critical to morphogenesis is the precise regulation of the activity of membrane receptors, which receive signals from the environment to trigger a spatio-temporally controlled cascade of events. Miss-regulation of receptor activities typically lead to malformations, fatal disease or malignant transformation. The tyrosine kinase FGFR1/Breathless (Btl) receptor plays a pivotal role during the formation of the tracheal system in *Drosophila*. The activation of Btl by binding of its ligand Branchless (Bnl) leads to the specification of terminal cells at the tip of tracheal tubes, which form the fine branches responsible to oxygenate the tissues. We found that Wengen (Wgn), a TNF receptor, is also required for terminal cell specification. Our results indicated that Wgn works in an unconventional manner during tracheal development, independent of its proposed canonical ligand, named Eiger (Egr) and its canonical signalling pathway, the JNK pathway. Our cellular approach indicated that Wgn is constitutively internalised and accumulates in endocytic vesicles, and colocalise with Btl in these vesicles. Our biochemical approach indicated that Wgn and Btl form a complex. Our genetic approach indicated that Wgn regulates the activity of Btl. Thus, we propose a model in which Wgn would regulate terminal cell specification by regulating the intracellular trafficking of Btl receptor modulating in this way its physiological activity. We have generated new tools to investigate in further detail Wgn activity in life conditions and the Btl/Wgn interactions. We are extending our analysis of Wgn requirements to other tissues in which Wgn is also expressed, in order to test whether this unconventional mechanism that we identified in the trachea is a general mechanism of activity of Wgn. This could suggest that the binding of TNFRs to unrelated proteins is a general strategy of TNFRs activity.

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*Speaker

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Keywords: trachea, FGFR, TNFR, morphogenesis, intracellular trafficking

Epithelium-egg shell interaction and embryo chirality during *Drosophila* gastrulation

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Gastrulation is a complex and well-coordinated process that, through a precise combination of tissue rearrangements and cellular movements, leads to the segregation of the germ layers. Both individual cells and tissues change their relative positions over time, being strongly influenced by the interaction with their surroundings. Of particular interest is the role played by the vitelline envelope, the innermost layer of the eggshell. Integrin-mediated attachment of the blastoderm to the vitelline envelope is required for the proper gastrulation of several species, including *Drosophila melanogaster*. In *Drosophila*, the disruption of the attachment results in a twisted gastrulation (TG) phenotype. To quantitatively study this phenomenon, we generated a high-resolution dataset of *scab* null mutant embryos through Selective Plane Illumination Microscopy. Moreover, exploiting the specificity of the TG phenotype, we imaged germ-band extension in different genetic backgrounds, looking for additional molecular players involved in the mechanism of attachment. Surprisingly, we found a high variability in germ band extension dynamics, even in the wild-type. This suggests a mechanical instability of the germ band, that could be compensated by the blastoderm-vitelline envelope interaction. We are preparing a theoretical model to formally study the forces at play during germ-band extension. While quantifying the TG phenotype, we also detected a bias in the handedness of the twist. When we compared tissue deformation on the two sides of the midline, we observed a consistent left-right asymmetry across the samples. Based on these data, we developed the idea that in *Drosophila* left-right asymmetry could be at least partially set up already in the blastoderm, while the earliest developmental process showing chirality reported so far is gut formation, occurring much later in development. We hypothesized that the Scab-mediated attachment could be necessary to keep the germ-band symmetric during its extension. We are now exploiting optogenetically-driven RNA silencing, among other techniques, in order to understand better the meaning of this left-right asymmetry and eventually find out its molecular basis.

Keywords: Integrin mediated attachment, Scab, variability, germ band extension, twisted gastrulation phenotype, chirality, Myo1D, SPIM

^{*}Speaker

Mitochondrial morphology dynamics regulate gastrulation in *Drosophila* embryogenesis

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Mitochondrial morphology dynamics have been recently shown to be important for embryogenesis and stem cell differentiation. We have been studying the role of mitochondrial morphology dynamics in regulating cell shape morphogenesis in *Drosophila* embryogenesis. We have previously found that mitochondria are small, abundant, and active in *Drosophila* embryos in the syncytial division cycles. They migrate apically during cell formation. Loss of mitochondrial fission protein Drp1 leads to a decrease in cell length and actomyosin contractility in cellularization. We find that a second apical mitochondrial migration event takes place post cellularization specifically in the ventral furrow cells during gastrulation. Loss of Drp1 causes mitochondrial clustering in the basal regions of the cell and prevents mitochondrial apical migration. This leads to a decrease in reactive oxygen species, loss of actomyosin contractility, and aberrant ventral furrow formation during gastrulation. This mitochondrial migration is likely to depend upon the Dorsal/Nfkb signaling pathway. We find that the overexpression of the transcription factor Dorsal leads to the spread of apical mitochondrial migration on the dorsal side of the embryo. Further, mitochondrial fission mutant embryos show an aberrant accumulation of factors activated in the Dorsal/Nfkb signaling pathway. This defect in the accumulation of signaling factors and actomyosin contractility is inhibited by additionally depleting mitochondrial ROS scavengers and abrogating electron transport chain components in Drp1-depleted embryos. Our data collectively show a crucial interaction of mitochondrial distribution and dynamics with the Dorsal signaling pathway in order to drive gastrulation in *Drosophila* embryogenesis.

Keywords: mitochondria, Drp1, gastrulation, actomyosin contractility, Dorsal, embryogenesis

*Speaker

Programmed disassembly of an Interplanar Amida Network coordinates 3D epithelial growth during pupal wing development

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The developing *Drosophila* pupal wing comprises apposed dorsal and ventral epithelia that are essentially identical to each other in size, shape and patterning. Pupal wing development reveals that dynamic morphological changes in 3D architecture take place during the first 24h after puparium formation. However, how the two epithelia coordinate growth with each other while physically separated during the inflation stage of pupal wing genesis, which coincides with a mitotic growth phase, is thus far unclear. By employing in vivo live imaging (5D imaging: three physical dimensions, time, and multiple imaging wavelengths), we found that both dorsal and ventral cells generate basal microtubule (MT) protrusions to form a cellular network between the two epithelia. The cellular network is composed of a 3D meshwork structure: the robust vertical MT protrusions, and thin lateral microfilament (MF) extensions. Although a part of the structure was previously called the transalar cytoskeletal array, we term it the Interplanar Amida Network (IPAN). Our data reveal that basal protrusions of the IPAN sustain a cellular network between dorsal and ventral epithelia during the early inflation stage. Then, programmed disassembly of the IPAN releases the cell-cell contacts. Thereafter, MTs reorganize to form mitotic spindles when cells progress to mitosis. Importantly, loss of cell-cell contacts impacts mitosis in both dorsal and ventral layers to support 3D tissue growth. Our results further indicate that MT protrusions initially nucleated by non-centrosomal MT organizing centers (MTOCs) degenerate, resulting in centrosomal MTOC-mediated mitotic spindle formation for mitosis. Overall, the IPAN provides a unique framework that coordinates 3D tissue development through changing cell shape.

Keywords: epithelia morphogenesis, cellular protrusion, three dimensional architecture, microtubule dynamics, non centrosomal microtubule organizing center

*Speaker

Role of mechano-gated ion channel NompC in epithelial morphodynamics

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Coordination of individual activities in epithelial tissue dynamics requires instantaneous transduction of internally generated forces. Neurosensory cells sense mechanical stimuli, such as touch and sound, using mechano-gated ion channels with exquisite sensitivity. We hypothesise that the same channels are involved in sensing mechanical stress in dynamic epithelia. Applying tissue-scale *in vivo* imaging techniques and optophysiological and mechanical perturbation, we investigated the role of the mechano-gated ion channel NompC in the spatiotemporal orchestration of cell behaviour in a highly dynamic epithelial tissue, namely the amnioserosa (AS) in *Drosophila*. Using membrane-bound (myristoylated) calcium (Ca²⁺)-sensor GCaMP7s, we can visualise the Ca²⁺-influx upon wounding. In wounding experiments in the amnioserosa, we observed *nompC*-dependent Ca²⁺ influx, indicating that NompC directly or indirectly controls cytosolic Ca²⁺ levels. *nompC* mutants showed incomplete germband retraction, loss of or ectopic canthi formation, tissue rupture, or unusually elongated AS cells. However, some mutant larvae completed development and hatched. To assess *nompC* function in cell coordination, we focused on cell dynamics in the elliptical phase of amnioserosa before dorsal closure. We recorded and completely segmented movies of wild-type and *nompC* mutants. Analysis of the obtained ‘dynome’-data revealed differences in several morphodynamic parameters of single cells and cell pairs. We found a gain of anticorrelated cell pairs at the expense of synchronised cell pairs in *nompC* mutants. On the cellular level, we detected an anisotropic orientation of microtubules towards the lateral epidermis, which becomes isotropic in the wild-type by the onset of dorsal closure but persists in *nompC* mutants. We assessed tissue scale mechanical tension by UV laser-induced junction ablation, detecting a strongly reduced initial recoil velocity in *nompC* mutants, specifically in lateral junctions. In contrast, recoil velocities in junctions along the embryonic axis were similar to the wild-type. In summary, our study reveals a specific function of the mechano-gated channel NompC in epithelial cell-cell coordination, which leads to isotropic tension distribution in the tissue and, thus, AS morphogenesis.

Keywords: Epithelial morphodynamics, Amnioserosa, Calcium, Mechanosensitive ion channel

^{*}Speaker

Single-cell behaviour and cell-cell interactions during anastomosis

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Organ morphogenesis and inter-organ communication are orchestrated by a great number of different cell behaviours, which ensure proper assembly, function and, subsequently, contribute to proper organismal homeostasis. Many of these cell activities comprise the generation of cells with specialized shapes and dynamic cell-cell interactions. To achieve close contact and interaction, cells migrate and express a variety of adhesion molecules, many of these belonging to the Ig-domain superfamily.

Organs like the vertebrate vascular system and the insect tracheal system are formed by interconnected tubular networks, which have a major role in inter-organ communication. In these systems, the formation of tubular connections or anastomoses is crucial to accomplish normal function.

Although much is known about the collective cell behaviours and genetic programmes involved in the formation of branched tubular structures, single-cell behaviours, and their contribution to the final organ supracellular structure, are less well-understood. Using the *Drosophila* tracheal system as a model, we have identified Teiresias (Tei) as a major player in cell-cell interaction and anastomosis. Tei is a putative transmembrane protein carrying multiple extracellular immunoglobulin-like repeats, previously reported to be required for neuronal feminization in *Drosophila*. *tei* mutant embryos correctly specify their tracheal fusion cells (FC) but fail to accurately anastomose and form a continuous tracheal network. Furthermore, developing axons in *tei* embryos fail to choose the right path, generating midline defects. Our results suggest that Tei is an important player in cell-cell communication. We will present data and discuss how this adhesion molecule can modulate interactive single-cell behaviours, such as the decision of when to start and stop migrating, the interaction with the appropriate partner cell and the establishment of cell-cell contacts during anastomosis.

Keywords: cell adhesion, anastomosis, cell communication, tubulogenesis, nervous system

*Speaker

Spatio-Temporal control of myoblast diversity in *Drosophila*

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Life is constantly in motion, as the Renaissance philosopher Michel De Montaigne once said. One common behavior animals use to find food, mates, or evade predators is locomotion. In animal appendages, the morphology of muscles is key in ensuring precise movement. These muscles are innervated by a unique wiring of motoneuron axon terminals that control the timing and intensity of muscle contraction. However, how muscles and motoneurons coordinate their development to establish these unique axon-muscle connections and maintain them throughout adult life remains largely unknown. During my thesis, I made a significant discovery regarding the genetic program that governs the morphology of adult muscles. To achieve this objective, I employed single-cell RNA profiling, computational tools, and genetic techniques to visualize and selectively modify the genotype of myoblasts during their development. Additionally, I utilized advanced microscopy techniques to analyze the impact of these genetic manipulations on muscle architecture. The findings from my research demonstrate that muscle progenitors, known as myoblasts, possess a naive state upon joining the epithelial cells of the leg disc. Subsequently, during the early stages of the mid larval stage, these myoblasts become organized into subpopulations that are gradually determined to produce specific muscles. This progressive determination of myoblasts occurs in two steps. Initially, myoblasts are determined to generate either proximal or distal muscles. Subsequently, myoblasts are gradually determined to produce unique adult muscles 24 hours prior to the onset of the fusion process. Currently, our research endeavors involve exploring the intrinsic and extrinsic factors that control this multistep regulation of muscle morphology. My research has enabled the identification of distinct subpopulations of myoblasts and the identification of specific genes that govern muscle diversity. This project has the potential to contribute novel knowledge and understanding of the intricate mechanisms involved in controlling the diverse array of muscles.

Keywords: development, muscles, single cell, morphogenesis

*Speaker

Tissue-extrinsic control of gut shape via sexually dimorphic trachea

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The 3D spatial arrangement of visceral organs is often typical and non-random, but little is known about how this is maintained and how it impacts individual organ function and inter-organ communication. Using micro-computed tomography we have developed methods to quantify 3D features of organ shape and position and reveal that the intestine has constant shape that is sexually dimorphic. Through a series of cell ablation experiments, we identified the tracheal system as a major determinant of curvature and the 3D arrangement of the midgut central loops. We find that the tracheal system is sexually dimorphic and that this is controlled by a new cell-extrinsic mechanism of sex differentiation involving the gut visceral muscle. Using sex reversal experiments in targeted tissues, we demonstrate that sex differentiation of trachea is partially responsible for the stereotypical shape of the male and female gut. We hypothesize that tracheal branches spanning across different gut regions, provide sutures that constrain gut shape. This previously overlooked role of trachea in sculpting the 3D spatial arrangement of organs may help explain signalling paradoxes and sex differences in organ function. For instance, we hope to show that this has implications for food transit and appositional signals between the gut and its adjacent organs

Keywords: Gut, Trachea, Organ shape, Sex determination, Inter organ signalling

^{*}Speaker

Transcription & chromatin

A precise balance of transcription factor levels and binding site affinities determines Hox specificity

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Hox proteins have similar binding specificities in vitro, yet they control different morphologies in vivo. This paradox has been partially solved with the identification of Hox low-affinity binding sites. However, anterior Hox proteins are more promiscuous than posterior Hox proteins, raising the question how anterior Hox proteins achieve specificity. We use the AP2x enhancer, which is activated in the maxillary head segment by the Hox TF Deformed (Dfd) together with its co-factor Extradenticle (Exd). This enhancer lacks canonical Dfd-Exd sites but contains several predicted low affinity sites. Unexpectedly, these sites are strongly bound by Dfd-Exd complexes and their conversion into optimal Dfd-Exd sites results only in a modest increase in binding strength. These small variations in affinity change the sensitivity of the enhancer to different Dfd levels, resulting in perturbed AP-2 expression and maxillary morphogenesis. Thus, Hox-regulated morphogenesis seems to result from the co-evolution of Hox binding affinity and Hox dosage for precise target gene regulation.

Keywords: Hox, binding affinity, Deformed, low affinity binding, converting low to high affinity binding

*Speaker

Building the Regulatory Fly Cell Atlas

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With the Fly Cell Atlas we recently obtained the gene expression profile for more than 250 annotated cell types across the entire adult fruit fly. Here, we set out to model the underlying gene regulatory programs that underly these transcriptomes, and to characterise the full landscape of genomic enhancers that control gene expression in the adult fly. To this end, we first generated a scATAC-seq atlas of adult fly, by extracting nuclei from the whole head and whole body, as well as from dissected testis and ovaries. We used a combination of 10x scATAC-seq, HyDrop-ATAC, and 10x sc-multiome, the latter to serve as a bridge to the scRNA-seq atlas. After quality control and filtering, our atlas contains 690,000 cells and 150,000 genomic intervals that are accessible in at least one cell cluster. We then used topic modelling to cluster cells by chromatin accessibility and used various integration techniques to transfer the cell type annotations from the scRNA-seq to the scATAC-seq atlas, including multi-ome bridging, integration of gene activity scores, and manual curation. This mapping was performed at two hierarchical levels: a broad annotation categorizes all cells into CNS neurons, sensory neurons, glia, muscle cells, epithelial cells, fat cells, tracheal cells, reproductive system, and gland cells. At higher resolution, this process resulted in > 100 annotated cell types. Next, we applied motif discovery to the differentially accessible regions for each cell type, and used the scRNA-seq to prioritise candidate TF combinations and target genes for each cell type using SCENIC+. Finally, we designed new architectures of convolutional neural networks (CNN) to train a large CNN across the adult fly, called DeepFly. This model takes enhancer sequences as input and predicts genome-wide and cell type specific chromatin accessibility. With DeepFly, we are currently mapping the landscape of enhancer codes across the entire adult fly, and newly identified enhancers will be validated in vivo using transgenic enhancer-reporter assays. The multi-ome Fly Cell Atlas and the accompanying gene regulatory networks and enhancer models will represent a unique resource to the Drosophila community, explaining how regulatory code of the fly genome translates into cell types.

Keywords: scATACseq, Deep Learning, Gene Regulation, Multiome, eGRNs

*Speaker

Drosophila TET does not act as a 6mA demethylase and essentially controls gene expression in a catalytic-independent manner.

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Members of the Ten Eleven Translocation (TET) family are well known for oxidating and demethylating 5-methylcytosine (5mC), a prevalent epigenetic mark in vertebrates. Yet, these epigenetic enzymes also seem to have other substrates and enzymatic-independent functions, which are less well characterized. *Drosophila* genome is largely devoid of 5mC and does not code for any cytosine DNA methyltransferase (DNMT) but presents a well-conserved *tet* gene. *Drosophila* thus represents a well-suited model to decipher the non-canonical (5mC DNA-independent) functions of TET enzymes. Recently, the presence of 6-methyladenine (6mA) in DNA has been reported in various eukaryotes and in *drosophila*, this epigenetic modification was shown to be erased by TET. Yet, the existence and significance of 6mA in metazoans remain controversial and the role of TET in 6mA oxidation is unexpected.

Here, we re-evaluated 6mA presence as well as TET function and mode of action in *drosophila*. Using LC-MS/MS measurements and strict axenic breeding conditions, we detected only traces of 6mA DNA (corresponding to a few hundred adenines per genome) in different tissues and developmental stages. Intriguingly, these levels were not increased in the absence of TET. In addition, SMRT-seq analyses suggest that TET loss does not affect 6mA deposition on the genome and we found no evidence that TET can directly demethylate 6mA DNA *in vitro*. Besides, we show that in contrast with TET expression, TET enzymatic activity is not required for adult fly emergence and survival. Focusing on the larval central nervous system, we further show that TET essentially controls its development in a catalytic-independent manner and our results suggest that it acts together with the Polycomb complex to regulate transcription.

Overall, we propose that TET is not involved in 6mA erasure and that it controls gene expression and fly development essentially in a catalytic-independent manner.

Keywords: epigenetic, Ten Eleven Translocation (TET), larval central nervous system (CNS),

^{*}Speaker

demethylation

Exploring the role of transcription in topology during early *Drosophila* embryogenesis

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Chromatin is folded and organised in the nucleus at different scales. Although 3D genome organisation and transcription are tightly linked, it is difficult to discern cause from consequence. While there are many examples of topology being essential for transcription, there is also emerging data that transcription can influence topology. For example, in mammalian cells lines, blocking or ectopically inducing transcription is sufficient to remove or create insulation at some loci, and RNA Polymerase II (Pol II) is required for both compartment and loop establishment following mitosis. However, studies have either focused on specific loci (by deleting or inserting a promoter) or used degrons or drugs to globally inhibit Pol II activity, with the limitation that degrons function over hours, while transcription occurs over minutes, and the application of drugs, especially in embryos, is difficult to control. To avoid secondary effects and precisely dissect the timing of events, here we used the optogenetic iLEXY system, which leads to extremely rapid (within minutes) depletion of nuclear factors in living embryos in response to blue light. We targeted M1BP (Motif1-binding protein), as it is required for TBP recruitment, the transcription of housekeeping genes and is enriched at TAD boundaries, allowing us to explore the role of transcription in boundary insulation. We show that throughout embryogenesis M1BP binds constitutively to the promoter of ~2900 genes, the majority of which are at TAD boundaries. We generated a knockin M1BP-iLEXY fusion, which is homozygous viable under safe light. Under blue light, M1BP is efficiently exported from the nucleus and results in complete embryonic lethality. Quantitative CUT&Tag demonstrates that depletion for one hour either during or after zygotic genome activation leads to a significant reduction in ~90% of M1BP peaks. For hundreds of genes the pre-initiation complex (PIC) is lost (indicated by the loss of TBP, TAF5, and Pol II itself) and transcription reduced. However, at other loci, although M1BP binding is lost, the PIC is unchanged. This provides a unique opportunity to simultaneously examine the function of M1BP versus the function of the PIC/transcription in topology, which we are currently exploring. I will present our results showing the functional impact of loss of (i) PIC assembly (when both M1BP and the PIC are removed) or (ii) M1BP alone, on boundary insulation and gene expression during zygotic genome activation in the early *Drosophila* embryo.

^{*}Speaker

Keywords: transcription, chromatin, topology, embryogenesis, 3D genome

Heterochromatin dynamics in DNA damage repair

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The eukaryotic nucleus consists of different chromatin domains each defined by specific molecular and biophysical properties that necessitate distinct chromatin responses to DNA damage. Silenced heterochromatin domains pose a particular challenge to the DNA-damage response machinery due to their compact chromatin features and, often, repetitive DNA content. The two main types of heterochromatin are constitutive heterochromatin, associated with pericentromeric repeats, and facultative heterochromatin, required for silencing developmental genes. Research from the past decade has revealed that repair of double-strand breaks (DSBs) in constitutive heterochromatin domains requires specific DSB repair responses.

We hypothesize that break-proximal chromatin changes are necessary to support the DSB repair dynamics in heterochromatin. By using locus-specific DSB systems in *Drosophila* animals, we study the role of histone modifiers in the repair of DSBs in both constitutive and facultative heterochromatin. Results from our lab suggest a role for specific histone-modifying activities at DSBs in both facultative- and constitutive-heterochromatin domains. Our data reveal that DSB repair in facultative heterochromatin depends on the removal of the silencing histone mark H3K27me3. Moreover, we find that DSBs in constitutive heterochromatin require the local gain of histone acetylation to initiate DSB-repair signalling. Together, these results suggest that the transient acquisition of a more open, ‘active’ chromatin state at both facultative- and constitutive-heterochromatic break sites is required for faithful DSB repair in these transcriptionally silenced domains.

Keywords: DNA damage repair, double, strand break repair, heterochromatin

*Speaker

Pri peptides temporally coordinate transcriptional programs during epidermal differentiation

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To achieve highly differentiated state, cells are undergoing multiple transcriptional processes whose coordination is not well understood. In *Drosophila* embryonic epidermal cells, Pri smORF peptides activate a transcriptional program leading to cell shape remodeling. Here, we show that they also temporally repress an extracellular matrix gene program, therefore coordinating several differentiation processes in epidermal cells. Through the transcription factor Ken, they prevent the activation of a cuticle gene network, to produce a soft cuticle specific to early larval stage. After long-lasting transcriptional repression, these genes are expressed at late larval stage to pigment and sclerotize the cuticle. These results uncovered a temporal switch to set up distinct structures of cuticle adapted to animal lifestyle and which might be involved in the evolutionary history of insects.

Keywords: epidermal differentiation, smORF peptide, transcription factor, cuticle

*Speaker

Re-evaluating enhancer modularity and boundaries with quantitative approaches in *Drosophila*

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Enhancers are cis-regulatory elements that control when, where, and how much transcription of their target genes is initiated from a corresponding core promoter. Classically, they are defined by their ability to drive spatiotemporal expression in reporter constructs that resemble the endogenous expression of their native target gene. However, these assays often overlook whether the tested fragment is sufficient to drive the native level of transcription or can confer robust expression under stressful conditions. To understand the function and evolution of enhancers, it is essential to define them by mapping the distribution of regulatory information across the entire DNA sequence required for normal endogenous gene expression. In this study, we used the pigmentation locus *yellow* in *Drosophila* as an experimental system to address the question of enhancer boundaries. In the species *Drosophila biarmipes*, it has been reported that the region upstream of the coding sequence contains the *wing blade*, *spot*, and *body* enhancers. While the original dissection of *yellow* regulatory sequences, testing the function of arbitrary segments, suggested that these enhancers were modular, a more systematic analysis of the same region revealed that the *wing blade* and *spot* enhancers were actually broader, extensively overlapping, and shared regulatory information. Given that the *body* and the *wing blade* enhancers drive activities in different tissues, are very old enhancers and had enough evolutionary time to have their sequences separated, we set to re-examine their modularity. To investigate the distribution of regulatory information driving expression in the fly abdomen, we used a series of constructs containing systematic dissection of the *yellow* regulatory region and conducted quantitative reporter assays in flies to assess changes in expression pattern and intensity. We found that the *body* enhancer boundaries are much wider than previously described and overlap with the other enhancers creating a complex regulatory region. Our work shows that, at least in the case of *yellow*, the notion of discrete and modular enhancers, depicted in textbooks as pearls on a string, is not accurate. Instead, we find that distinct regulatory activities are borne from intertwined enhancers, spanning much larger regions (4 kb) than is generally thought for enhancers (100-1000 bp). These findings lead us to reconsider by which mechanisms regulatory elements achieve functional modularity.

Keywords: enhancer, transcriptional regulation, pattern formation

*Speaker

Selective requirement for CBP catalytic activity during zygotic genome activation

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The p300/CBP acetyltransferase is a conserved co-activator of gene expression that establishes H3K27ac and H3K18ac histone marks. During development, the enrichment of these marks on enhancers and gene promoters is a reliable sign of gene expression, although the actual function of acetylation is unclear. We aimed to reveal CBP acetylation-dependent roles in zygotic genome activation (ZGA) and development of early *Drosophila melanogaster* embryos. We used CRISPR/Cas9 to engineer a catalytically dead CBP (*nejire*) allele, and studied germline clone embryos obtained by FLP-FRT mediated mitotic recombination where the maternal load consists of only mutant CBP. These embryos successfully developed until gastrulation, showing that ZGA appears to be acetylation-independent. Interestingly, whole-mount in situ hybridization revealed that pair-rule genes such as *even-skipped* (*eve*) and *fushi tarazu* (*ftz*) display consistent defective patterns, with some stripes missing while others persist. Among the strongly affected stripes are *eve* stripe 2 and the fourth stripe of *ftz*. Complementing these studies, we optogenetically inactivated CBP. Unexpectedly, CRY2-CBP remained chromatin bound in blue light, whereas histone acetylation was strongly reduced. Similar selective pair-rule stripe phenotypes were observed in these embryos. We conclude that only some developmental enhancers depend on CBP-mediated acetylation for activity during embryo development.

Keywords: histone acetylation, zygotic genome activation, p300/CBP, enhancer

*Speaker

Stonewall links chromatin organization at the nuclear periphery to female germline stem cell fate in *Drosophila*

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The association of genomic loci to the heterochromatin compartment at the nuclear periphery is proposed to facilitate cell-type specific gene repression, leading to distinct cell fates. While identifying proteins that position genomic loci at the nuclear periphery is pivotal to unraveling the link between nuclear architecture and cell fate, these proteins remain relatively unknown in *Drosophila*. In this study, we performed a FISH-based RNAi screen targeting ~1000 genes to discover novel regulators of nuclear architecture in *Drosophila* Kc167 cells. Among our hits, we identified Stonewall (Stwl), a MADF-BESS domain containing DNA-binding protein, as a novel candidate mediating the association of chromatin to the nuclear periphery. We further observed that Stwl is enriched at the nuclear periphery in these cells and quantitative mass spectrometry revealed interactions with multiple nuclear envelope (NE) proteins. Previous studies have shown that Stwl is essential for germline stem cell (GSC) maintenance in the *Drosophila* ovary, as *Stwl* mutation results in GSC loss, while *Stwl* overexpression leads to an increase in GSC number. However, the mechanism by which *Stwl* promotes GSC fate is poorly understood. Based on our screen, we hypothesized that Stwl might repress GSC differentiation genes through positioning them at the nuclear periphery. To test this hypothesis, we first identified direct targets of Stwl in a GSC-enriched population using RNA-sequencing and CUT&RUN. Strikingly, Stwl binds loci encoding known regulators of GSC differentiation, such as *benign gonial cell neoplasm (bgn)* and *meiotic-P26 (mei-P26)* and represses the expression of these genes. Furthermore, the de-repression of these differentiation genes in Stwl depleted GSCs was associated with re-localization of these loci from the nuclear periphery to the interior. Finally, light and electron microscopy in Stwl-depleted GSCs revealed discontinuities in the nuclear lamina during interphase with these regions lacking evident heterochromatin compartments. Thus, our data reveal Stwl as a novel mediator of gene positioning at the nuclear periphery in *Drosophila* ovarian GSCs. Through this mechanism, Stwl promotes a cell-specific gene expression program ensuring GSC maintenance and female fertility. Since there are at least 16 MADF-BESS family members in the *Drosophila* genome, we propose that other MADF-BESS proteins may play similar roles in mediating cell-specific gene expression in other tissues.

*Speaker

Keywords: Stonewall, Germline Stem Cell, Cell Fate

Stem cells & regeneration

Deciphering gene regulatory networks in early spermatogenesis by multimodal sequencing

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Stem cells are vital for organismal life, as they can differentiate into various cell types while self-renewing at the same time. Therefore, understanding stem cell communication and behavior is crucial to understand tissue homeostasis. For this, the adult *Drosophila* testis serves as an excellent model. At the niche, two stem cell types are anchored that self-renew and divide to differentiate. The germline stem cells go through mitotic and meiotic differentiation, while the somatic cyst stem cells divide once and encapsulate the germline to support spermatogenesis. This functional unit of cells forms its own microenvironment to govern the tight signaling interactions initiating transcriptional programs critical for spermatogonial differentiation.

In an effort to understand this process, we simultaneously mapped in single nuclei RNA and ATAC profiles of an enriched spermatogonial cell population. Leveraging both modalities, we built enhancer-driven gene regulatory networks, allowing us to explore the regulatory logic behind early spermatogenesis.

We identified known and novel TFs both active and crucial in early spermatogenesis, that we perturbed *in silico* with established computational tools, and functionally validated by CRISPR-mediated knock-outs. We will further characterize cell communication by mapping ligand-receptor expression and transcriptional responses at the niche and throughout differentiation. The integrated chromatin accessibility profile will allow us to unravel the drastic chromatin conformational changes throughout spermatogenesis on a genome wide level, so far poorly characterized in the field. Additionally, by identifying differential accessible regions (DARs) we will generate cell- and stage-specific genetic tools for more targeted cell manipulation, thereby leveraging the combined profiling of both single cell gene expression and chromatin accessibility maps.

Keywords: testis, single cell, RNA, ATAC, early spermatogenesis

^{*}Speaker

Exploring chromatin state transitions in the *Drosophila* intestinal lineage

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Adult tissues rely on resident stem cells for maintenance and repair throughout the entire life of the organism. Differential gene expression is central to the capacity of stem cells to self-renew and differentiate into more specialized cells and chromatin organization plays essential role in transcriptional regulation. Whereas genome-wide chromatin state characterization on stem cells during development or on cultured stem cells have provided understanding on its role in maintenance of stemness and fate specification, chromatin changes underlying gene expression in an adult stem cell lineage *in vivo* in the context of a homeostatic tissue are less well understood. Cell type-specific genome-wide profiling of 5 chromatin-associated factors (RNA Pol II, Brm, Pc, HP1, and H1) in the intestinal lineage allowed us to define by mathematical modeling based on the combinatorial binding levels of each of these factors along the genome, 7 major chromatin states. We then examined changes in chromatin states during lineage determination. Our data reveal that stem cell-enriched genes transition from an active chromatin state in the stem cells toward distinct repressive states in the other cell types of the lineage depending on the differentiation paths. Indeed, a repressive HP1-enriched state is more prominent in enteroendocrine (EE) cells than in enterocytes (ECs), where the histone H1-enriched Black state is favored. Strikingly, terminal differentiation genes related to the physiology and metabolic functions of differentiated cells transition from a primed H1-enriched Black state in ISCs to an active chromatin state in both ECs and EEs. This highlights the importance of such conserved Black state, lacking most chromatin marks and chromatin associated factors, as a reservoir for lineage specific genes in adult stem cells. Finally, we uncovered the role of histone linker H1 in EE fate priming and not in EC priming indicating specific mechanisms of chromatin regulation within multipotent lineages. Overall, this work highlights the complexity of chromatin state transitions underlying transcriptional regulation during lineage specification *in vivo* in a homeostatic tissue.

*Speaker

Keywords: adult stem cells, intestine, chromatin states

Genetic, epigenetic and environmental factors induce a mitosis-dysplasia positive feedback loop via DNA replication stress during aging

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Dysplasia develops in the aging *Drosophila* intestine due to the accumulation of clusters of heterogenous intestinal stem cell-like (ISC-like) cells, but the nature of this phenomenon remains unclear. Using a combination of treatments and genetic, epigenetic and functional transcriptomic analysis we find that the DNA damage response markers, ATM/ATR and γ H2Av, are expressed along the *Drosophila* midgut. The ubiquitousness and inducibility of these markers by a multitude of ISC-controlling genetic factors and treatments, including those directly involved in DNA replication stress (DRS), indicate that mitosis and dysplasia are inextricably linked to DRS. We find that mitosis, DRS and dysplasia operate in positive feedback loop producing dysplastic progenitors characterised by increased ploidy. Dysplastic ISC-like cells start clustering in young adults as a function of the mitotic index, which in turn is subject to treatment, genetic and epigenetic background, anteroposterior regionality and sex, and promote tumour formation in aged flies. Notch is differentially expressed and epigenetically regulated along the midgut and between sexes. We devised a new tool to monitor the epigenetic downregulation of the Notch locus and found that spontaneous Notch locus silencing promotes dysplasia. Finally, enterocyte (EC) resistance to apoptosis counteracts dysplasia by compromising compensatory ISC proliferation. Thus, midgut dysplasia is controlled by the rate of mitosis and concomitant DRS, which in turn are controlled by genetic background, epigenetic differences along the anteroposterior axis, sex, EC health, and dietary and pharmacological interventions.

Keywords: Dysplasia, stem cells, DNA replication stress, mitosis, aging

*Speaker

Mechanosensory regulation of intestinal regeneration in *Drosophila* through the vascular stem cell niche

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Long-term maintenance of organismal homeostasis, under varying environmental conditions, relies on tight coordination of tissue intrinsic and systemic signals. The gastrointestinal tract is an ideal model system for studying complex interactions between stem cells and their niche. The vasculature is a prominent constituent of multiple stem cell niches and its role in the intestinal microenvironment remains largely understudied. We have recently uncovered a novel chemically driven crosstalk between the adult *Drosophila* midgut and the surrounding vasculature-like tracheal system, which is essential for intestinal regeneration following damage (Perochon *et al.* 2021, NCB). New observations suggest the presence of distinct sub-populations of *piezo* expressing tracheal cells activated by physical/biomechanical cues and contributing to gut/tracheal communication during intestinal regeneration. We are using an interdisciplinary approach, combining *Drosophila* genetics, scRNA-seq, live imaging, and computational image analysis to understand the importance of mechanical forces in regulating the tracheal microenvironment and stem cell adaptations during intestinal regeneration. We have identified how these forces are sensed and then, integrated by the tracheal tissue. We also have characterized the signalling pathways that are activated in this context. Our results provide insight into how the vascular niche integrates mechanical stimuli, exerted by the intestinal epithelium upon damage, to regulate intestinal regeneration. Ultimately, we aim to discover fundamental mechanisms to drive intestinal repair through manipulation of the vascular stem cell niche.

Keywords: intestinal stem cell, vascular niche, regeneration, biomechanics

*Speaker

Peer pressure: adhesion-dependent tension in the glial niche regulates neural stem cell proliferation

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Neural Stem/progenitor Cells (NSCs) exist in a niche, a complex and dynamic cellular microenvironment supporting their functions. The niche must respond to NSC needs while mediating the impact of local and systemic inputs to support neurogenesis. In particular, the intricate structure of the niche, rich in direct cell-cell contacts and adhesions, has the potential to be a critical regulator of NSPC behaviour. However, how structural interactions between the NSCs and their niche control neurogenesis remains poorly explored.

The *Drosophila* developing, larval brain harbours genuine NSC niches that contain common players and recapitulate core features of the vertebrate microenvironment. Here, a specific subpopulation of glial cells, the cortex glia, are known to be essential for neurogenesis and intimately enclose each NSC and its neuronal progeny within a membrane chamber. The precise encasing of each individual NSC chamber pinpoints the existence of tightly controlled interactions between CG and NSCs lineages.

We first performed transcriptional analysis of cortex glia along chamber morphogenesis, which identified an enrichment in surface immunoglobulins. We choose to focus on a specific immunoglobulin whose partner is expressed in NSCs. We discovered that these binding partners act as adhesion complexes bringing together NSC and cortex glia membranes. Further, the independent knockdown of these immunoglobulins in either cortex glia or NSCs resulted in a

*Speaker

remarkable phenotype in NSCs, which displayed altered nuclear shape and slower cell cycle progression. Strikingly, we found that such immunoglobulin-mediated adhesion impinges on NSC proliferation by regulating the cortical tension in the cortex glia. Altogether, these findings highlight a mechanism by which tension cues in the niche are relayed to the stem cells and regulate neurogenesis. This work demonstrates that the niche exerts mechanical constraints directing NSC behaviour at an individual level.

Keywords: neural stem cell, niche, architecture, cortical tension, proliferation

Ramping it up a Notch: Midgut injury accelerates stem cell differentiation by modulating a fate-determining lateral inhibition circuit

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Efficient organ regeneration requires the rapid replacement of damaged cells by new cells in order to restore healthy physiological function. In many organs, the main source of regenerative cells comes from injury-activated stem cell divisions. However, stem cell progeny are undifferentiated and thus initially incompetent to contribute to organ function. Here, we examine the live differentiation kinetics of injury-born progeny in the *Drosophila* adult midgut, in which a Delta-Notch lateral inhibition circuit controls the decision between stem cell and terminal enteroblast fates. Real-time *in vivo* imaging reveals that nascent enteroblasts in bleomycin-injured guts activate Notch nearly two times faster compared to their counterparts in healthy guts. Mathematical modeling suggests that these accelerated Notch kinetics can arise by increasing the threshold of Delta inhibition by Notch, which would suspend lateral inhibition and ultimately manifest as a failure to downregulate Delta in Notch-activated cells. Consistent with this hypothesis, we observe aberrant Delta expression in Notch-activated enteroblasts in injured but not healthy guts. Injury-induced perdurance of Delta is accompanied by nuclear exclusion of the Notch transcriptional corepressor Groucho, implying that injury-induced spatial restriction of Groucho may inhibit it from repressing Delta expression. Altogether, our results reveal how injury-induced modulation of Delta-Notch signaling circuitry accelerates cell differentiation to efficiently restore organ structure and function.

Keywords: midgut, Notch, Delta, live imaging, stem cell differentiation

^{*}Speaker

Regional and cellular heterogeneity of intestinal tryptophan metabolite signaling

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Stem cells can self-renew or differentiate to maintain tissue homeostasis, and their dysregulation is associated with pathological states like cancer and tissue decline during aging. Nutrients are known to affect proliferation and differentiation of intestinal stem cells (ISCs) in both mammals and *Drosophila*, but the underlying mechanisms remain incompletely understood. Moreover, although the intestine is known to be functionally compartmentalised along the anterior-posterior axis, possible regional differences in ISC regulation have been largely overlooked. By high-resolution imaging of the entire *Drosophila* midgut coupled to an automated pipeline that allows us to quantify intestinal cell types along the midgut, we discovered that ISCs display prominent tissue heterogeneity in their response to essential amino acids. Tryptophan in particular caused strong and region-specific ISC proliferation. By further screening the endogenous pathways downstream of tryptophan for ISC regulation phenotypes, we found that serotonin-precursor 5HTP induced the strongest ISC proliferation in the posterior midgut. While investigating this regional difference in ISC serotonin sensing, we found that old enterocytes in the posterior midgut use serotonin as a signal to attract nearby progenitor cells, and to promote their differentiation into enterocytes. Genetically preventing serotonin signaling between enterocytes and their precursor cells inhibited differentiation of progenitor cells into enterocytes and blocked epithelial renewal specifically in the posterior midgut. Conversely, genetically forcing increased serotonin signaling in the progenitors leads to increased progenitor differentiation into enterocytes in the same region. Taken together, these results demonstrate that the ISCs display region-dependent differences in their potential to sense and respond to tryptophan and its metabolites, and that these differences can underlie regional regulation of biological processes such as epithelial renewal.

Keywords: intestinal stem cells, epithelial renewal, tryptophan metabolism

*Speaker

Somatic cells support germ cell survival by shuttling glycolytic products

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Tissue development and function depends on proper communication among different cell types. Recent work has suggested that in addition to classical intercellular signaling transduction pathways, cells can also communicate by exchanging metabolites. This is particularly relevant in the case of germ cell development, which requires the support of neighboring somatic cells. However, the molecules involved in soma-germ communication remain poorly characterised.

In the *Drosophila* testis, a cluster of post-mitotic cells forms the niche that maintains two different stem cell populations. Germline stem cells (GSCs) give rise to gonialblasts, which undergo a series of incomplete, transit-amplifying divisions to form germ cell cysts that will later undergo meiosis. Somatic cyst stem cells (CySCs) give rise to post-mitotic cyst cells. Each germ cell cyst is fully enclosed by two cyst cells to support the germline throughout its differentiation process. Previous work has shown that somatic cyst cells engulf the germline and establish septate junctions, thus cutting off the germ cells from the surrounding environment. We hypothesized that the isolation of the germline might prevent it from directly receiving basic nutrients and that the cyst cells could provide metabolic support to the germline.

We previously showed that somatic cells increase the expression of glycolytic enzymes during differentiation, leading us to ask whether somatic glycolysis was involved in support of the germline by cyst cells. We knocked down enzymes implicated in glycolysis in somatic and germ cells and studied germ cell survival. Autonomous knockdown of glycolytic enzymes had no visible phenotype in the germline, indicating that glycolysis is dispensable in germ cells. However, somatic knockdown of glycolysis genes led to non-autonomous cell death of germ cells. Next we sought to establish how metabolites are shuttled between cells. We performed an RNAi screen to knock down monocarboxylate transporters expressed in the soma and found that Silnoo is required non-autonomously for germ cell survival, suggesting that Silnoo transports metabolites from soma to germ cells.

Altogether, our data suggest that glycolysis in cyst cells supports germ cell survival and imply that cyst cells provide glycolytic products to germ cells. This shows remarkable conservation with mammalian spermatogenesis and provides the basis for asking how somatic support cells can transmit nutritional information to the germline.

*Speaker

Keywords: testis, growth, development, signaling, glycolysis, metabolism, support, transport

Understanding female-specific predisposition to genetically-induced intestinal tumours

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Epidemiological data highlight significant *sex bias* in *cancer* incidence and survival. However, the influence of sex on the tumor formation remains poorly understood. To better understand the cellular processes and to identify molecular actors involved in tumorigenesis according to sex, we study a model of genetically induced tumors in the adult midgut by inactivation of the Notch (N) receptor in intestinal stem cells (ISC). For a reason still unknown, the incidence of these N- tumors is high in females, but limited in males. In *Drosophila*, the simple ectopic expression of sex determinant *transformer* (*traF*) in males allows the "feminization" of tissues. By expressing *traF* in various organs or cell types, we determined that the sex does not act autonomously at the ISC or at the gut level. Interestingly, we show that the only "feminization" of a neuronal population is sufficient to trigger tumor formation in males. Conversely, targeted ablation of these neurons in female efficiently decreases tumorigenesis. We observed that these neurons are peptidergic and innervate the midgut from the central nervous system. These results suggest that there are important physiological differences between the female and male intestines controlled by the sexual identity of a dedicated neuronal population and sex-specific signal(s). This sex-specific regulation contributes to augmented susceptibility to tumor appearance in females.

Keywords: stem cell, gut, somatic sex, tumour

*Speaker

Unraveling heterogeneity and dynamics of muscle stem cell niches through integration of single-cell transcriptomics and advanced imaging

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The size and heterogeneity of the muscle stem cell (MuSC) pool have a significant impact on tissue homeostasis, regenerative capacity, and the resistance of muscle tissue to external environmental factors. For healthy and disease-free muscle tissues throughout life, it is critical to understand the mechanisms that determine the size of the MuSC pool during development and its maintenance in adulthood, as well as the factors that contribute to its change. To this end, we combine single-cell and spatial transcriptomic methods with cell type-specific CRISPR gene editing and imaging approaches to assess both molecular and cellular phenotypes using *Drosophila* as powerful in vivo model. We have recently integrated available single-cell RNAseq datasets from the larval muscle stem cell niche of three different laboratories into a unified dataset, providing us with a spatially resolved gene expression map of muscle stem cell populations and their niche with greatly improved resolution. To enable rigorous quantitative analysis of MuSC populations, we have developed a sophisticated image-processing pipeline, encompassing a deep-learning based approach for precise cell segmentation and a state-of-the-art random-forest algorithm for accurate MuSC subpopulation classification. Leveraging these advancements, we have successfully quantified the entire pool sizes of MuSC subpopulations and extracted intricate cellular features from diverse MuSC subpopulations. Together our data provides new insights into the biology of muscle stem cell niches during development resolving MuSC heterogeneity and pool dynamics. This research paves the way for future investigations aimed at deciphering the complex relationship between developmental processes and the functioning of adult MuSCs.

Keywords: muscle stem cells, spatial transcriptomics, advanced imaging

*Speaker

Signalling

Activation of the Innate Immune System Accelerates Tissue Growth in a *Drosophila* Tumor Model.

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The Toll pathway is a conserved signaling cascade that regulates the innate immune response to infection. Apart from its role in innate immunity, Toll signaling has been shown by our lab to negatively regulate growth during development and to be required for the elimination of less fit cells through cell competition. In mammals, the existence of a link between innate immunity, TLR signaling (homologous to the *Drosophila* Toll pathway) and tumor growth has been extensively studied.

However, the role of TLR signaling in mammalian tumors remains controversial: Pathway activation is suggested to be either pro or anti-tumorigenic. Our study aims to better understand the predicted dual-role of the TLR pathway in tumor growth. To this end, we compare the effect of Toll pathway manipulation in different *Drosophila* tumor models.

Here we show that Toll signaling promotes growth after hyperactivation of Ras signaling (overexpression of *RasV12*) in the developing eye disc. RasV12-transformed eye discs show substantial changes in gene expression after Toll activation as revealed by bulk RNA sequencing. Induced genes are closely associated with growth controlling pathways, such as JNK and Hippo/Yki signaling. The activation of Toll signaling further inhibited retinal cell differentiation in RasV12-transformed eye discs raising the possibility that the cells are trapped in an early developmental stage.

Our results suggest that, once activated in cells predisposed by the oncogenic factor RasV12, Toll signaling no longer promotes cell death to maintain tissue homeostasis as shown during cell competition, but acts a potent oncogene driving tissue hyperplasia. We propose a model, in which Toll signaling primes cells for oncogene-mediated overgrowth through suppression of differentiation in the eye/antennal disc. To elucidate the interplay of molecular players involved in the regulation of tissue growth following Toll activation, we investigate candidate pathways in various tumor and tissue contexts.

Overall, we anticipate that our study will contribute to the understanding of the role of the TLR signaling pathway in mammalian tumor growth.

*Speaker

Keywords: Development, Growth, Disease model, Cancer, Signaling, Innate Immunity

Fat and sugar control of taste perception

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Animals maintain weight over time, suggesting a strict intake regulation of the high caloric macronutrients, fat and sugar. In most animals over consumption of sugar also suppresses sweet taste perception and sweet food intake. Here, we show that along suppression of sweet sensation sugar overconsumption in *Drosophila* also increases fatty acid perception. We further show that excess fat intake in return favours sugar perception and suppresses fat sensation. Using genetics, we discover that the sugar signal is Hedgehog secreted from the gut to the hemolymph in response to dietary sugar, which both suppresses sugar taste sensation and enhances fatty acid taste perception. We further demonstrate that high-fat diet induces secretion of the Leptin ortholog Upd2 from adipose tissue into the haemolymph. The released Upd2 triggers JAK/STAT signaling in the taste neurons that increases sweet perception and suppresses fatty acid perception. The link between Upd2 and taste regulation and the contrasting function of Hedgehog suggest that sweet and fatty taste sensitivity at any given moment is a product of feeding and nutritional history. The shared taste regulation thus, can balance information flow to the brain, and reduce superfluous information processing, reducing the cost to sort and filter complex stimuli to modulate feeding decisions.

Keywords: Fat and sugar control of taste perception

*Speaker

Glutamate receptors interact with transcription factors to regulate synaptic scaling

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Neural networks can globally regulate their neurotransmission properties in response to changes in presynaptic activity through a homeostatic mechanism known as synaptic scaling. We have identified that synaptic scaling also occurs in *Drosophila* motor synapses, where the ratio of high-conductance GlurIIA containing glutamate receptor complexes and lower conductance GlurIIB receptor containing complexes are altered in response to presynaptic neurotransmission. Investigating the mechanisms through which this process occurs, we discovered a key role for the C-terminal domain of glutamate receptors which can act as a signalling scaffold. We found that amino acids in this region of glutamate receptors are phosphorylated by activity-dependent kinases. Surprisingly, we further found that this phosphorylation induced the sequestration of a BMP family transcription factor to synapses. We show that these glutamate receptor-transcription factor interactions are essential for the process of synaptic scaling, via regulation of transcriptional output in the postsynaptic nucleus. Overall, our data shows that synaptic activity, via neurotransmitter receptors, can immediately and directly regulate gene expression through transcription factors, revealing a novel mode of neuronal activity-dependent signalling.

Keywords: Neuromuscular Junction, Neurotransmission, BMP, Glutamate Receptors

*Speaker

Investigating the role of autophagy and Snap29 in C9orf72-linked ALS/FTD

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Disruptions of autophagy are observed in several neurodegenerative diseases, including *C9*-linked amyotrophic lateral sclerosis/frontotemporal dementia (ALS/FTD). *C9*-linked ALS/FTD is caused by a *G4C2* hexanucleotide repeat expansion (HRE) in the *C9orf72* locus and constitutes the most common genetic cause of the diseases. Among other disruptions, the RNA and Dipeptide Repeat (DPR) species that are generated by the *G4C2* HRE appears to impair the nuclear pore complex, thereby disrupting the nucleocytoplasmic transport of TFEB, the master regulator of autophagy and lysosomal biogenesis. How TFEB mislocalisation is connected with mTOR signalling and autophagy regulation during *C9*-linked ALS/FTD pathogenesis, however, remains unclear. We therefore aim to address this question using well-established *Drosophila melanogaster* models of *C9*-linked ALS/FTD. In line with previous findings, we observe a reduction in nuclear localisation of Mitf (*Drosophila* TFEB) upon overexpression of *G4C2* repeats, as compared to controls. Surprisingly, in the adult head, Mitf appears to accumulate at the protein level, predominantly in the inactive, cytoplasmic form. These results suggest that proteasomal degradation of inactive Mitf or indeed its inactivation - mediated by mTOR - may additionally be disrupted by *G4C2* toxicity. We are further investigating these possibilities and exploring genetic modulators of Mitf in the context of *G4C2* toxicity. Importantly, we find that reducing levels of the SNARE protein *Snap29* leads to alteration in Mitf localisation and regulation. Further, by performing a genetic interaction in the adult fly eye, we show that reducing *Snap29* levels either by RNAi or by a heterozygosity, strongly suppresses *G4C2* toxicity. We are currently dissecting how Snap29 may prevent *G4C2*-induced toxicity and disruptions of Mitf activity. We are also interested in ascertaining whether such effects involve the regulation of autophagy or mTOR signalling.

Keywords: Autophagy, C9orf72, ALS, Snap29, Mitf, mTOR

^{*}Speaker

Notch induced neural tumors exhibit differentiation capacities during allograft propagation

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Overactivation of Notch signalling in larval brains results in overgrowths characterized by aberrant propagation of stem-like neural cells. This initial tumorigenic insult induces the expansion of Type I and II neuroblasts and the de-differentiation of intermediate GMC and INP progenitors and early neuronal cells. Allograft injections of primary hyperplastic brain lobes in the abdomens of host flies result in fast tumor spread eventually killing the fly. Transcriptome, ATAC-seq and CUT&Tag chromatin profiling as well as phenotypic characterization of transplanted Notch-induced tumours, demonstrate that the vast majority of tumor cells maintain their stem cell-like characteristics. However, we discovered that cancer cells also retain their differentiation capacity. This is evident by the re-appearance of transcription factors essential for neuronal maturation and the presence of extensive axonal structures within the tumor mass. Thus, although suppression of the differentiation program appears to be essential for the initial step of Notch induced neural transformation, during tumor progression cancer cells manage to re-initiate an aberrant neuronal program. This dys-differentiation tumor state, between neural and neuronal cell fates, may contribute to tumor evolution and reveal potential vulnerabilities of cancer progression.

Keywords: Notch signaling, cancer, chromatin, differentiation, neuroblasts

*Speaker

Regulation of Fat signalling and tissue growth by deubiquitylating enzymes

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Regulation of tissue growth is influenced by many signals, including polarity cues. The Hippo signalling pathway restricts tissue growth and receives inputs from the planar cell polarity-controlling Fat signalling pathway. Fat signalling components restrict tissue growth via several mechanisms that ultimately control the activity of the pro-growth transcriptional activator Yorkie. Fat signalling modulates the activity of the Yorkie inhibitory kinase Warts, as well as the function of the FERM protein Expanded, which promotes Hippo signalling and also directly inhibits Yorkie. Although several Fat pathway activity modulators are known to be involved in ubiquitylation, the role of this post-translational modification in the pathway remains unclear. Moreover, no deubiquitylating enzymes have been described in this pathway. Here, using an *in vivo* RNAi screening approach, we identify the deubiquitylating enzyme Fat facets as a novel regulator of Fat signalling and tissue growth. Fat facets interacts genetically and physically with Fat signalling components and regulates transcription of Yorkie target genes. Thus, we uncover a role for reversible ubiquitylation in the strict control of Fat signalling and, by extension, in the regulation of tissue growth.

Keywords: Hippo signalling, ubiquitylation, Fat signalling, tissue growth

*Speaker

Role of autophagy in blood cell differentiation

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Drosophila larval hematopoiesis occurs mainly at the lymph gland, where blood cell progenitors differentiate into two major cell types: plasmatocytes that are analogous to mammalian macrophages, and represent around 95% of total blood cells; and crystal cells that share some features with mammalian megakaryocytes, and represent the remaining 5%. A third cell type, the lamellocytes, develops only upon wasp egg infection. In this work we have investigated the regulation of crystal cell differentiation, which is known to depend on the Notch pathway. We found that autophagy inhibition in blood cell progenitors results in increased crystal cell differentiation through a mechanism that depends on increased levels of the Notch receptor. We show that Notch activation during hematopoiesis largely depends on the endocytic pathway, and that autophagy sets a limit to this activation by promoting Notch lysosomal degradation. Over-activation of the IIS-TOR pathway provokes inhibition of autophagosome biogenesis, which in turn prevents the formation of Notch-containing amphisomes, being the latter necessary for Notch lysosomal destruction. Reduction of Notch lysosomal degradation shifts the balance towards Notch gamma secretase-dependent processing at late endosomal membranes, resulting in increased activation of the Notch pathway, and increased crystal cell differentiation. Our work defines a mechanism by which autophagy regulates cell differentiation by establishing a connection between the IIS-TOR and Notch pathways.

Keywords: Blood cells, hematopoiesis, autophagy, Notch

^{*}Speaker

Searching with a purpose - Effects of Notch activity on the behaviour of its transcription complex

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During development cells receive a variety of signals that coordinate their fates. One example is the Notch signalling pathway which is essential in many development processes. It regulates expression of many target genes through the core transcription factor *Suppressor of Hairless*, *Su(H)*. In order to understand how Notch activity brings about changes in transcription factor behaviour that lead to transcriptional changes, we have probed Su(H) behaviours in real time, using in vivo Single Molecules Localisation Microscopy. Tracking the behaviour of sparsely labelled, endogenous, Halo-tagged Su(H) in *Drosophila* larvae salivary glands, we implemented two different trajectory analysis methods to characterise sub-populations of Su(H) characterized by different motion characteristics in Notch-Off and Notch-On conditions. Both methods independently reveal that in Notch-OFF conditions, only a small fraction of Su(H) molecules exhibit ‘bound’/‘subdiffusive’ behaviour, with highly anisotropic and compact trajectories. Notch activation increases this fraction and, in addition, the proportion of molecules exhibiting exploratory behaviour in the nucleus. These properties are shared by the co-activator Mastermind. To analyse directly what happens at a Notch-regulated gene, we use a strain in which the Enhancer of Split complex (*E(spl)-C*), a highly Notch responsive locus, carries a fluorescent tag. Activation of target genes in Notch-ON conditions is accompanied by increased binding of Su(H) and Mastermind at the target locus. Furthermore, both Su(H) and Mastermind, exhibit characteristic of guided exploration near target enhancers. We suggest therefore that the Su(H) activator complex acquires an exploratory behaviour, favouring local searching and retention close to its target enhancers. This change in the way Su(H) interacts with the chromatin can explain how it efficiently increases its occupancy at target sites in Notch-ON conditions.

Keywords: Development, Notch, Transcription, Single Molecule Localisation Microscopy, Halo labelling, Transcription factor dynamics

*Speaker

The master cell cycle regulator CDK1 regulates Ras-MAPK-dependent cell fate choice during Drosophila eye development

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The delicate equilibrium between cell proliferation and differentiation is fundamental to proper organ development and tissue homeostasis, with imbalances potentially leading to tumorigenesis, tissue degeneration, or accelerated ageing. While cell proliferation is governed by evolutionarily conserved proteins known as cell cycle regulators (CCRs), their traditional role in coordinating proliferation and differentiation was thought to be secondary, primarily acting as effectors under transcriptional control by developmental pathways. However, emerging evidence highlights a more pivotal role for CCRs, as they directly influence cell type-specific factors through a post-translational mechanism, intricately linking cell differentiation processes to cell duplication. Despite its profound biological and clinical implications, this metazoan-specific function of CCRs remains largely unexplored. Using the Drosophila eye imaginal disc as our model - a system renowned for its high spatiotemporal coordination between cell proliferation and differentiation - we conducted a comprehensive RNAi or over-expression screen of all CCRs. This led to the identification of 42 CCRs whose genetic manipulations disrupt proper fate specification or differentiation of retinal cells. Among these, Cyclin-dependent kinase 1 (CDK1), a master CCR regulating the G2/M cell cycle transition, emerged as a central focus of our study. Notably, CDK1 depletion results in a defect in the suppression of R8 photoreceptor fate, leading to excess R8 formation and the failure of R2/5 differentiation, reminiscent of the phenotype observed upon EGFR-Ras-MAPK inactivation in pro-neuronal cells. Further genetic analyses suggest that CDK1 regulates these fate specification processes by influencing the activity of ETS transcription factors downstream of MAPK, potentially through phosphorylation. This finding suggests that CDK1 may play a role in integrating the cellular responsiveness to EGFR-Ras-MAPK signalling to the progression into mitosis. Reports of CDK-dependent phosphorylation in mammalian RTK pathway components, including ETS transcription factors, suggest a conservation of this regulatory mechanism across species. These novel roles of CDK1 in Drosophila retinal differentiation, indicating differential roles of CDKs in the regulation of various extracellular signaling pathways, underscore the high conservation of cell cycle control mechanisms. Thus, these findings on Drosophila CCRs may have broader implications for understanding similar mechanisms in mammalian systems.

Keywords: cell cycle, CDK, mitosis, differentiation, cell fate choice, signalling, EGFR, Ras, MAPK, ETS proteins, phosphorylation, eye development, retinal differentiation

*Speaker

The mechanosensitive channel Piezo delays epidermal wound closure to ensure effective inflammatory response and restoration of epithelial integrity

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Wound healing entails a fine balance between re-epithelialisation and inflammation, so that the risk of infection is minimised, tissue architecture is restored without scarring, and the epithelium regains its full functionality. How the two events are orchestrated *in vivo* remains poorly understood, largely due to the experimental challenges of simultaneously addressing mechanical and molecular aspects of the damage response. We use live imaging of ventral epidermis of stage 15 *Drosophila* embryo following laser induced aseptic wound, as a model to study the tissue repair process. We uncovered the role of the mechanosensitive ion channel Piezo during wound healing. We observed that loss of epidermal Piezo leads to weakened inflammatory response due to dampened Ca²⁺ dependent ROS production and consequent reduced haemocytes recruitment at the wound site. Moreover, we observed that loss of Piezo drives a faster healing kinetics. Mechanistically, Piezo acts as a molecular brake on wound closure dynamics by activating a Nos-cGMP axis that ultimately suppresses myosin cable activity, thus restraining the re-epithelization rate. Despite loss of Piezo appears initially beneficial, by suppressing the inflammatory response and facilitating wound closure, it is severely detrimental to the long-term effectiveness of repair. In fact, wounds inflicted to Piezo knock-out embryos become a permanent point of weakness within the epithelium, leading to impaired barrier function and consequent high mortality rate. In summary, our work uncovers a central role for Piezo in regulating epithelial cell dynamics and immune cell responsiveness during damage repair *in vivo*, to ensure the re-establishment of a fully functional epithelial barrier.

Keywords: Wound healing, Piezo

*Speaker

RNA biology

ELAV autoregulates to safeguard the neuronal 3' UTR landscape under normal and stress conditions.

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The formation and maintenance of neural cells and circuits requires the coordinated regulation of gene expression at each step of RNA metabolism. In *Drosophila*, the highly conserved RNA-binding protein ELAV mediates alternative polyadenylation (APA) to generate mRNAs with longer 3' UTR sequences specifically in the nervous system. These neuronal 3' UTRs (nUTRs), which can reach several kilobases, are enriched in regulatory elements that confer additional regulatory potential to the mRNA. Interestingly, the gene *elav* contains a nUTR, whose synthesis is mediated by ELAV protein itself.

In this study, we demonstrate that ELAV protein expression is tightly regulated via the *elav* nUTR. We show that ELAV protein levels can withstand drastic variations in *elav* copy numbers; this regulation is abolished in vivo in the absence of the *elav* nUTR. We demonstrate that a negative feedback loop maintains optimal ELAV levels in neurons. The nUTR-less *elav* mRNA is translated into ELAV protein; ELAV in turn promotes the formation of *elav* nUTR, which produces less protein. Polysome profiling in *drosophila* brains shows that the nUTR-containing *elav* mRNA displays less association to translating polysomes.

We studied the physiological role of *elav* autoregulation. Stress conditions induced ELAV degradation and triggered the regulatory loop in flies exposed to starvation. During stress, deletion of the nUTR resulted in loss of ELAV autoregulation, impairment of ELAV target gene expression, and reduced fly survival. Our study provides insight into a critical mechanism in the maintenance of ELAV protein and the preservation of gene expression and neuronal functions in a fluctuating environment

Keywords: Alternative polyadenylation (APA), untranslated regions (UTR)

*Speaker

Endogenous retrotransposon activity in the *Drosophila* intestine - towards the mechanisms of action of selfish DNA in the soma

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^{1,3}

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A growing body of research uncovers the importance of selfish DNA in somatic lineages throughout development and adult life. Endogenous retrotransposons, transposable elements that propagate via a copy-and-paste mechanism involving an RNA intermediate, occupy large portions of all eukaryotic genomes. A great majority of their multiple copies remains silenced in somatic cells, nevertheless, some are transcribed, and a small fraction retains its ability to mobilize, often in a tissue specific manner. Because of the highly repetitive nature of retrotransposons, identification of the precise active copies is often challenging. Consequently, the mechanisms that drive their somatic activity are not well understood.

In our previous work (Siudeja, van den Beek et al, 2021) we provided sequencing-based evidence of somatic retrotransposon mobility in the intestinal tissue of *Drosophila melanogaster*, which can lead to tumor suppressor inactivation and formation of gut neoplasia in aged midguts. Here, I will present our ongoing efforts towards revealing the mechanisms of action of these selfish elements. Using short- and long-read DNA and RNA sequencing, we identified the first fly "hot" donor locus of an endogenous retroviral element *rover*, highly active in the tissue. We then dissected the transcriptional landscape and local sequence and chromatin environment of all fixed *rover* copies present in the genome. This analysis offered insights into how locus-specific features allow active retrotransposon loci to escape repression, produce functional transcripts and mobilize in a somatic lineage.

Using this model system, my newly established team aims to further dissect the modes of retrotransposon regulation in the soma and the interplay between selfish genetic elements and tissue homeostasis *in vivo*.

*Speaker

Keywords: midgut, transposable element, retrotransposon, DNA sequencing, RNA sequencing, long, read sequencing, chromatin

Germ granule higher-order organization coordinates their different functions.

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Biomolecular condensates are membrane-less compartments that allow the coordination and efficiency of biochemical reactions in the cell by concentrating substrates and enzymes in a confined space. They are formed by phase separation, a demixing process that results from multivalent interactions between proteins and nucleic acids. RNA-Protein (RNP) condensates, formed by RNAs and RNA Binding Proteins (RBPs), are hubs for post-transcriptional regulation. Several types of RNP condensates were found to be not homogeneous and composed of multiple immiscible phases. However, whether these different phases contribute to their biological functions remains a key question. Germ granules are RNP condensates essential for germ cell specification and differentiation, involved in the coordination of germ cell mRNA localization and translational regulation. They represent an outstanding model to study the relationships between organization and functions of RNP condensates. Using Stimulated-Emission-Depletion (STED) super-resolution microscopy, we show that *Drosophila* germ granules have a core/shell organization, with their main protein components enriched in the shell phase. Single molecule imaging of live translation reveals that the translation of *nanos* mRNA, a germ cell mRNA essential for antero-posterior patterning and germ cell formation, occurs at the shell and immediate periphery of the granule, but not in the core. Interestingly, decreasing translation leads to relocalization of *nanos* mRNA towards the core. Finally, altering germ granule core/shell organization leads to a strong decrease in *nanos* mRNA translation. These data reveal a strong connection between germ granule organization and functions. We propose a model in which germ granule higher-order organization reflects a functional compartmentalization: translational activation is restricted to the shell phase that is permissive to translation, whereas translational repression occurs in the core where mRNAs are highly concentrated.

Keywords: RNP condensates, Germ granules, mRNA, Translation, *Drosophila*

*Speaker

Single molecule imaging and modelling of mRNA decay dynamics in the *Drosophila* embryo

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Regulation of mRNA degradation is critical for a diverse array of cellular processes and developmental cell fate decisions. Many methods for determining mRNA half-lives rely on transcriptional inhibition or metabolic labelling. Here we use a non-invasive method for estimating half-lives for hundreds of mRNAs in the early *Drosophila* embryo. This approach uses the intronic and exonic reads from a total RNA-seq time series and Gaussian process regression to model the dynamics of premature and mature mRNAs. We show how regulation of mRNA stability is used to establish a range of mature mRNA dynamics during embryogenesis, despite shared transcription profiles. Using single molecule imaging we provide evidence that, for the mRNAs tested, there is a correlation between short half-life and mRNA association with P-bodies. Moreover, we detect an enrichment of mRNA 3' ends in P-bodies, consistent with 5' to 3' degradation occurring in P-bodies for at least a subset of mRNAs. Building upon this, we are now investigating whether mRNA degradation could be regulated spatially in the early embryo. By integrating live and fixed imaging data, we can infer mRNA degradation rates for cells at different positions within an expression domain. I will present preliminary modelling results and P-body colocalisation analysis, which suggest that mRNA degradation plays a role in delineating gene expression patterns in early embryogenesis.

Keywords: embryogenesis, mRNA degradation, mathematical modelling, gene expression, post, transcriptional regulation, P, bodies

^{*}Speaker

Translation regulation by HBS1 in retinal development and disease

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Nearly 30% of autosomal dominant Retinitis pigmentosa (adRP), an age-dependent retinal degeneration disease, is caused by mutations in the vision protein Rhodopsin-1 (Rh1). These mutations frequently result in misfolding of Rh1, leading to the activation of the Integrated Stress Response (ISR) pathway. We recently demonstrated that loss of photoreceptors in a *Drosophila* model of adRP is exacerbated by the loss of the ISR kinase, *Perk*, and its downstream effector, *Atf4*. When activated by ER stress due to misfolding proteins, Perk phospho-disables the eIF2 complex to limit mRNA translation. Paradoxically, these inhibitory conditions induce the translation of the transcription factor Atf4 to restore ER homeostasis. To discover translation factors that enable such paradoxical Atf4 synthesis, we performed a *Drosophila* RNAi screen wherein we identified the translation termination factor, Hbs1, as a potential regulator of the *Atf4* mRNA 5' leader. Hbs1 is a GTPase which forms a heterodimeric complex with Pelota (also known as DOM34). Together, this complex aids in ribosome recycling post-termination. Consistent with the protective roles for Atf4 in adRP, Hbs1 mutants also showed exacerbated retinal degeneration in a *Drosophila* adRP model. Interestingly, we also found retinal degeneration in aging *Hbs1* mutants, which is reminiscent of retinal degeneration defects seen in human patients with HBS1. These data prompted us to examine the role of Hbs1 in eye development. Electroretinograms (ERG) from young *Hbs1* mutants showed a substantial reduction of on and off transients, and a drastic loss of photoreceptor potential with age. These defects are indicative of disruption in phototransduction between the retina and lamina neurons. TEM imaging of *Hbs1* mutants reveals loss of pigment and support cells, which may explain the phototransduction defects seen with ERG. We are currently determining the specific cell types in which Hbs1 is required, and whether Hbs1 mediates photoreceptor development via regulation of the *Atf4* 5' leader. Altogether, our study shows an important role for translation regulation by Hbs1 in eye development, as seen by TEM, and in retinopathies, as seen with our adRP model.

Keywords: mRNA translation, Hbs1, Retinitis pigmentosa, Atf4, stress

*Speaker

Physiology & metabolism

C-terminal binding protein couples redox-dependent sugar sensing to enteroendocrine cell fate regulation

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Sugars are essential macronutrients for life. How cells read and integrate signals about the presence of sugars remains insufficiently understood. As NAD⁺/NADH redox balance is influenced by sugar catabolism, we hypothesized that availability of NADH might serve as a proxy to inform about the availability of sugars. Thus, proteins holding NAD(P)-binding motifs might play a role in controlling cellular and physiological functions in response to dietary sugar. To test this hypothesis, we selected ~150 genes based on the presence of NADP(H)-binding motif in the proteins they encode and performed an RNAi screen on larval sugar tolerance. Among the hits, we identified the transcriptional cofactor C-terminal binding protein, CtBP, an NADH putative sensor, to be necessary for survival on high sugar diet. *ctbp* mutant larvae show deregulated expression of intestinal transcripts and physiological changes in midgut function. Most importantly, CtBP deficient animals show reduced numbers of several enteroendocrine (EE) cell subtypes. EE cell-specific knockdown of CtBP leads to reduced EE cell numbers and sugar intolerance, demonstrating a cell autonomous regulation of EE cell fate by CtBP. We found that NADH-dependent CtBP homooligomerization, a prerequisite for its function as a transcriptional cofactor, is indeed nutrient-dependent in EE cells being increased on high sugar diet. Moreover, we provide evidence that CtBP mediates its control on EE cells through physical interaction with Prospero, the key determinant of EE cell identity. CtBP and Prospero share a significantly overlapping set of direct downstream target genes. Thus, our data implies that the interplay between CtBP and Prospero allows dynamic control of EE cell fate in response to changes in nutrient intake.

Keywords: Sugar sensing, redox sensing, enteroendocrine cells, NADH, CtBP, Prospero

*Speaker

Drosophila blood cells regulate sugar homeostasis through fructose-sensing receptor

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The primary goal of immune cells is to protect the host from harmful pathogens or to cope with environmental stresses. In addition to the conventional immune functions, recent studies have suggested that blood cells also play a role in metabolic regulation through systemic interactions with other tissues. However, the mechanistic details underlying metabolic function at an organismal level have not been adequately elucidated. Here, we found that gustatory receptor 43a (Gr43a), a chemosensory receptor for fructose sensation, is expressed in *Drosophila* larval hemocytes and gauges fructose levels in the hemolymph. Knocking-down of *Gr43a* in larval hemocytes induces sugar levels in the hemolymph, while decreasing intracellular sugar levels in hemocytes. Increased hemolymph sugar levels caused by loss of *Gr43a* promotes insulin secretion in the brain insulin-producing cells (IPCs), which induces insulin signaling in the fat body to accumulate lipids. Thus, our results demonstrate that *Drosophila* hemocytes directly control internal sugar homeostasis by sensing the level of circulating sugars via Gr43a for systemic metabolic regulation.

Keywords: *Drosophila melanogaster*, hemocytes, metabolism, immunity, regulation, sugar sensing receptor

^{*}Speaker

Neuronal triglyceride metabolism regulates sex differences in whole-body energy homeostasis

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Biological sex is a key regulator of body fat across species. In *Drosophila*, females store twice as much body fat as males and break down fat more slowly during starvation. We previously showed that neuronal loss of triglyceride lipase *brummer* (*bmm*) blocked the sex difference in fat breakdown. Because we now reproduce these sex-specific effects in flies with neuronal loss of additional genes related to intracellular triglyceride metabolism and storage, our data suggests the sex-specific regulation of neuronal triglyceride contributes to male-female differences in body fat. Yet, the underlying mechanism(s) remain unclear, as much remains to be discovered about the intracellular regulation and function of triglyceride in neurons. Here, we use neuronal expression of a GFP targeted to lipid droplets, a specialized organelle dedicated to triglyceride storage, to visualize neuronal lipid droplets and reveal sex-, diet-, and age-dependent effects on lipid droplet abundance. We further show *bmm* and related genes influence the abundance of neuronal lipid droplets, and our unbiased analysis of the brain lipidome showed that neuronal loss of *bmm* caused significant and sex-specific changes to brain lipids. Because loss of *bmm* in one group of ~18 neurons reproduced phenotypes associated with reduced activity in these neurons only in males, our findings suggest a model in which triglyceride plays a sex-biased role in supporting neuronal activity. This reveals a previously unrecognized role for triglyceride in supporting neuron function, and identifies new sex- and cell type-specific roles for many genes involved in regulating intracellular triglyceride metabolism.

Keywords: Sex difference, lipid droplet, neuron, triglyceride

*Speaker

Store now, use later: the temporal allocation of amino acid resources during development

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Food availability fluctuates in nature and all organisms have evolved systems to store nutrients. This evolutionary adaptation is particularly critical to ensure resources for energy-consuming life transitions, non-feeding developmental periods or survival in unpredictable environments. Although extensive research has addressed the question of fat and carbohydrate storage, animals also depend on amino acid fluxes for growth and tissue renewal, yet protein storage remains an under-explored topic. Holometabolous insects such as *Drosophila* offer an interesting model to tackle this question. Indeed, these animals spend an extended period of development during which massive tissue remodelling takes place without feeding, therefore questioning the presence of amino acid resources needed for proper development. Using a combination of genetic and molecular approaches, we highlighted the spatial and temporal control exerted on the expression of hexamerins, a group of insect-specific albumin-like storage proteins. We studied the mechanism by which hexamerins ensure amino acid availability through shuttling between the hemolymph and the fat body. We show that hexamerins serve as reliable amino acid stores during metamorphosis and are required for building adult structures, contributing to organ function and final body size. Finally, genetic perturbations of hexamerin production and/or shuttling at the larval stage lead to increased larval growth, a loss of adult body allometry and a deterioration of adult fitness, exemplifying the importance of proper temporal allocation of amino acid resources during development.

Keywords: stored amino acids, resource allocation, hexamerins

*Speaker

The *Drosophila* gene *sima*/Hif1alpha is an essential regulator of the larval glycolytic program

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The rapid growth that occurs during *Drosophila* larval development requires a dramatic rewiring of central carbon metabolism to support biosynthesis. Previous studies from our lab and others demonstrated that this exponential growth phase is preceded by a dramatic metabolic switch, which results in the transcriptional up-regulation of several metabolic pathways, including those that encode enzymes involved in glycolysis and the pentose phosphate pathway as well as Lactate Dehydrogenase. The resulting metabolic program exhibits the hallmark characteristics of aerobic glycolysis and establishes a physiological state that supports biomass accumulation, similar to that seen in tumor cells. Studies in the fly so far have only discovered a single transcription factor involved in this process - the *Drosophila* Estrogen-Related Receptor (dERR), which is absolutely required to up-regulate carbohydrate metabolism in preparation for larval growth. Here we describe our discovery that Sima, the sole *Drosophila* ortholog of the hypoxia inducible factor-1 alpha (Hif1a), is also essential for promoting glycolysis in larvae. Using CRISPR/CAS9 generated *sima* alleles, we discovered that the classic *sima* p-element insertion mutation *sima*(KG07607) represents a weak hypomorph that exhibits very mild metabolic defects. In contrast to this canonical allele, our novel null mutations exhibit a mid-L2 lethal phase and phenocopy the developmental and metabolic defects observed in *dERR* mutants. Moreover, RNA-seq analysis of *sima* mutants reveal a dramatic downregulation in glycolytic genes, with the overall transcriptional profile mimicking that of the *dERR* mutant. Subsequent metabolomics studies revealed that, when compared to control larvae, *sima* mutants exhibit a severe block in glycolytic metabolism. Considering that Sima and dERR are capable of physically interacting, our studies suggest that these two ancient transcription factors cooperatively regulate the larval metabolic program.

Keywords: Hif1alpha, Estrogen Related Receptor, glycolysis, larval development

*Speaker

The macrophage genetic cassette *inr/dtor/pvf2* is a nutritional status checkpoint for developmental timing.

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A small number of signaling molecules, used reiteratively, control differentiation programs, but the mechanisms that adapt developmental timing to environmental cues are less understood. We report here that a macrophage *inr/dtor/pvf2* genetic cassette is a developmental timing checkpoint in *Drosophila*, which either licenses or delays biosynthesis of the steroid hormone in the endocrine gland and metamorphosis according to the larval nutritional status. Insulin-Receptor/dTor signaling in macrophages is required and sufficient for production of the PDGF/VEGF family growth factor Pvf2, which turns on transcription of the sterol biosynthesis Halloween genes in the prothoracic gland via its receptor Pvr. In response to a starvation event or genetic manipulation, low Pvf2 signal delays steroid biosynthesis until it becomes Pvr-independent, thereby prolonging larval growth before pupation. The significance of this developmental timing checkpoint for host fitness is illustrated by the observation that it regulates the size of the pupae and adult flies

Keywords: Macrophages, nutrient sensing, ecdysone, developmental timing

*Speaker

The peptide hormone CG14075/Marmite, secreted from the corpora cardiaca, regulates the starvation-dependent response in *Drosophila*

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Organisms adapt by skillfully changing their physiological state in response to environmental changes. In the model organism, *Drosophila melanogaster*, insulin-like peptide-producing cells (IPCs) and glucagon-like hormone, Adipokinetic hormone (AKH)-producing cells (APCs), called corpora cardiaca, are known to regulate physiological responses in response to feeding (Nässel and Zandawala, *Prog Neurobiol.* 2019). However, while many studies have elucidated the function and regulation of IPCs, APCs are only known to produce AKH and to be regulated by several neuropeptides. Therefore, it remains to be elucidated how APCs receive complex information such as nutrition and hunger and output this information. To this end, we attempted to narrow down the genes expressed in APCs using RNA-seq and found that, in addition to *AKH*, a gene, *CG14075*, is predominantly expressed in APCs.

To understand the function of *CG14075*, we generated *CG14075* KO flies. We found that *CG14075* KO or *CG14075* RNAi in the APCs resulted in increased sensitivity to starvation and promoted the consumption of glycogen and trehalose in response to starvation. Consumption was significantly enhanced from 24 to 48 hours after starvation, suggesting that *CG14075* has a function in the mid-to-late-starvation period. Furthermore, we found that the loss of *CG14075* resulted in significant changes in the metabolic profile, as confirmed by LC-MS/MS. Since *CG14075* is a secreted factor with a signal peptide sequence, we explored the profile of *CG14075* secretion in response to starvation. We found that the amount of *CG14075* in hemolymph increased significantly 18-36 h after starvation and that the *CG14075* secretion was suppressed by sugar refeeding.

Interestingly, AKH secretion was increased 6-12 hours after starvation and induced lipolysis, suggesting that although AKH and *CG14075* are secreted by the APCs, the mode of secretion and its physiological effects are different. Recently, *CG14075* was named Marmite (Mmt) and reported as an evolutionarily conserved neuropeptide that functions in the brain (Francisco et al. *bioRxiv.* 2022). Collectively, our results indicate that APC produces a novel evolutionarily conserved hormone-like peptide, *CG14075*/Mmt, not only AKH, and that *CG14075*/Mmt improves

*Speaker

fitness for starvation by regulating glycogen and trehalose metabolism during mid-starvation.

Keywords: Hormone, Metabolism, Feeding, Starvation, Adipokinetic hormone

The sex and reproductive plasticity of intestinal muscles

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The adult *Drosophila* gut undergoes striking anatomical and functional changes to adapt to environmental challenges or changes in internal state. Previous work has shown that the intestinal epithelium differs between males and females at the levels of organ size, metabolism and proliferative capacity. One major reason for these sex differences is reproduction: in females, mating increases intestinal stem cell (ISC) proliferation increasing intestinal size. As the intestinal epithelium enlarges, how do the intestinal-visceral muscles ensheathing the midgut react? Do they adapt or contribute to reproductive intestinal plasticity? We have observed that the gut muscles differ between the sexes: females have more muscle cells and wider myofibrils inside each muscle cell. Strikingly, muscle-specific sex reversals alter myofibril width, and uncover a role of muscle sexual identity in controlling gut size. Furthermore, cellular and functional analyses show that the intestinal muscles of adult females increase in size and elongate their sarcomeres postmating, which is associated with changes in intestinal transit and peristaltic activity. Mechanistically, previous studies have shown that reproductive resizing of the intestinal epithelium is driven by the increased activity of Juvenile Hormone (JH) signalling on ISCs and enterocytes. In contrast, we have observed that JH signalling is specifically reduced in gut muscles postmating, and that preventing muscle remodelling precludes reproductive remodelling of the midgut reducing fecundity. Therefore, JH ensures reproductive output by acting in opposite ways in the intestinal epithelium and the muscles that surround it. Our findings underscore the importance of a previously unrecognised plasticity of intestinal muscles, and shed light on how adult somatic organ plasticity is coordinated across different tissues within the same organ.

Keywords: plasticity, muscle, intestine, sex differences, hormones

^{*}Speaker

Y chromosome toxicity does not contribute to sex-specific differences in longevity

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Men and women are phenotypically different. Height, weight, disease prevalence or life expectancy are sex-biased, but the origin of these differences is still not well understood. Some studies suggest that differences in longevity are due to the intrinsic presence of the sex chromosomes, and in particular due to the Y chromosome structure. A correlation between Y chromosome number and longevity was observed, since life expectancy is reduced in individuals with supernumerary Y chromosomes. In the animal kingdom, heterogametic sexes (the males in mammals or some insects, the females in birds or reptiles) have shorter lifespan, and this led to the hypothesis of a "toxic effect" of the Y (or W) chromosome. These chromosomes, mostly composed of simple repeated sequences and transposable elements, are transcriptionally silenced in large heterochromatic domains. In *Drosophila*, we measured that the Y chromosome represents 13% of the male genome and these large heterochromatin structures are absent in females. During aging, heterochromatin marks are lost, transposons become derepressed and transpose, potentially generating deleterious mutations and faster aging. It suggests that the Y chromosome-associated toxicity might impact the male physiology according to its size. This led us to test whether Y chromosome size has any consequences on sex-biased phenotypes and longevity. To this aim, we developed an innovative CRISPR/Cas9 strategy in *Drosophila* using gRNAs to target specific repeated sequences of the Y chromosome. With this method, we generated a library of Y chromosomes with different sizes or complete loss. Using this library in combination with a reporter gene, we demonstrated that the size of the Y chromosome can affect heterochromatin maintenance in the whole genome. However, we measured how Y chromosome presence or size modifies *Drosophila* lifespan and to our surprise, we revealed that the amount of Y chromosome heterochromatin does not change fly lifespan. The hypothesis of the Y chromosome-associated toxicity cannot explain sex differences in lifespan. Instead, we could show that sex-biased life expectancy is essentially controlled by the sex determination pathway itself, through the female-sex determinant, *transformer*.

Keywords: Y chromosome, sex differences, sex chromosomes, Y toxicity, lifespan, CRISPR/Cas9, heterochromatin

^{*}Speaker

marmite defines a novel conserved neuropeptide family mediating nutritional homeostasis

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Neuropeptides play a key role in regulating physiology and behavior, including feeding. While animals modify their food choices to respond to the lack of specific nutrients, the mechanisms mediating nutrient-specific appetites remain unclear. Here, we identified *marmite* (*mnt*), a previously uncharacterized *Drosophila melanogaster* gene encoding a secreted peptide that controls feeding decisions. We show that both *mnt* mutants and neuronal knockdown of *mnt* specifically increased the intake of proteinaceous food, whereas neuronal *mnt* overexpression reduced protein appetite. *mnt* expression is also higher in animals maintained on amino acid rich food, suggesting that *mnt* encodes a protein-specific satiety signal. *Mnt* is expressed in a small number of neurons in the adult nervous system, with a single pair of neurons modulating protein appetite. Finally, sequence and phylogenetic analysis showed that *mnt* is part of an ancient and conserved family of neuropeptides, including the poorly understood vertebrate *neuropeptides B* and *W* genes. Functional experiments showed that *mnt* and vertebrate *NPB* and *NPW* modulate food intake in both flies and mice. Therefore, we discovered an ancient family of neuropeptides involved in controlling feeding across phyla.

Keywords: Neuropeptide, Feeding behavior, Nutritional homeostasis, Conservation, Protein appetite

*Speaker

Immunity & symbiosis

13C-labeled glucose and trehalose metabolism in larval hemocytes responding to parasitoid wasp

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Rapid activation of immune processes is associated with increased nutrient consumption by immune cells. Previously, using a model of infestation of *Drosophila* larvae by a parasitoid wasp, we have shown that increased demands for nutrients by immune cells are associated with a systemic metabolic switch whereby immune cells become privileged in nutrient acquisition within the organism. Several recent studies that have used single-cell transcriptomics of hemocytes in the same model have suggested possible metabolic changes in hemocytes. However, hemocyte metabolism itself has not yet been investigated. We have established a metabolomics analysis of hemocytes *in vivo* using ¹³C stable-isotope tracing. We found that hemocytes indeed consume more carbohydrates during infection and increase ATP production by glycolysis, which ends in lactate. Most striking, however, is the repeated oxidation of glucose-6-phosphate in the so-called cyclic pentose phosphate pathway (PPP) and the formation of NADPH. Cyclic PPP has only been demonstrated experimentally once in immune cells, in mammalian neutrophils. By silencing the first enzyme of the PPP pathway, Zwischenferment (*Zw*), we have shown that cyclic PPP, and thus NADPH, is required for efficient lamellocyte differentiation during wasp infestation (likely related to the role of NADPH in reductive biosynthesis), but also for parasitoid killing (related to the role of NADPH in ROS generation). Expression studies have further suggested the importance of trehalose metabolism in lamellocytes. Using an expression reporter for the cytoplasmic form of trehalase (*Treh*) and by tracing ¹³C-labeled trehalose, we found that lamellocytes do indeed metabolize trehalose, in contrast to hemocytes in the absence of infection, which can only utilize glucose. Again, trehalose is metabolized by lamellocytes in cyclic PPP, but is required for antioxidant production. Knocking out trehalase in hemocyte clones allowed us to elucidate the possible different roles of NADPH production by cyclic PPP. On the one hand, hemocytes must produce toxic radicals (the role of plasmatocytes adhering to the egg) and concentrate them in a capsule around the egg (formed by lamellocytes), but on the other hand, the capsule also protects the host from this toxic reaction (via antioxidant production by lamellocytes). Although inactivation of trehalase in half of the lamellocytes increased resistance, it also reduced the fitness of the surviving fly. The tuning of immune cell metabolism is clearly of great evolutionary and ecological importance.

*Speaker

Keywords: immunity, metabolism, ^{13}C stable isotope, metabolomics, hemocyte, trehalose

An antimicrobial peptide family evolutionarily adapted to control bacteria present in the host ecology

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Immune genes are among the most rapidly-evolving genes in the genome. As host-encoded immune effectors, antimicrobial peptides (AMPs) combat pathogens and shape the microbiome in plants and animals. Though their microbicidal properties have been studied for decades, technical difficulties prevented genetic dissection of their precise importance *in vivo*. Thanks to CRISPR technology, our group and others have begun disentangling the complex roles of AMPs in an *in vivo* context. We could show that AMPs were globally important for regulating the *Drosophila* microbiome, particularly the mutualist gut microbe *Acetobacter*. However, a surprising finding has been that many AMPs seemingly contribute little to defence against specific pathogens, with just single genes being relevant to a given infection in many cases. At present, it is unclear why such specific AMP activities might exist, or how exactly the host antimicrobial peptide repertoire is adapted to be an effective regulator of the microbiome. In this study, we characterize the function and evolution of the Dipterecin antimicrobial peptide family of Diptera. Using mutations affecting the two *Diptericins* (*Dpt*) of *Drosophila melanogaster*, we reveal the specific role of *DptA* for the pathogen *Providencia rettgeri* and *DptB* for the gut mutualist *Acetobacter*. Strikingly, presence of *DptA*- or *DptB*-like genes across Diptera correlates with the presence of *Providencia* and *Acetobacter* in their environment. Moreover, *DptA*- and *DptB*-like gene sequence predicts host resistance against infection by these bacteria across the genus *Drosophila*. This work provides a clear-cut case of rapid evolution following gene duplication that produced immune novelty, where both the host immune genes and target pathogens could be identified. Importantly, our study explains the evolutionary logic behind the bursts of rapid evolution of an antimicrobial peptide family, and reveals how the host immune repertoire adapts itself to changing microbial environments.

Keywords: Antimicrobial peptides, Dipterecin, Microbiome, Evolution, Immunity, Pathogen

*Speaker

An experimental framework to identify microbe-derived metabolites promoting host health

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Host-microbe interactions exist in a delicate balance between mutualism and conflict. This is keenly illustrated in the gut where mutualistic host-microbe interactions are crucial for host health. Mutualistic host-microbe interactions are mediated via a range of mechanisms. One important mechanism for host-microbe interactions is through the production of a diverse milieu of metabolites which affect host health. However, we lack a mechanistic understanding of which microbes, and microbe-derived metabolites, promote host health. Here, we develop an assay utilizing the fruit fly, *Drosophila melanogaster*, to distinguish microbes and microbe-derived metabolites which promote host health by measuring fly survival in a sub-optimal nutrient landscape supplemented with microbe-derived metabolites. We identified that metabolites produced by two *Lactobacillus* species, and two *Acetobacter* species promote survival in flies. Intriguingly, these findings were dependent on the microbiome status of the host, as *Lactobacillus*-derived spent media extends the survival of antibiotic treated flies, whereas *Acetobacter*-derived spent media did not. Moreover, we identify lactic acid and acetic acid promote host survival, as lactic acid strongly enhances fly survival whereas acetic acid has microbiome-dependent effects on host survival. Together, our work provides an experimental framework to identify the microbes and microbe-derived metabolites which promote host health in vivo. Ultimately, our work should empower mechanistically principled interventions utilizing microbe-derived metabolites to promote host health.

Keywords: Microbiome, Host, Microbe

*Speaker

Drosophila host defense against secreted virulence factors: how a few immune-response secreted peptides protect the host against distinct mycotoxins, a bacteriocin, and a bacterial protease carried by outer membrane vesicles

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The current paradigm in *Drosophila* innate immunity is that upon sensing infections the Toll and/or Immune deficiency NF-kappaB pathways are activated in the fat body and induce the expression of antimicrobial peptide (AMP) genes. The AMPs secreted in the hemolymph attack the invading microbes. This model is supported by genetic data, at least as regards Gram-negative bacterial infections. The situation is less clear with respect to Gram-positive bacterial and fungal infections that are countered by the Toll response, which regulates the expression of some 250 genes. Toll-dependent families of short secreted peptides include Bomanins and BaramicinA-derived peptides. Interestingly, the deletion of 10 *Bomanin* genes at the 55C locus largely phenocopies the Toll mutant susceptibility phenotype to these pathogens.

We have reported that *Aspergillus fumigatus*, which kills only Toll-deficient flies and 55C deletion flies, does not proliferate nor disseminate in wild-type or Toll mutant flies, being controlled by melanization. We have identified two *Af* mycotoxins that kill solely immuno-deficient flies and contribute to *Af* virulence. Restrictocin is a ribotoxin that efficiently cleaves 28S RNA *in vivo* only in immunodeficient flies; verruculogen affects the nervous system and induces tremors in all flies: only wild-type flies are able to recover from seizures. These different noxious effects are countered by distinct sets of Bomanins specific to each mycotoxin, with BomS6 expressed in neurons playing a primeval role in the recovery from tremors (Xu *et al.*, EMBO Reports, 2022).

Independently, we have demonstrated that *BaraA* mutants are susceptible only to *Enterococcus faecalis* and to *Metarhizium robertsii*, an entomopathogenic fungus. BaraA-derived peptides

*Speaker

protect the host from the action of EnterocinV, a bacteriocin secreted by *E. faecalis*, and from DestruxinA, a pore-forming mycotoxin. The silencing of *BaraA* in glial cells leads to a heightened sensitivity to DestruxinA and to EnterocinV (Huang *et al.*, PNAS, 2023).

We now report that two genes previously annotated as lncRNAs encode secreted peptides that are required in the host defense against bacterial outer membrane vesicles and the associated PrtA metalloprotease. Unexpectedly, they genes are also required in the host defense against EnterocinV and DestruxinA while *BaraA* is involved in the host defense against PrtA. Thus, at least three proteins mediate the protection against biochemically strikingly distinct secreted virulence factors of prokaryotic or eukaryotic origin.

We conclude that an important aspect of host defense against infections is the ability to protect the organism from the action of various secreted microbial virulence factors.

Keywords: innate immunity, host defense against infections, mycotoxins, bacterial toxins, outer membrane vesicles, Toll pathway, *Metaphizium robertsii*, *Enterococcus faecalis*

Endosymbiont-induced nutritional immunity protects the host from infections

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Host sequestration of essential micronutrients, like iron, to restrict pathogen access to vital nutrients is a conserved defense mechanism termed nutritional immunity. Insect symbionts are known to protect their hosts against pathogens via several mechanisms. However, whether nutritional immunity contributes to endosymbiont-mediated protection has not been studied, although some of the endosymbionts are known to induce nutritional immunity response in the host. Here, we used *Drosophila melanogaster* and its endosymbiont *Spiroplasma poulsonii* as a model to test the potential role of endosymbiont-induced iron sequestration in host protection from infections. First, we found that *Spiroplasma*-infected flies are more resistant to several pathogens, particularly to *Staphylococcus aureus* and *Rhizopus oryzae*. Our transcriptomic analysis showed upregulation of Toll pathway-regulated genes, melanization response, and key iron sequestration gene *transferrin 1* in *Spiroplasma*-infected flies. Consistent with RNA-seq results, we observed increased melanization and iron sequestration in *Spiroplasma*-infected flies. Next, we showed that the protective effect of *Spiroplasma* was not present in melanization-deficient and in *tsf1* mutant flies impaired in iron sequestration. Thus, *Spiroplasma*-induced melanization and iron sequestration are key mediators of endosymbiont protective effects against infections. Overall, our results support a model where *Spiroplasma* induces the Toll pathway which consequently triggers downstream defense reactions melanization and iron sequestration. Currently, we are investigating how *Spiroplasma* induces the Toll pathway and the effect of endosymbiont on intestinal immunity and homeostasis.

Keywords: symbiont, immunity, infection, melanization, iron sequestration, Spiroplasma

*Speaker

Immune cells maintain internal oxygen homeostasis in *Drosophila*

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Hemocytes of *Drosophila* larvae are divided into three types: plasmatocytes occupy 95 % of the total hemocytes and mainly play a role in phagocytosis. The second type is crystal cell that is involved in the melanization process through non-enzymatic polymerization via pro-phenol oxidase to phenol oxidase conversion. The third type is lamellocyte, which gives rise only during stress responses, such as infestation, and functions in pathogen encapsulation. Despite their role in innate immunity, hemocytes do not function in oxygen delivery. Instead, insects express Cu²⁺-mediated hemocyanin as an oxygen transfer protein. In this study, we found that the distribution of circulating hemocytes dynamically changes according to ambient oxygen concentration. In particular, hemocytes interact with the trachea under hypoxic conditions. We found that PPO2 in crystal cells maintains the overall body oxygen level by changing the crystal-to-cytosol transition. Therefore, *Drosophila* lacking crystal cells are hypoxic, which can be restored by hyperoxia. Overall, our results demonstrate a novel function of crystal cells in oxygen homeostasis.

Keywords: PPO2, Oxygen, Hypoxia

^{*}Speaker

Microbiota-mediated suppression of a gut-derived decretin's expression promotes *Drosophila* larvae systemic growth upon malnutrition

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Our previous findings unveiled that the association of undernourished germ-free (GF) *Drosophila* larvae with selected commensal bacteria, including *Lactiplantibacillus plantarum* WJL (*Lp*WJL), supports juvenile growth *via* promoting the systemic release and activities of *drosophila* Insulin-like peptides (dILPs), the analogous systemic endocrine mechanisms of vertebrate Insulin and Insulin-like Growth Factors. Recent work in multiple model systems revealed that gut endocrine functions, mediated by the enteroendocrine cells (EECs), are also impacted by nutrition and microbiota. Despite these advances, how poor nutrition alters EEC functions in the context of juvenile growth and how a selected microbial intervention participates in the restoration of fine-tuned EEC functions remain elusive. Here, we show that a subset of EECs in the midguts of *Drosophila* larvae specifically respond to low-nutrition diet by regulating Limostatin (Lst), a decretin hormone induced by starvation that inhibits dILPs output and secretion. Upon malnutrition, gut-derived *lst* was unaltered in *Lp*-associated larvae in contrast to normal fed larvae, whereas its transcript levels were markedly upregulated in GF condition. Intriguingly, both low-yeast diet and microbiota had no effect on the expression of corpora cardiaca (CC)-derived *lst*, which was previously demonstrated as a nutrient-responsive decretin hormone in *Drosophila* adults. Furthermore, using *lst1* null allele, we found that *lst*-deficiency led to the accelerated larval systemic growth upon undernutrition. However, this phenotype was not observed after knocking down *lst* in CC, which implies that the promotion of systemic growth is specifically attributed to the downregulation of gut-derived *lst*. Together, our findings indicate that *Lp*WJL suppresses the malnutrition-triggered upregulation of gut-derived *lst*, which in turn prompts *Drosophila* larvae systemic growth through the regulation of Insulin-like signalling by the gut-derived decretin Lst.

Keywords: Microbiota, Limostatin, decretin, dILPs, systemic growth, *Drosophila* larvae

*Speaker

The gut microbiome controls gastrointestinal transit in *Drosophila*

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The intestinal microbiota plays a crucial role in the regulation of host nutrition, metabolism, and behavior. In the last decade, *Drosophila melanogaster* has emerged as an ideal model to investigate how the microbiome exert these effects. *Drosophila* are conventionally fed with diets containing preservatives, such as propionic acid and methylparaben. While these xenobiotics prevent food decay, they may have side-effects on the microbiome and ultimately mask its impacts on host physiology. We observed that removing preservatives from the diet leads to an increase in gut bacterial density. This dietary intervention is also associated with a marked accumulation of food in the gastrointestinal tract. These observations suggest that the gut microbiome controls transit, and that this effect can be perturbed by xenobiotics. We identified bacterial species that regulate transit by performing feeding and defecation assays, microbiome profiling and tissue imaging. We are currently exploring the mechanisms through which bacteria control this function in their hosts. Our studies show that symbionts can auto-aggregate on the surface of the ingested food, and we are currently testing if the formation of biofilm and changes in the structure of the bolus are responsible for delaying transit. Our studies could therefore open new perspectives on how the microbiome regulates transit, a vital process that support nutrition in both hosts and their microbes.

Keywords: Microbiome, Transit, Physiology, Metabolism

*Speaker

The twilight zone between transposons and endogenous retroviruses

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Endogenous retroviruses are abundantly embedded within host genomes and provide a unique snapshot of multiple past viral infections. They are thought to be evolutionarily related and structurally similar to LTR retrotransposons, yet retroviruses primarily differ by a canonical Envelope gene crucial for infectivity. We have discovered a group of active transposons in the *Drosophila* ovary that mimic retroviral behavior - demonstrating infectivity traits - despite the absence of an Envelope-coding gene. We further identified an alternative infectivity gene encoded in the genomes of these transposons, potentially substituting the Envelope's role in enabling cell-cell transmission. These findings, which introduce the concepts of infectious transposons or Envelope-less retroviruses, necessitate reconsideration and redefinition of the conventional boundaries between transposons and viruses.

Keywords: Endogenous retroviruses, Transposons, Infectivity, Envelope

*Speaker

cGAS-like receptor-mediated immunity: the drosophila perspective

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In mammals, the enzyme cGAS senses the presence of cytosolic DNA and synthesizes the cyclic dinucleotide (CDN) 23-cGAMP. This CDN binds to and activates the protein STING to trigger immunity. We recently discovered in the model organism *Drosophila melanogaster* two cGAS-like receptors (cGLR1 and cGLR2)) that activate STING-dependent antiviral immunity several CDNs, in addition to 23-cGAMP. Using an accurate and sensitive mass spectrometry method, we identified an unexpected diversity of CDNs produced in a cGLR-dependent manner in response to viral infection in *D. melanogaster*. For example, the CDN 2'3'-cdiGMP is the most potent STING agonist identified so far in *Drosophila melanogaster*. We next explore CDN-mediated immunity in 14 different *Drosophila* species covering 50 million years of evolution, in which we have identified 61 cGLRs. *In silico* analysis of cGLRs reveals that some of them may sense nonnucleic acid ligands. We aim to shed light on the evolution of cGLRs in flies and provide a basis for the understanding of the function and regulation of this emerging family of PRRs in animal innate immunity.

Keywords: cyclic dinucleotides, secondary messenger, cGAS, like receptors, STING pathway, virus, innate immunity

^{*}Speaker

POSTER Presentations

by topics

Cell biology

A Multiplayer game: how heteroplasmy transmission is regulated.

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Mitochondrial diseases caused by mutations in the mitochondrial genome (mtDNA) affect 1 in 5000 individuals. There are multiple copies of mtDNA in an organism and different mtDNA variants can coexist, a phenomenon called heteroplasmy. The level of mutant mtDNA often determines the onset and severity of mtDNA linked disorders. Therefore, limiting the amount of detrimental mtDNA is vital for organismal health.

The nuclear genome is known to play an important role in governing mtDNA maintenance and transmission. However, very little is known about its influence on the transmission of co-existing mitochondrial genomes. To identify nuclear factors that impact the competition among mtDNAs, we performed the first genome-wide haploid-insufficiency screen using *Drosophila* which stably transmits two mitochondrial genomes enforced by purifying selection benefiting one healthy genome and a selfish advantage favouring a detrimental mutant. This screen identified multiple nuclear loci that show a dosage effect on the heteroplasmy dynamics over generations. We mapped one locus to the catalytic subunit of mtDNA polymerase – *PolG1*. Interestingly, the reduction of PolG1 level (but not the rest of mtDNA replication machinery) significantly enhances the purifying strength that restrict pathogenic mtDNA mutations in offspring without altering the total mtDNA copy number (Chiang et al, Curr Biol 2019).

To extend the scope of our study, we established cultured *Drosophila* cells heteroplasmic for the same mtDNA variants. We are performing a genome-wide RNAi screen, which revealed some exciting modifiers of the heteroplasmy ratio already. We will carry out functional studies to reveal how they regulate heteroplasmy dynamics during somatic divisions. Our studies, using both *Drosophila* and cultured cell models, will gain a better understanding of heteroplasmy transmission during development.

Keywords: mtDNA, Heteroplasmy

*Speaker

A functional role of POU/Oct transcription factor in mitosis

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POU/Oct proteins are conserved transcription factors that have been implicated in many biological processes, such as stem cell fate determination, cell differentiation, immunity, tissue growth and regeneration. Here, we augment functional roles of *Drosophila* POU/Oct factor Nubbin (Nub), a homolog of mammalian Oct1 and Oct2, in cell proliferation. The *Drosophila nub* gene encodes two independent proteins, Nub-PB and Nub-PD. Comparative analysis demonstrates that the Nub-PD isoform is crucial for timely mitotic progression in S2 cells. Nub-PD knockdown in S2 cells display intriguing mitotic defects characterized by defective spindle organization and a significant increase in multinucleated cells. Here we combine genetics, immunostaining and live imaging methods to analyze the function of Nub-PD during mitotic nuclear divisions of transcriptionally silent syncytial pre-blastoderm staged embryos. Several mitotic errors were detected in *nub* mutant embryos, such as asynchronous divisions, defective chromosome segregation, and abnormal spindles, consistent with our cell culture results. Furthermore, we demonstrated a dynamic localization of the Nub-PD during mitotic divisions in pre-blastoderm embryos and S2 cells. Nub is localized to chromatin in prophase, then moves to the mitotic spindles at the onset of metaphase and in the end of mitosis (telophase), it is again localized to midbody in S2 cells. Interestingly, this dynamic localization of Nub-PD protein is dependent on intact spindle microtubules. Together, our findings propose a novel and previously unappreciated role for POU/Oct factors during mitosis.

Keywords: Transcription factor, Mitosis, Syncytial blastoderm embryo

*Speaker

Actin binding proteins, in concert, regulate *Drosophila* follicle cell migration

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From gastrulation to organogenesis, as well as being linked to morphogenesis and wound healing, collective cell migration is essential for several developmental processes. On contrary, erroneous cell motility might accelerate the spread of metastatic cancer and inflammatory illnesses. The *Drosophila* egg chamber (EC) starts as a spherical tissue during early ovary development which with maturation elongates to create an elliptical egg shape. In each egg chamber, the outer follicular epithelial cells migrate in a sheet-like, direction-independent pattern. A phenomenon known as "Molecular corset" occurs during this migration, in which the circumferential arrangement of actin fibres in the follicular cells and the basement membrane together form a mechanical constriction to the egg chamber, resulting in the ellipsoidal shape of a mature egg. Actin polymerization, to form stable actin filaments are one of the primary requirements for a cell to start migration. The primary focus of our research is to unveil the function of different Actin Binding Proteins (ABPs) in this atypical follicle cell migration. We have screened many ABPs, which gave us few promising candidates that at low expression level affects the *Drosophila* follicular cell migration resulting in the formation of misshaped egg. In addition, a novel built-in redundancy was also observed between actin bundling protein Singed (sn) and actin binding protein Vinculin (Vinc). Individual knockdown of *singed* and *vinculin* did not substantially change the egg's aspect ratio. However, double knockdown of *singed* and *vinculin* significantly altered the aspect ratio of *Drosophila* EC. Further, the depletion in the Filamentous actin (F-actin) level of *singed* and *vinculin* double knockdown follicle cells hinted us that the altered aspect ratio of *singed* and *vinculin* double knockdown eggs were result of erroneous follicle cell migration. Our experimental outcomes revealed that *singed* and *vinculin* exhibit redundancy in modulating F- actin not only in *Drosophila* ovarian follicle cells but also in Border cells. Furthermore, the genetic screening also revealed that individual knockdown of Arp 2-3 complex genes and "capping protein β " (*cpb*) gene have severely changed the egg phenotype. Our findings indicated that the Arp 2-3 complex and capping protein β control the F actin concentration at the apical side of the migrating follicular cells.

Keywords: collective cell migration, molecular corset, actin binding proteins, singed, vinculin, Arp

*Speaker

2, 3 complex, cpb, F, actin

Alström syndrome proteins are novel regulators of centriolar cartwheel assembly and centrosome homeostasis in *Drosophila*

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Centrioles are highly conserved microtubule-based structures that play major roles in cell division and cell signalling by nucleating the cilium. Several pathologies are associated with alterations in their number or their ability to build cilia, including cancers and ciliopathies. Alström syndrome is a rare ciliopathy linked to mutations in one gene, *alms1*, encoding a centriolar protein whose function remains unclear. Our work shows that Alms1a and Alms1b, the two ALMS1 proteins found in *Drosophila*, are general regulators of centriole duplication and centrosome homeostasis.

Using the innovative expansion microscopy (U-ExM) technique, we characterised Alms1a and Alms1b localisation with unprecedented resolution. We showed that they both localise at the proximal end of centrioles with spatiotemporal differences. Alms1a is a pericentriolar material protein (PCM) loaded at the proximal end of centrioles at their onset of assembly in all tissues analysed, including male germline, neuroblasts and embryonic blastoderm, while Alms1b is only detected on mature centrioles in a subset of these tissues.

We showed that acute loss of Alms1a and b, using RNAi, is associated with complete centriole duplication failure in all three analysed tissues, whereas chronic loss of these proteins in a double KO mutant leads to centriole disengagement and reduced PCM recruitment, at least in the male germline. This shows that centriole duplication is a highly buffered process sustained by strong compensatory mechanisms in mutant situations.

In this regard, we placed Alms1 in the molecular hierarchy of centriole duplication. This highly controlled and conserved process involves the phosphorylation of Ana2 (STIL in mammals) by Sak/Plk4 leading to Sas-6 recruitment and cartwheel assembly which drives procentriole formation. We showed that acute loss of Alms1a and b does not alter Sak/Plk4 recruitment but impairs Ana2 amplification at the duplication site, resulting in the absence of Sas-6 recruitment.

Altogether our work demonstrates that Alms1a and b are novel players in the initiation of cartwheel formation and PCM assembly in *Drosophila*. It further suggests that Alms1a and b are involved in the fine-tuning of Ana2 activity at procentrioles.

^{*}Speaker

Keywords: centriole duplication, Alms1, expansion microscopy, Alström syndrom

Are different astrocyte morphologies dictated by the distinct neuron-types they associate with?

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Building a functional nervous system requires the coordinated development of the correct numbers and types of neurons and glia. Astrocytes are the most common glial cell-type and are essential for synapse development and function. Their complex arborised morphology allows for specific and close contact to different neurons and neuronal parts, including synapses. Indeed, astrocyte function is intimately linked to their morphology. While at least some aspects of astrocytic morphology are known to be regulated by neuronal signals across species, including worms, flies, fish and mice, how diverse astrocyte morphologies arise is not yet clear. Many in the field have speculated that morphologically distinct astrocytes may be functionally specialised to the specific circuits or neurons they support, but knowing which astrocytic features reflect cell intrinsic or extrinsic properties is not known. Astrocytes of the adult *Drosophila* optic lobe take on eight stereotypical morphologies (morphotypes) that populate distinct neuropils and neuropil layers, however, in contrast to neurons, seven of these morphotypes were transcriptionally indistinguishable within the limits of present sequencing depths (Lago-Baldaia *et al.*, revisions submitted to *eLife*). We hypothesise that the different astrocyte morphotypes arise in response to extrinsic signals through association with distinct neurons in space and time. In this project, we test whether altering the neuron-type composition has a corresponding effect on the morphological diversity of astrocytes. Taking advantage of the field's extensive knowledge of the neuron-types of the optic lobe, we use gene expression systems and whole-animal mutants to manipulate neuron-type composition during development and analyse astrocyte morphology in the adult. This work is the starting point for further research into the regulators of astrocyte morphology and morphological diversity.

Keywords: Astrocytes, Morphology, Development, Cell communication

*Speaker

Autophagy-mediated unconventional secretion of cholesterol

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Cholesterol contributes to the physical properties of cellular membranes (the plasma membrane as well as internal structures), and differences in cholesterol abundance from membrane to membrane affect their function. The maintenance of proper intracellular cholesterol balance is therefore critical to normal health and is tightly regulated. Cholesterol also acts as a precursor molecule for steroid-hormone synthesis. The prothoracic gland (PG) produces a large burst of ecdysone as the trigger for metamorphosis, which accordingly requires a large amount of sterol precursor molecules, which are stored until needed within intracellular lipid droplets. We previously found that autophagy, a pathway through which intracellular components are degraded for reuse, can mobilize sterols from these droplets into the steroid-biosynthetic pathway. A contemporaneous report showed that, before critical weight, autophagy induced by starvation instead leads to the ejection of cholesterol from the PG cells, as a way to prevent inappropriate steroidogenesis and maturation. The choice between these two routes, and the mechanism by which cholesterol is ejected, are an interesting topic for study. As our model system we use PG cells in which cholesterol buildup in endosomes, lysosomes, and autolysosomes is induced by loss of the vesicular sterol exporter Npc1a. This leads to overactivation of Tor and developmental arrest. We have found that ecdysone signaling, which marks the transition from pre-critical weight to post-critical weight, appears to regulate the fate of autophagosed lipids: expression of a dominant-negative form of the ecdysone receptor rescues the Npc1a-loss-induced cholesterol buildup, TOR activity, and arrest, and manipulating certain trafficking steps suppresses this rescue. Conversely, performing the opposite trafficking manipulations in the absence of dominant-negative EcR can also rescue these phenotypes. The ecdysone receptor is orthologous with mammalian LXRs, which regulate many aspects of cellular lipid metabolism; thus, our findings – that intracellular cholesterol can be mobilized and removed from the cell through an unconventional autophagy-mediated secretory route, regulated by EcR/LXR – findings may contribute to interventions against cholesterol-promoted disorders including atherosclerosis and cancer.

Keywords: cholesterol, sterols, ecdysone, prothoracic gland, autophagy

*Speaker

Blood-brain barrier integrity and sexual dimorphisms during macrophage invasion of the *Drosophila* nervous system

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The central nervous system (CNS) of *Drosophila* can be considered as an immune-privileged organ. It is separated from the remaining body by the blood-brain barrier (BBB), which is formed by glial cells and the occluding septate junctions established between surface glial cells. The BBB allows ion and metabolite homeostasis and prevents the invasion of pathogens. Using a novel infection model we could recently demonstrate the infiltration of macrophages into the CNS across an intact BBB during early metamorphosis. Nevertheless, dye uptake experiments revealed a slight but significant increase in BBB permeability during immunity induction. This might hint to the possibility that macrophage transmigration across the BBB leads to transient opening of the otherwise closed septate junctions, and in addition suggests a paracellular route of invading macrophages. To further study how macrophages enter the brain, we initiated an electron microscopic analysis. First results support the notion of a paracellular invasion route. Macrophages come in close contact with occluding junctions of surface glial cells. We will discuss further approaches to dissect how macrophages can regulate opening of occluding septate junctions during their transmigration across the glial barrier. Interestingly, we found sex specific differences in migration across the BBB, with males being more affected than females. First experiments hint to sex specific factors within the glial cells of the BBB as a reason for different migration rates. The group of B. Dauwalder performed single cell sequencing of those cells, revealing sexual dimorphisms in transcription levels of various proteins. To decipher the underlying molecular mechanisms, we are utilizing sequencing data from male and female BBB forming glial cells. Taken together, we show that our model can be used to study especially sex dependent differences of glial cells in vivo.

Keywords: BBB, Blood, brain barrier, sex dimorphism, hemocytes, macrophages

*Speaker

Cells talking to each other: Interplay of EGFR and JNK signalling in the *Drosophila* testis.

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Tissue homeostasis and repair relies on the balance between stem cell self-renewal and differentiation, guided by short-range communication between cells and their microenvironment. In our lab, we use the *Drosophila* testis to understand how squamous somatic cyst cells (CCs) support the differentiation of the germ cells they encapsulate to eventually give rise to mature sperm. Previously, we have shown that loss of the cortical polarity proteins Dlg, Lgl and Scribble or clathrin-mediated endocytic (CME) components in CCs, results in EGFR upregulation, leading to cell non-autonomous death in the neighbouring germ cells.

The Jun N-terminal Kinase (JNK) signalling pathway has been implicated in regulating apoptosis and in mediating cell competition in the tumorous context. On the other hand, reactive oxygen species (ROS) have been linked to JNK signalling in response to cellular stress, while high levels of ROS can disrupt germline differentiation via EGFR signalling. Here we show that EGFR overactivation in CCs, also upon loss of *dlg*, *scribble*, *lgl* or CME components, leads to upregulation of JNK signalling and the MAP kinase p38, while ROS is activated in the neighbouring germ cells that are destined to die. Knocking down the JUN kinase *basket* (*bsk*) in CCs with increased EGFR signalling levels, can partially rescue the phenotype of germ cell loss, suggesting that germ cell death is mediated by the coordinated action of JNK pathway and ROS. Using scanning electron microscopy (SEM) we also looked at the ultrastructure of the squamous CCs to elucidate how these differentiated, non-dividing, flat epithelial cells build an internal structure and compartmentalization that facilitates close-range communication with the germline and coordinated tissue responses during spermatogenesis.

Our work aims to shed light on how squamous CC-germline communication promotes spermatogenesis and fertility, while it activates alternative pathways that protect the germline when intrinsic cellular events and homeostasis are disturbed. Understanding the complexity and makeup of squamous testis CCs, will uncover conserved regulatory strategies and entry points to eventually model squamous cell function across diverse organisms, and human diseases like squamous cell carcinomas.

Keywords: squamous epithelia, germline, signaling, cell communication, testis, EGFR, JNK, ROS, dlg

*Speaker

Cellular mechanisms and genetic regulation of neuronal migration in the *Drosophila* optic lobe

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How different cell types arise from the same stem cell and how they implement different terminal features such as morphology, behaviour and function is a longstanding question in developmental biology. Asymmetric cell division plays an important role during this process, as it allows sister cells to acquire different fates. Here we present the case of a *Drosophila* optic lobe lineage that produces Lamina wide field (*Lawf*) neurons and two closely related types of glia, epithelial and marginal (*eg/mg*) glia from a common progenitor. Neuronal versus glial fate choice is implemented by Notch signalling, with NotchON cells becoming glia and NotchOFF cells acquiring neuronal identity. Both *Lawf* and *eg/mg* glia undergo cell migration after specification, following different trajectories and using different modes of locomotion. Here, we aim to identify the cellular mechanisms that regulate *Lawf* and *eg/mg* glia migration, as well as how they orient and direct their movement in the complex three-dimensional environment of the developing visual system of *Drosophila*. Finally, we use single cell transcriptomics to search for candidates that link the acquisition of *Lawf* and *eg/mg* glia cell identity with their specific migratory behaviour. Interestingly, *Lawf* is the only neuronal type described to migrate actively as a postmitotic neuron in the optic lobe of *Drosophila*. This makes *Lawf* neurons an ideal model to understand the genetic basis of neuronal migration, which would likely be generalizable to other organisms.

Keywords: Neuronal migration, optic lobe, cell fate, glia, neurodevelopment

*Speaker

Characterization of a new ciliary-like structure in *Drosophila* neurons

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In Mammals, each neuron exhibits a primary cilium involved in environmental sensing and signal transduction. Neuronal primary cilia are crucial for sensory functions, homeostasis and development. Their alteration is associated with a broad spectrum of developmental pathologies termed ciliopathies as well as with adult-specific neurodegenerative diseases such as Alzheimer and amyloid lateral sclerosis. Surprisingly, primary cilia have not been identified in *Drosophila* CNS neurons so far.

Our lab recently discovered never-described ciliary-like structures rich in F-actin, but devoid of microtubules, in adult *Drosophila* CNS neurons. As indicated by 3D reconstructions from high-resolution confocal imaging, these structures (1-2 per cell) are nucleated hundreds of nm below the plasma membrane, a result we are currently confirming through correlative light-electron microscopy (CLEM) and ultrastructural analyses. Furthermore, they are enriched in very specific non-canonical myosins.

Through a time course analysis, we found that actin-rich cilia-like structures are already formed during late developmental stage but undergo dramatic elongation upon aging, reaching up to 2 μ m in length in 35 day-old flies. To identify regulators involved in their formation and/or elongation, we performed a selective candidate RNAi screen, thus uncovering 11 regulatory genes, 6 of them known for their function in F-actin regulation. These results highlight the role of F-actin in ciliary structure morphogenesis. Moreover, the different mutant phenotypes that we have obtained will allow us to both address the role of cilia-like formation and to investigate the impact of their elongation upon aging.

Overall, this work has uncovered new actin-rich membrane protrusions that elongate upon aging in the adult *Drosophila* brain and may share analogy with mammalian primary cilia. Ongoing work will help better understand their function.

Keywords: Actin, Neurons, Aging

*Speaker

Cic non-autonomously promotes neural stem cell differentiation as a transcriptional repressor and activator in the cortex glial niche

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Gliomas comprise 80% of primary brain tumours, yet new therapies to improve patient outcomes haven't emerged in over 30 years. Although we have an extensive catalogue of brain cancer mutations (e.g. The Cancer Genome Atlas), we lack functional data for mutations predicted by GWAS to drive glioma. Further to this, despite brain cancer likely arising from glioma stem cells, inter-tumour interactions of significance to renewal and differentiation are unknown. For example, the HMG-box transcription factor CIC, first identified in *Drosophila*, is frequently mutated in low grade oligodendroglioma. Previous studies in flies and humans suggest Cic/CIC functions as a transcriptional repressor downstream of receptor tyrosine kinase signalling and that this might provide a mechanism for tumour initiation and progression driven by CIC loss-of-function. Here, we demonstrate *Cic* knockdown (KD) specifically in the cortex glial niche of the larval brain drives overproliferation of the neighbouring neural stem cells. To determine how Cic functions cell non-autonomously to drive neural stem cell expansion, we determined genome-wide binding (Targeted DamID) and intersected with differentially expressed genes for Cic KD vs control (RNA-seq) specifically in cortex glia. Despite previous reports classing Cic as a transcriptional repressor, we demonstrate Cic functions equally to control glial niche-neural stem cell signalling as a transcriptional activator. In addition, analysis of the transcriptome of the neural stem cell lineage associated with Cic-depleted glia, revealed upregulation of proliferation genes and downregulation of neuronal differentiation factors, consistent with the observed neural stem cell overproliferation. Thus, our data demonstrate Cic functions non-autonomously in the glial niche to prevent excessive renewal of neural stem cells. Given the conservation of Cic function, these observations may inform inter-tumour signalling defects between glioma stem cells and neighbouring glia of significance to CIC-driven oligodendroglioma.

Keywords: Cancer models, stem cells, microenvironment, stem cell niche

*Speaker

Cohesin gene depletion cause tumor-like formations in *Drosophila melanogaster* type II neuroblasts during development

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Pathogenic mutations in cohesin genes are causative of a number of cancers including Medulloblastoma, a congenital brain tumor with a poor survival rate. Our preliminary bioinformatic analysis showed that 5% of Medulloblastoma patients have loss of function mutations in *Stag2*, a core component of the cohesin complex, possibly leading to haploinsufficiency. As there are currently no *in vivo* models of Medulloblastoma considering cohesin activity, we aim to use *Drosophila melanogaster* to study the role of *Stromalin* (*SA*), the *Stag2* ortholog in *Drosophila*, during brain development. To this end, we used RNAi-mediated knockdown of *SA*, specifically in clusters of type II neuroblast (NB-II) in the central nervous system. Using different antibodies to mark the maturity of cells in NB-II clusters, we found that *SA* downregulation prevents cluster maturation in larval brain. Surprisingly, adult brains of *SA* KD animals revealed masses of undifferentiated cells, in contrast to control animals in which larval NB-II mature before adulthood. We also followed the NB-II lineage depleted of *SA* across development, and found that it expands with aging, suggesting that depleted NB-II might undergo tumorigenesis. These observations suggest that *SA* has a tumor-suppressive role, and its mutations may predispose for neoplastic transformation. This preliminary model of cohesin-dependent brain tumor development could represent an innovative model to discover and test new drugs. We will further study it in combination with other genetic lesions typical of Medulloblastoma patients.

Keywords: Cohesin, Medulloblastoma, Brain Tumor, Neuroblast, Development

*Speaker

Die or eat your neighbors: Induction of apoptosis or phagocytic activity in epithelial cells is a consequence of who dies first

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Apoptosis is executed by the action of caspase proteases, which when activated above a lethal threshold, lead to a cell death and rapid clearance by professional (macrophages) or non-professional (neighboring cells) phagocytes. Phagocytosis of apoptotic cells involves recognition of the dying cell, internalization into phagosomes, and final degradation following fusion of the phagosomes with lysosomes. While macrophages are cells specialized in clearing apoptotic cells and other large particles, some nurse cells are also capable of acquiring a phagocytic fate, such as glia and Sertoli cells that clear dying neurons and germ cells, respectively. However, much less is known about how some cells within a tissue composed of similar cells are selected to acquire a phagocytic activity. Here, I present our unpublished data about the mechanisms involved in the acquisition of phagocytic activity in a simple tissue of largely homogenous epithelial cells experiencing an identical stress. We show that following ionizing irradiation, some epithelial cells within the *Drosophila* wing imaginal discs do not die, but become positive to Lysotracker, a processes that requires the main phagocytic receptor Draper (CED-1 homolog). Indeed, while the cells still die in the absence of Draper, apoptotic clearance is severely impaired. Significantly, we also show that acquiring phagocytic activity requires a non-autonomous signal emanating from the dying adjacent cells. Overall, our data supports a model in which cells that stochastically first reached the lethal threshold of caspase activity, dictate survival and a phagocytic fate in neighboring cells that lag behind in reaching that threshold.

Keywords: Cell death, Phagocytosis

*Speaker

Discovery of regenerative clustering of Enteroblasts and Discs Large as a novel regulator of the adult *Drosophila* midgut homeostasis using a morphometric analysis

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Maintenance of epithelial barrier function is essential for animal health. The adult *Drosophila* midgut provides a powerful model system for studying epithelial homeostasis. It consists of a monolayered epithelium of absorptive enterocytes (ECs) and secretory enteroendocrine cells, underpinned by stem cells and intermediate progenitor enteroblasts (EBs). With the "made-to-stock" model proposed by Antonello et al., 2015, we now know that EBs are the functional reservoir of regeneration in the midgut. Following damage, EBs differentiate into polarised ECs through a Mesenchymal-Epithelial Transition (MET). While much is known about the transcription factors and signalling pathways that drive EB MET, the mechanical aspects involved have received less attention. To tackle the relationship between gene function and the underlying cell mechanics, we established an analysis pipeline that quantifies morphometric and spatial distribution metrics. Using this system, we first revealed an unexpected clustering of EBs at various stages of their MET process in 60% of the wildtype midguts assayed. Since the presence of clusters correlated with the formation of new ECs we termed these "MET regenerative clusters". Based on their presence we classify midguts into two functional categories: "quiescent" or "regenerative". To identify genes that potentially mediate cluster formation, we performed a small candidate screen with RNAi in a paraquat-induced stress background, which implicated several members involved in mechanosensing. In addition, knockdown of SJ components produced significant deviations in EB morphology and distribution patterns when compared to wildtype. Discs Large (*dlg*) was selected for further study. Dlg belongs to the MAGUK family of scaffolding proteins and is known to regulate Septate Junction (SJ) formation, cell polarity and proliferation. *dlg* knockdown in EBs produced cells that were rounder, larger, and more aggregated. Subsequent follow-up experiments revealed that loss of *dlg* resulted in a multilayered epithelium, and that deficient cells often had junctional defects. Normal Dlg function was also required for EB quiescence and MET, as *dlg* knockdown produced EBs with precocious expression of the EC-marker Pdm1. Finally, the presence of *dlg* mutant clones disrupted normal tissue architecture and was associated with stress responses, such as JAK/STAT and JNK activation. Together, our results have demonstrated the ability to extract meaningful biological information out of currently underutilised image data and uncovered a novel role of Dlg in EB MET. These results demonstrate the utility of the adult midgut as a model to expand our understanding of homeostatic MET.

*Speaker

Keywords: midgut, enteroblast, MET, mesenchymal, epithelial transition, discs large, dlg

Dissecting the mechanism driving the swimming cell migration of *Drosophila* pupal fat body cells.

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During many years, pupal fat body cells (FBCs) were considered to float passively across the pupal hemolymph. However, we found that these giant cells are motile and actively migrate under physiological and pathological conditions during pupal development. FBCs appear to use an adhesion-independent swimming mode of cell migration, which allows cells to move through liquids without close contact to any substrate. Here we explore the mechanism underlying the migration of pupal FBCs and use them as an *in vivo* model to study the still ill-defined swimming cell migration mode. Like other migrating cell types, FBCs need to generate internal forces and transmit them to their environment to power their migration. To generate internal forces, migratory FBCs produce cortical actin waves which propagate towards the cell rear. We found that these actin waves are coupled with actomyosin contraction in the cell rear. By using a newly developed high throughput screening method, we discovered that the small Rho GTPases and several of their effectors regulate FBC migration by controlling actin wave dynamics. To further understand, how the FBCs transmit their forces, we are also investigating the effect of the local environment on FBC migration and study if their migration could be coupled with a flow of transmembrane proteins at their cell surface. Altogether our data reveal the novel mechanism underlying the FBC swimming migration. It also establishes the pupal FBC as an *in vivo* model that can provide valuable insights into *in vivo* swimming cell migration.

Keywords: Swimming Migration, Small Rho GTPases, Actin, Fat Body Cells

^{*}Speaker

Dissecting the molecular mechanism that mediates the process of Fat Body Remodeling

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Dissecting the molecular mechanism that mediates the process of Fat body Remodeling
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Epithelial-to-mesenchymal transition (EMT) is a process where epithelial cells lose polarity, adhesion, and gain invasive and migratory properties of mesenchymal cells. This process is crucial for embryogenic development and tissue regeneration but is also involved in diseases (e.g. cancer metastasis). Fat body cells (FBCs) in *Drosophila melanogaster* undergo a process similar to EMT, called fat body remodeling (FBR), during metamorphosis. However, little is known about the molecular mechanism driving FBR. Moreover, although FBR is in many ways similar to EMT, (e.g. cell-cell and cell-basement dissociation followed by gain of cell motility), unlike an epithelial tissue which exhibits apicobasal polarization with a basement membrane only on the basal side, the fat body faces a basement membrane on both sides. Additionally, it is still unknown whether the fat body tissue is polarized. Thus, it remains to be tested whether FBCs undergo an EMT-like process during FBR. In this study I have first tried several different approaches to live image the wildtype process of FBR both *in vivo* and *ex vivo* to study the morphological changes occurring during FBR in more detail. Additionally, my experiments testing for a potential cell polarity show that polarity proteins are present in the fat body tissue of a 3rd instar larvae and are asymmetrically localized, suggesting that the fat body tissue is in fact polarized. Moreover, the asymmetry of several of the polarity proteins gets lost at 3hr APF during the process of FBR, suggesting that the fat body is polarized when cells are still in a sheet and this cell polarity is lost during FBR. These results may potentially suggest that FBR can be classified as an EMT-like model. In addition, my project provides a novel model to easily screen for genes regulating this potential EMT-like process which might also be involved in cancer, arthritis, and cardiovascular diseases, etc., thereby enhancing our understanding on EMT-related disease mechanisms and therapeutics.

Keywords: Fat body remodeling, EMT, Cancer

*Speaker

Dynamics and contribution of Actin cytoskeleton in 3D cell intercalation independent on Myosin II

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During organogenesis, cells change their shape and size in response to mechanical signals and forces. Cell intercalation is one of the different mechanisms driving cell rearrangement. During this process, distant cells in a tissue become neighbours. Thus, forces at play must be generated and controlled in time and space and they are controlled in part by the dynamics of the acto-myosin network cytoskeleton associated with the adherent junction (AJs).

The 2D cell intercalation has been extensively analysed and largely rely on forces generated by the actomyosin network. However, the 3D cell intercalation allowing most of the organs structure is less investigated. To explore in vivo 3D cell intercalation, we study the respiratory organ, so called tracheal system, of drosophila embryo as a model system. Interestingly in this model, cell intercalation does not require Myosin II.

Using genetic experiments, cell imaging and quantitative image analysis, we are investigating the contribution of the actin network to forces required for 3D intercalation. We have highlighted a specific actin network dynamic during tracheal morphogenesis, which is gradually enriched and stabilized at the AJs. We have revealed a pulsatile behavior of actin at tricellular AJs (tAJs), place of force transmission in response to mechanical stress. We have identified the regulators involved in this actin pulsatile dynamic. We have also characterized actin subnetworks during this process and determined their contribution on tension exerted on AJs during cell intercalation. Using a new genetic tool to depolymerize actin in vivo only in tracheal cells, we have also revealed the contribution of actin at each step of 3D cell intercalation and at each position within the group of migrating cells. We intend to propose a new model of 3D cell intercalation independent on Myosin II.

Keywords: Cell intercalation, Actin dynamics, Tricellular junction, in vivo 3D model, tracheal system

*Speaker

Experience-Dependent Glial Pruning of Synaptic Glomeruli During an Early-Life Critical Period

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Critical periods are important early-life time intervals when initial sensory experience remodels brain circuit synaptic connectivity to optimize environmental input. In the *Drosophila* juvenile brain, we focus on the precisely-mapped olfactory brain circuit, which has an extensively characterized, manageably short (<1 week) critical period. In this circuit, single receptor olfactory sensory neuron (OSN) classes synapse onto single projection neurons extending to the brain learning/memory mushroom body. Critical period odorant experience drives synapse pruning in this circuit, which is reversible only during this brief window. Glial phagocytosis is well characterized in *Drosophila* injury models and early developmental remodeling, suggesting one possible pruning mechanism. In these other contexts, glial phagocytosis occurs via Draper (Megf10) engulfment receptors that signal via Basket (JNK) and AP1 (Jun/Fos) to induce transcription of Cheerio (FlnA, F-actin linking signaling scaffold) and matrix metalloproteinases (extracellular matrix (ECM) remodeler). Here, we find glia mediate experience-dependent synaptic pruning of Or42a OSN glomeruli following odorant exposure only during the early-life critical period. Glia infiltrate synaptic glomeruli neuropil in response to early odor experience, and utilize Draper-mediated Basket signaling to prune OSN synapses. Downstream of Draper and Basket signaling, we find that glial Cheerio expression is induced by critical period odor experience, and that glial Cheerio is required for synaptic pruning. We find this F-actin crosslinking protein allows glia to regulate their actin cytoskeleton to enable experience-dependent synaptic pruning only during the early-life critical period. This work has been supported by National Institutes of Mental Health R01 MH084989 to K.B. with presentation supported by Gisela Mosig Fund and Vanderbilt Graduate School travel grants to N.N.

Keywords: Glia, Synaptic Pruning, Critical Period

*Speaker

Exploring actomyosin regulation in epithelia by systematic characterization of RhoGEF/GAP dynamics

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Epithelial cells are characterized by a stereotypical polarity and junctional organization, which maintenance is key to ensure epithelial function. During development, epithelial tissues undergo several cell and tissue remodelling processes including cell division, cell rearrangements and apoptosis. It is now well established that the actomyosin cytoskeleton is at the core of the interplay between epithelial organization and cell dynamics. However, how the actomyosin cytoskeleton is spatiotemporally regulated in developing epithelia remains poorly understood. Here, to investigate actomyosin regulation *in vivo*, we focused on the regulation of RhoGTPases by RhoGEF and RhoGAPs. Therefore, we generated a transgenic library of the 48 *Drosophila* RhoGEF/GAP tagged with GFP, and used it to perform a localization screen of all RhoGEF/GAP during epithelial morphogenesis. Thereby, we built an exhaustive map of RhoGEF/GAP localizations, revealing several new candidate regulators of RhoGTPases from interphase to cytokinesis in developing epithelia.

Furthermore, we characterized the function of two RhoGEFs, Cysts and RhoGEF4, which by regulating different RhoGTPases play distinct roles in mechanosensing and junction formation during the multicellular process of epithelial cytokinesis.

Together, we expect that the RhoGEF/GAP library and localization map we provide will open the way to study actomyosin regulation during cell and tissue dynamics in different developing and homeostatic contexts.

Keywords: epithelia, actomyosin, RhoGEF/GAPs, localization screen, junction formation

*Speaker

Functional characterization of Immune induced DNases in *Drosophila melanogaster* host defense

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Deoxyribonucleases are enzymes which catalyze the cleavage of phosphodiester linkages in the DNA backbone, thus degrading DNA. The genome of *Drosophila melanogaster* encodes three Dnases: (1) Caspase-Activated Dnase (CAD), (2) Dnase II and (3) Stress-Induced Dnase (SID). Both Dnase II and SID are strongly expressed in the larval fat body and are transcriptionally induced after microbial infection, suggesting a role in *Drosophila* innate immunity. To characterize the role of Dnases in *Drosophila* innate immunity, we generated null mutants for *Dnase II* and *SID* genes. *Dnase II* mutants show a delay in development, reduced lifespan, strong locomotor defect and an overloaded microbiota at early adult stage. The double mutant *DnaseII,SID* is homozygous lethal. Curiously, *Dnase II* mutant flies are not susceptible to microbial or *Drosophila* DNA injection. However, *Dnase II* mutant flies are susceptible to Gram-negative and Gram-positive systemic bacterial infection, suggesting a role for *Dnase II* in disease tolerance. *Dnase II* and *SID* mutant larvae show a strong JNK pathway activation. *Dnase II* mutant larvae also show activated IMD signalling after injury, which can be rescued by *Relish* or *STING* knock-out. After injury, we observe the accumulation of apoptotic bodies in hemocytes of *Dnase II* deficient larvae. This suggests an incapacity to digest phagocytosed apoptotic bodies, leading to a subsequent activation of immunity. Collectively, our work provides the first functional characterization of *Dnase II* and SID enzymes in *Drosophila melanogaster* immunity using a full knock-out approach.

Keywords: Immunity, Dnase, apoptosis, disease tolerance, infection, efferocytosis

*Speaker

Glial gap junction protein is affected in a Progressive Myoclonus Epilepsy *Drosophila melanogaster* model

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North-Sea Progressive Myoclonus Epilepsy (NS-PME) is a rare genetic disease that presents at an early age with progressive movement disorder, scoliosis and epilepsy. NS-PME is caused by a single recessive mutation in the Golgi SNAP receptor 2 gene (GOSR2). The protein encoded by GOSR2 is involved in vesicle fusion at the Golgi. However, what the consequences are at the cellular level is still largely unknown. In our lab, we have created a NS-PME *Drosophila melanogaster* model by RNAi-mediated knockdown of *membrin*, the GOSR2 ortholog in fruit-flies, and we found that ubiquitous knockdown leads to progressive heat-induced seizure-like behaviour in adult flies, which got progressively worse as the flies aged. Interestingly, specific knockdown of *membrin* in glia, but not in neuronal cells, resulted in a similar phenotype. This led us to the questions how do glia affect seizures in NS-PME. Previously, it was shown that loss of gap junction proteins in specific glia can cause a heat-induced seizure phenotype similar to what we found in NS-PME. Therefore, we hypothesized that the glial function in NS-PME is related to glial expression of gap junction proteins. We tested this hypothesis by immunostaining and Western blot analysis of a gap junction protein in *Drosophila melanogaster* brains and heads with and without glial knockdown of *membrin*. We found that the gap junction protein is both changed in expression pattern and reduced in level in NS-PME flies compared to control. This shows that gap junction protein expression is indeed affected in our *Drosophila melanogaster* model for NS-PME. To corroborate the importance of gap junction protein in preventing seizures in NS-PME, we tested glial overexpression of gap junction proteins in the background of glial *membrin* knockdown and we found that this overexpression almost completely rescues the heat-induced seizures phenotype. With these findings we show, in a *Drosophila melanogaster* model of human disease, that the expression of gap junction proteins is affected by glial *membrin* knockdown and that glial expression of these proteins plays an important role in preventing seizures.

Keywords: Gap junction protein, Progressive Myoclonus Epilepsy, Epilepsy, Glia

*Speaker

How cellular metabolic reprogramming shapes nutrient utilization by the germline and impacts female fertility

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Cells process nutrients via metabolic pathways to maintain tissue and organismal homeostasis. In multicellular organisms, cell differentiation leads to specialization in metabolic programs. In the context of development, metabolic reprogramming is thought to be prewired to guide specific developmental outcomes. Likewise, metabolic reprogramming has been shown to be a hallmark of aging and the alteration of metabolic pathways may accelerate the process and the onset of age-related diseases. The mechanisms regulating the implementation of specific metabolic programs, how these programs relate to cell and organ function, how they impact whole-animal physiology, and how factors such as dietary nutrient availability, the microbiome or aging impacts these at a molecular and functional level remains an open question in biology. We have recently shown that in the *Drosophila* female germline, cells undergo a process of metabolic reprogramming which regulates both ovary function and animal's nutrient preferences. Egg production depends on the upregulation of a particular branch of carbohydrate metabolism, the pentose phosphate pathway, in the germline. This process also leads to a specific increase the animal's appetite for sugar, the key nutrient fueling this metabolic pathway and ensuring female fertility. Our findings show that cellular metabolic programs of a small set of cells are not only critical for organ function but also have a global impact on animal physiology. These findings have generated a novel paradigm to understand how specific metabolic programs are implemented and modulated to regulate cellular, tissue and whole-animal functions.

Keywords: metabolic programs, germline, fertility, inter, organ communication, diet, oogenesis, nutrient appetite, pentose phosphate pathway

*Speaker

Hox/Slbo, a transcriptional repressor/activator tandem controlling developmental autophagy in *Drosophila* fat body

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Autophagy is an evolutionary conserved cellular process that takes place in most cell types in metazoans, facilitating cell homeostasis maintenance in normal and stress conditions. Autophagy is also part of the animal's developmental program, nevertheless the regulatory mechanisms underlying the control of developmental autophagy are poorly characterized. Our team has established that the *Drosophila* Hox transcription factors, well known master regulatory proteins controlling animal body plan diversity, also control autophagy. To further delineate the transcriptional regulatory control of autophagy, we took a transcriptomic approach, and identified the Slbo transcription factor. Slbo displays a mirror temporal expression dynamic to that of Hox proteins, which results from mutually suppressive Hox/Slbo cross regulatory interactions. Slbo knock down and forced expression established Slbo as a potent autophagy inducer. Further functional characterization indicates that Slbo modulates cell adhesiveness in preparation for fat body remodeling. Our results indicates that Hox/Slbo transcription factors constitute a transcriptional repressor/activator tandem required to time autophagy.

Keywords: Autophagy, Development, Transcription Factor, Fat body

^{*}Speaker

Identification of Sun as a novel regulator of autophagy termination in *Drosophila*

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Autophagy is a degradative process that plays a crucial role in maintaining cellular homeostasis in eukaryotic cells. Dysregulation of autophagy has been associated with diseases, including cancer and neurodegeneration. Despite the extensive knowledge about the autophagy machinery as well as autophagy induction and execution, the molecular mechanisms underlying the termination phase remain poorly understood. To fill this gap, we used *Drosophila* as a model system and employed GFP/mCherry-Atg8a to elucidate the *in vivo* dynamics of autophagy termination. We performed an *in vivo* genetic screen and identified Stunted (Sun) as a novel regulator of autophagy termination. Sun has a role in mitochondria metabolism, inter-organ communication, and regulation of insulin signaling. However, the link between Sun and the autophagy pathway is missing and is the focus of our investigation. In the control fat body, the number of autophagosomes increases during nutrient starvation and subsequently decreases when nutrients are replenished. When *sun* is downregulated, we observe a similar increase in the number of autophagosomes during nutrient starvation. However, even after nutrients restore, the process of clearing the autophagosomes is hampered in the larval fat body, gut, and S2 cells. These findings are consistent with elevated levels of the autophagy receptor Ref(2)P. Interestingly, the nutrient-dependent reactivation of mTOR is also impaired in the fat body carrying decreased levels of *sun*, hinting at a critical role of Sun in autophagy termination. Altogether, these findings offer new insights into the molecular regulation of autophagy termination, potentially providing mechanisms that could be targeted in the treatment of cancer and neurodegeneration.

Keywords: autophagy, autophagy termination, Atg8a, Stunted, fat body

*Speaker

Identification of force generators regulating cell shape and cell identity through a genetic screen of GAPs and GEFs in the *Drosophila notum*

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While the expression of the genetic program is instructing a cell its identity, biomechanical cues such as mechanical cues including matrix elasticity for example, could affect the cell identity. This questions the impact of cellular forces regulating cell shape on cell identity. The actomyosin cytoskeleton is a well-established force generator in the plane of adherens junctions (AJs). Indeed, actin and the non-muscle type II myosin (Myo II) have the property to organize into contractile minifilaments able to impose forces on cell cortex, hence membrane deformations. Within a cell, two pools of Myo II can be distinguished: the junctional myosin at the cortex and the medial myosin in the center of the cell at the AJs level. To perform its functions, actomyosin activity is tightly controlled by Rho. Rho exists in two states, the activated state, bound to GTP and the inactivated state, bound to GDP. This molecular switch is activated by GEFs (Guanine nucleotide exchange factor) and inactivated by GAPs (GTPase activating proteins).

The objective of this work is to identify GEFs and GAPs that regulate Rho activity and consequently actomyosin network contractility in *Drosophila notum* epithelial cells that may impact their shape in the plane of the AJs and their identity.

The notum of the *Drosophila* is composed of two different cell populations: the epidermal cells (EPI) and the sensory organ precursor cells (SOP) that exhibit a different cell shape in the AJs plane. A screening of GAPs and GEFs was performed using non-invasive confocal microscopy live imaging on *Drosophila* by expressing RNAi in fly lines expressing sqh::GFP and Baz::mscarlet. Images were analysed in an automatic and unbiased manner using a python written program. This program is automatically segmenting the cells using CellPose pretrained cytoplasmic model thanks to the Baz::mscarlet signal to create masks allowing the quantification of Myo II within the cells as well as the perimeter, the area, the number of neighbors, the shape of the cells and the myosin ratios. The results of the screening that led to the identification of candidates regulating Myo II ratios, cell shape, and cytokinesis will be discussed.

*Speaker

Keywords: Myosin, mechanical forces, screening, automatic quantification, shape of the cells, adherens junctions

Identification of the mechanisms underlying microtubule cytoskeleton reorganization and centrosome elimination in programmed polyploid cells

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Most animal cells are diploid, containing all chromosomes in two copies. In contrast to diploidy, polyploidy is the presence of more than two copies of all chromosomes. While polyploidy is a strategy used by animals and plants to increase metabolism, barrier function and regenerative capacity, it has been shown in recent years that many tumors are tetraploid. Importantly, tetraploidy in human tumors is thought to lead to genetic instability but the mechanisms driving this relationship are only now starting to be elucidated.

Most physiological polyploid cells reorganize their cytoskeleton leading to drastic changes, the most important being the elimination of centrosomes and the reorganization of the microtubule (MT) cytoskeleton. In physiological polyploid cells, MTs are nucleated from the nuclear envelope. However, the requirement of MT cytoskeleton remodeling and need for centrosome elimination remains to be understood.

To address these questions, The Basto lab has screen for mutations that affect cytoskeleton remodeling in a physiological model of polyploid cells- the salivary glands of *Drosophila melanogaster*. We have identified a mutation in the non-muscle myosin II regulatory light chain (NMMMyo2), - spaghetti squash (sqh)-, which affects both processes: centrosome elimination and remodeling of the MT cytoskeleton. Using genetic tools, live imaging approaches, super-resolution microscopy combined with tissue clearing and electron microscopy techniques, our work is revealing the steps involved in NMMMyo2 function related to centrosome elimination. A detailed temporal and dynamic review along salivary gland development from embryogenesis till late larval stages is revealing the morphogenetic events that lead to centrosome elimination. Further, using different mutations, we have uncoupled the different roles of NMMMyo2 in centrosome elimination and MT cytoskeleton remodeling. Our work provides for the first-time insights into the mechanisms responsible for centrosome elimination in physiological polyploid cells.

Keywords: Polyploidy, Centrosome elimination, Microtubule cytoskeleton, Non muscle myosin II

*Speaker

Impact of Diet on Fwe-dependent cell competition in Disease and Aging

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Cell competition is a surveillance mechanism that can be mediated by Fitness-fingerprints, the so-called Flower. Flower isoforms are detected, and translate the relative fitness of cells into distinct fates through the Flower code – where winner cells express FlowerUbi and loser cells express FlowerLose isoforms, tagging them for elimination upon azot activation. Impairments in the mechanism potentiate the appearance of diseases, like cancer or aging-related pathologies. The same mechanism was described in humans, in a cancer context where four human flower isoforms (hFwe) were identified, and more recently in a *Drosophila* Alzheimer's Disease (AD) model. My goal is to evaluate the involvement of cell competition in AD and aging but also how this mechanism can be modulated by external factors, such as diet.

The results show that there is a functional conservation of hFwe, with hFwe1 and 3 behaving as loser isoforms in a cell-type specific manner, in which hFwe1 behaves as the only lose isoform in neurons, in a *Drosophila* model. The same conservation pattern is detected in the Alzheimer's Disease model, where expression of hFwe1 in optic lobes leads to the impairment of cell competition and a decrease in cell death, with effects in locomotion being expected. Considering the previous results, I am using *Drosophila* as a genetic model to evaluate if diets with different nutritional patterns are able to abolish or potentiate cell competition mediated by hFwe isoforms, in disease and aging contexts. After manipulating Sucrose and Protein content, the results show modulation in azot expression and cell death, in aging and disease scenarios.

Together these results will allow us to identify key players in the progression of aging and AD, advocating for a potential role of diet to prevent AD progression by tackling fitness-fingerprint mediated cell competition.

Keywords: Cell competition, flower, azot, Alzheimer's disease, nutrition

*Speaker

Insights from Secretory Cells Dynamics: Regulation of Female Reproductive Tract Secretions in *Drosophila*

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In many species, seminal fluid conveys crucial signals to the female reproductive tract (female-RT) upon copulation, shaping an environment that supports successful gamete interaction and offspring health. This paradigm holds true in *Drosophila melanogaster*, where the orchestration of the female-RT environment integrates male-derived factors and maternal contributions for efficient gamete handling. This complex interplay is directed by two pivotal players: the spermathecal secretory cells (SSC) which encase the spermatheca and provide support for long-term sperm storage, and the female accessory glands (femAG). Notably, disruption of both SSC and femAG development results in female sterility. Very little is known about the composition and functions of SSC and femAG secretions, and it is unclear how they regulate female-RT activities.

To determine whether SSC- and femAG-derived secretions are involved in modulating female-RT activities, we first performed GO term enrichment analysis of genes expressed in the spermatheca. We found significant post-mating changes in some members of the Ras-related in brain (Rab) family of GTPases, regulators of core secretory and endocytic machinery. Given the primary role of Rab GTPases as regulators of membrane trafficking, we next examined the expression and subcellular localization of different Rabs in SSC and femAG of virgin and mated females. Our findings suggest that mating induces changes in the SSC and femAG secretory/endocytic activity at the apical region, potentially causing the release of their contents into the lumen and other regions of the female RT. Additionally, the secretory activity of SSC and of femAGs involve different sets of Rab components related to the female's mating status. Moreover, we found that mating induces changes in SSC trafficking that alter the release of extracellular vesicles (EVs) from the SSC. We therefore proceeded to test whether silencing different Rabs affects the SSC trafficking system; disturbance of Rabs expression was found to result in reduced EV release from the SSC, confirming the impact of trafficking network impairment on secretion.

Our results indicate that mating induces changes in the SSC and femAG trafficking network. These changes alter secretory output, possibly targeting regions essential for the initiation and regulation of post-mating processes.

*Speaker

Keywords: reproduction, fertility, secretion, rab, extracellular vesicles

Integrated Stress Response signaling in adipose tissue acts as a systemic regulator of reproduction

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Reproduction is an energy-intensive process requiring systemic coordination. However, the inter-organ signaling mechanisms that relay nutrient status to modulate reproductive output are still unresolved. Here, we use *Drosophila melanogaster* as a model to establish the Integrated Stress response (ISR) transcription factor Activating Transcription Factor 4 (Atf4) as a fat tissue metabolic sensor that instructs oogenesis. First, we found that Atf4 in the fat contributes to a process called vitellogenesis, whereby yolk proteins and lipids synthesized in somatic cells are trafficked to maturing oocytes. We found that loss of Atf4 in the fat causes decreased expression of the lipase Brummer, yielding decreased yolk abundance in both adipocytes and oocytes as well as death of maturing follicles. Second, we saw that depletion of *Atf4* in the fat body blunts oogenesis recovery after amino acid deprivation and re-feeding, suggestive of a nutrient sensing role for Atf4. Finally, we discovered that Atf4 in the fat promotes egg-laying behavior (ovulation); decreased ISR signaling in fat tissues caused retention of excess mature oocytes. A candidate-based RNAi screen revealed that Atf4 in the fat regulates synthesis and secretion of a fat body-derived neuropeptide, CNMamide, which modulates neural circuits that promote egg-laying behavior (ovulation). Thus, we posit that ISR signaling in fat tissue acts as a "metabolic sensor" that instructs female reproduction: directly, by impacting yolk lipoprotein production and follicle maturation, and systemically, by regulating ovulation.

Keywords: reproduction, oogenesis, Atf4, stress, starvation, Brummer, CNMa

*Speaker

Interaction of mitochondrial fusion with electron transport chain function in the differentiation of *Drosophila* neural stem cells

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Mitochondria are central regulators of neural stem cells differentiation, and crucial for both neurodevelopment and adult neurogenesis. Neural stem cells (NSCs) or neuroblasts (NBs) in *Drosophila* require regulation of mitochondrial fusion and fission along with the activity of the electron transport chain to meet the metabolic demands of differentiation. However, the mechanisms by which mitochondrial morphology, and activity together regulate NSC differentiation is not well understood. In this study, we explore the interaction between mitochondrial morphology and electron transport chain complex I in vivo in NSC differentiation. We found that the inhibition of OxPhos by depleting different subunits of complex I did not affect the number of type II neuroblasts in the brain but reduced the number of intermediate precursor cells, ganglion mother cells and neurons in each type II neuroblast lineage. Complex I depletion led to a decrease in mitochondrial activity as measured by a decrease in mitochondrial membrane potential. This was coincident with a profound increase in ROS production. The decrease in numbers of lineage cells was coincident with a decrease in proliferation in the lineage. Nuclear cyclin E levels were also decreased in complex I mutant NBs, leading to delay in the G1/S transition. Interestingly, the proliferation and differentiation defects as well as mitochondrial activity in complex I mutant NBs could be restored by fused mitochondrial morphology obtained on additional depletion of fission protein Drp1, suggesting that mitochondrial fusion can maintain the mitochondrial activity for differentiation in type II NB lineage. Together, this study reveals a role of mitochondrial morphology in regulating the mitochondrial activity during differentiation. Our ongoing experiments further focus on the mechanisms by which mitochondrial fusion can alleviate the defects in complex I electron transport chain mutants to promote differentiation in neuroblasts.

Keywords: *Drosophila*, Neural stem cells, Differentiation, Mitochondria, Electron transport chain, Reactive oxygen species

^{*}Speaker

Interaction of the sorting nexin 25 homolog Snazarus with Rab11 balances endocytic and secretory transport and maintains the ultrafiltration diaphragm in nephrocytes

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Proper balance of exocytosis and endocytosis is important for the maintenance of plasma membrane lipid and protein homeostasis. This is especially critical in human podocytes and the podocyte-like *Drosophila* nephrocytes that both use a delicate diaphragm system with evolutionarily conserved components for ultrafiltration. Here we show that the sorting nexin 25 homolog Snazarus (Snz) binds to Rab11 and localizes to Rab11-positive recycling endosomes in *Drosophila* nephrocytes, unlike in fat cells where it is present in plasma membrane/lipid droplet/ER contact sites. To characterize this new function of Snz in the highly endocytic nephrocytes, we applied a wide variety light- and electronmicroscopy-based approaches such as live imaging, *ex vivo* tracer uptake, channel diffusion assay and tannic acid impregnation. Loss of Snz leads to redistribution of Rab11 vesicles from the cell periphery and increases endocytic activity in nephrocytes. These changes are accompanied by defects in diaphragm protein distribution that resemble those seen in Rab11 gain-of-function cells. Of note, co-overexpression of Snz rescues diaphragm defects in Rab11 overexpressing cells, whereas snz knockdown in Rab11 overexpressing nephrocytes or simultaneous knockdown of snz and tbc1d8b encoding a Rab11 GAP lead to massive expansion of the lacunar system that contains mislocalized diaphragm components: Sns and Pzd/ZO-1. We find that loss of Snz enhances while its overexpression impairs secretion, which, together with genetic epistasis analyses, suggest that Snz counteracts Rab11 to maintain the diaphragm via setting the proper balance of exocytosis and endocytosis.

Keywords: nephrocytes, Snx25, Snz, Rab11, endosomal maturation, recycling pathways

*Speaker

Investigating the role of metabolites in Minute cell physiology and cell competition.

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Mutations in ribosome protein (*Rp*) or *Minute* genes and in ribosome biogenesis factors result in debilitating diseases known as ribosomopathies. Cells heterozygous mutant for *Rp* genes (*Rp*/+) also get eliminated by cell competition when mixed with wild-type cells. The competitive elimination of *Minute* cells is thought to act as a quality control mechanism to eliminate subfit cells and is also likely relevant to cancer. Recent studies in *Drosophila* have focused on how *Rp*/+ cells experience stress pathway activation and transcriptional changes as an adaptation to single copy loss of *Rp* genes. Such transcriptional profiles reveal that *Rp*/+ cells have differential expression of several metabolic enzymes. Our recent work finds that some of these enzymes act as modulators of *Minute* cell competition and that the corresponding metabolites show altered levels in *Minute* cells. This suggests that metabolites can act as mediators or modulators of *Minute* cell competition.

Keywords: cell competition, metabolism

*Speaker

Investigation of the function of Snx21 in *Drosophila melanogaster*

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The sorting nexin (SNX) family contains proteins with a wide variety of domain structure, so their functions are diverse - they are involved in the function of membrane transport pathways, signaling processes, and the movement of organelles. Sorting nexins are characterized by the presence of the phospholipid-binding PX domain, which typically binds phosphoinositides, most commonly the early endosome-enriched phosphatidylinositol 3-monophosphate. Exact functions of many Snx proteins are still unknown, for example only one publication on human SNX21 provides experimental data concerning its endosome-associated scaffold function: in mammalian cells SNX21 recruits the huntingtin (Htt) protein to early endosomes and interacts with several members of the septin family through its C-terminal PX-associated B domain.

To explore the function of Snx21 in *Drosophila*, I performed loss-of-function experiments using two independent RNA-interference constructs in garland cells and salivary glands. The endosomal system of garland nephrocytes represents an excellent experimental model to study proteins with putative endosomal functions. Using immunofluorescence we found that *snx21* knockdown leads to a disordered endosomal pattern in garland cells. In the salivary glands, after the secretion of the adhesive material stored in the so-called glue granules, a portion of these granules remain in the cytosol and fuse with lysosomes to become crinosomes (a degradative compartment). Interestingly, knockdown of *snx21* causes a minor degradation defect because of a potential fusion defect between glue granules and lysosomes.

To further analyze Snx21 function, we generated a null mutant allele as well as the GFP- and GST-tagged Snx21-constructs. With GST-tagged Snx21 we performed lipid flotation assay and found the lipid interactor partners of Snx21. Snx21-GFP localizes to early and late endosomes in garland nephrocytes and to glue granules in salivary glands. Immuno-precipitation followed by mass spectrometry showed the interaction of Snx21 with known regulators of vesicle trafficking and surprisingly, many RNA-binding nuclear proteins also. Besides understanding the role of Snx21 in vesicular trafficking, analysis of its potential involvement in nuclear processes is an exciting new direction of our work.

Keywords: vesicular transport, phospholipid, binding domain, sorting nexins, *Drosophila*

*Speaker

Kinesin-1 controls nuclear migration by modulating centrosome activity

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The accurate control of the position of the nucleus is essential for many cellular contexts. The forces applied to position the nucleus vary according to cell type, but all originate from elements of the cytoskeleton. They can generate forces directly through polymerization or by the involvement of molecular motors. An important issue is to understand how different cytoskeletal cues can collaborate to accurately position the nucleus. The *Drosophila* oocyte is a perfect system to study this process *in vivo* in a developing context. The oocyte is a large polarized cell in which the nucleus is asymmetrically positioned. This positioning is mandatory for the polarity axis establishment of the future embryo. It presents an ideal system to study how different microtubule networks coordinate to generate the forces necessary for positioning the nucleus. We have previously found that at least two independent MT networks cooperate to position the nucleus (Tissot et al; 2017). We have recently established that the correct migration of the nucleus relies on a prepositioning step to the centre of the oocyte. This prepositioning of the nucleus is coordinated to a process of centrosome clustering located at the posterior of the nucleus. This clustering requires a reduction of centrosomes activity, indeed maintenance of a high level of Polo-kinase at centrosomes prevents centrosome clustering and impairs nuclear positioning. Furthermore, we have identified that the MT associated motor Kinesin 1 is critical in this process. In absence of Kinesin-1, centrosome clustering is impaired and the nucleus fails to position and migrate properly. In absence of Kinesin-1, essential component of the pericentriolar material such as SPD2 is increased at the centrosomes, suggesting that Kinesin-1 associated defects result from a failure to reduce centrosome activity. In accordance with this, depleting centrosomes rescues the nuclear migration defects induced by Kinesin-1 inactivation. Our results highlight a new function for Kinesin-1 in the nuclear positioning process which is independent of its known function in moving the nucleus as a cargo. Our results indicate that Kinesin-1 controls nuclear migration in the oocyte by modulating centrosome activity.

Keywords: Cytoskeleton, microtubules, nucleus positioning, centrosomes, kinesin

*Speaker

Linking the cell cycle to membrane oscillations in *Drosophila* syncytial cleavage division cycles

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In *Drosophila melanogaster*, as in other insects, the first cell divisions during embryonic development occur without cytokinesis. At the beginning of the 10th division, the embryo enters the syncytial blastoderm stage, where most nuclei are located at the periphery and continue to divide. At the beginning of each prophase, actin-rich membrane furrows move in between the spindles and retract during each telophase, thereby interference of dividing nuclei is prevented. The link between the cell cycle to these cortical actin and membrane oscillations is unknown. The localization of actin regulatory proteins is coupled with membrane transport through the recycling endosome (RE). RE transport requires the small GTPase Rab11 and Nuclear Fallout (Nuf), a Rab11 effector and adaptor protein to the Dynein microtubule motor. This transport mechanism is responsible for membrane growth during cellularization at cycle 14. We found that *drop out* (*dop*) encoding the single homologue of human MAST kinases, is required for this membrane growth. In a quantitative proteomic approach, we identified Ser401 of dynein-light intermediate chain (Dlic) as a potential substrate of Dop. Overexpression of Rab11, Nuf or phosphomimetic variants of Dlic suppressed membrane growth defects in *dop* mutant embryos. This led us to a model in which the phosphorylation of Dlic-Ser401 promotes its interaction with Nuf to control Rab11 dependent transport. This phosphorylation may also regulate Nuf-dependent transport of actin activators during syncytial divisions. To test this possibility, a phosphoablative version of Dlic was overexpressed, which resulted in defective syncytial divisions. However, these divisions exhibited phenotypes resembling Nuf-related defects and spindle assembly checkpoint (SAC) abnormalities. Thus, the phosphorylation of Ser401 might not only be important for Nuf-dependent transport but also for SAC silencing. We propose that the phosphorylation of Dlic is involved in linking the cell cycle to furrow formations during syncytial divisions.

Keywords: syncytial division cycles, Dynein, Transport, MAST, Kinases, Nuf, spindle assembly checkpoint

*Speaker

Manipulating myosin activity in *Drosophila* using an analogue sensitive allele of Rho kinase

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Actomyosin regulation is critical for controlling tissue behaviour during development and cell division. A key regulator of non-muscle Myosin II activity is Rho kinase. Rho kinase has been studied using mutations, RNAi knock-down, dominant negative constructs, optogenetic tools to activate or inactivate upstream regulators such as Rho as well as commercially available inhibitors. Here we report the generation of an analogue sensitive allele of Drok (*Drosophila* Rho kinase) to expand the toolkit allowing manipulating myosin II activity *in vivo* in a specific and acute manner. We benchmark the performance of commercially available Rho kinase inhibitors often developed for mammalian Rho kinase against the analogue sensitive allele in contractility assays in the fly as well as on cytokinesis regulation and polarity of asymmetrically dividing neuroblasts. We found that atypical protein kinase C (aPKC) inhibition causes rapid contraction of cultured wing discs. We used this assay to test the efficiency of DROK inhibition brought about in different ways in suppressing contractility. We further tested the effect of acute and specific Drok inhibition on neuroblasts, which yields astonishing phenotypes including the generation of binucleated neuroblasts and anucleated ganglion mother cell and provide evidence that Drok signalling regulates correct neuroblast polarisation.

Keywords: Rho kinase, Analogue sensitive, Myosin, Contractility, Neuroblast

*Speaker

Mapping the nanoscale organisation of cAMP signalling at synapses in *Drosophila* motor neurons

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Synapses are complex computational platforms that transform their functionality through synaptic plasticity. The membrane proteins G protein-coupled receptors (GPCRs) play a major role in modulating the strength of synapses via the second messenger cAMP. cAMP is per se very diffusible however, however within the cells it is becoming increasingly clear that cAMP signals are confined within cellular compartments. In *Drosophila* motor neurons we demonstrated that the localisation of PDE, enzymes that degrade cAMP, are crucial to the confinement of cAMP signals within a single bouton {Maiellaro, 2016}. Specifically, PDE localisation around the synaptic boutons blocks the diffusion of cAMP from bouton to bouton. Interestingly, each bouton contains 20-50 active zones, which are the sites of neurotransmitter release. Each active zone exhibits independent activity and regulation {Melom, 2013;Guerrero, 2005}. Therefore, mechanisms must exist that allow the confinement of cAMP to these domains. Additionally, the organization of components of cAMP signaling including PDE at the nanometer scale at the synaptic site is largely unknown. In this work, we test the hypothesis that the localisation of PDE at each active zone is the mechanism by which cAMP can modulate the activity of each active site independently. To this end leveraging on the power of *Drosophila*, we employ fluorescence resonance energy transfer (FRET) and Stochastic Optical Reconstruction Microscopy (STORM) to study the spatiotemporal dynamic of cAMP at each active zone and to map the nanodomain organization of PDE within active sites.

Keywords: synaptic plasticity, cAMP, GPCR

*Speaker

Masking phosphatidylserine in adult *Drosophila* brain prevents developmental neuronal phagoptosis and rescues neurodegeneration

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In mammals and *Drosophila*, removal of surplus neurons is critical for normal development of the central nervous system. However, neuronal loss is one of the main features of neurodegenerative diseases. Phosphatidylserine (PS) is an important "eat me" signal for phagocytic glia on apoptotic neurons, pruned neuronal processes and synapses, and stressed live neurons in different pathologies. To study the role of PS exposure in the adult brain, we used a recently generated model of neurodegeneration based on the adult-stage neuronal knock down of *Drosophila* S-phase kinase-associated protein1 (Skp1) homolog, SkpA, a component of the ubiquitin E3 ligases. This model flies show motor dysfunction and reduced life span accompanied by loss of dopaminergic and GABAergic neurons in the adult brain. Based on our previous observations, we hypothesized that activated phagocytic glia remove stressed live neurons exposing PS on their surfaces through non-autonomous cell death process phagoptosis. To reduce glial phagocytic activity and prevent neuronal loss, we expressed in adult brain neurons a truncated version of Milk fat globule epidermal growth factor-8 (MFG-E8), a secreted glycoprotein which binds PS but does not mediate engulfment. This protein completely rescued both types of neurons, dopaminergic and GABAergic, in the *skpA*-induced neurodegeneration model, significantly improved motor ability and expanded the life span. Moreover, we discovered that in wild type emerging flies near 20% of dopaminergic neurons are removed through phagoptosis as part of normal brain development, exposing the important physiological role of glial phagocytosis. Our findings suggest that investigating and timely manipulating glial phagocytosis could serve as a valuable strategy to fight neurodegeneration.

Keywords: neurodegenerative diseases, "eat me" signal, SkpA, glial phagocytic activity

*Speaker

Mechanism regulating the sub-cellular localisation of a novel RhoGEF isoform

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Cytokinesis is essential for the partitioning of cellular content into two daughter cells. It relies on the formation of a contractile ring composed of actin and myosin filaments at the plasma membrane between the two segregated pools of chromatids. The constriction of the ring through myosin activity drive cleavage furrow ingression, generating two physically separated daughter cells with equal genome content. The activity of the small GTPase Rho1, localized at the plasma membrane, is essential for this process, as it promotes actin nucleation and myosin activation. Rho1 activation is catalyzed by its guanine nucleotide exchange factor called Pbl-A in *Drosophila*. Rho1 must be tightly controlled spatio-temporally to ensure successful cytokinesis. The mechanism by which Rho1 is activated at the right place on the plasma membrane to initiate cytokinesis is conserved, involving the enrichment of Pbl-A at the equator of the cell via its interaction with the microtubule-associated protein RacGAP50C.

Our team has identified a novel Pbl isoform, called Pbl-B, produced from the alternative splicing of one large exon and expressed at similar levels than Pbl-A in most tissues. Interestingly, live imaging of dividing cells expressing Pbl-A or B tagged with GFP, revealed that Pbl-A was enriched both at the equator of the cells and at the furrow throughout ingression, consistent with its interaction with RacGAP50C. In contrast, Pbl-B remained cortical until telophase where it accumulated rapidly in the nucleus prior to Pbl-A. Analysis of dividing cells expressing solely Pbl-A or Pbl-B in *Drosophila* neural stem cells revealed that both isoforms act in concert to promote robust cytokinesis and preserve daughter cell size asymmetry.

The molecular pathway regulating Pbl-B sub-cellular localization is not known. While Alphafold prediction did not show major differences in secondary structure between Pbl-A and B, the additional 458 amino acids in the Pbl-B sequence may contribute to the difference in dynamics between the two isoforms. To determine this regulation, we developed a structure-function approach. We studied the localisation of GFP-Pbl-B truncated constructs in S2 cells. This approach allowed us to identify an additional nuclear localisation sequence as well as key negatively charged residues essential for Pbl-B cortical and nuclear localisation. *In vivo* studies confirmed the importance of these residues for the sub-cellular localisation and function of Pbl-B during cell division.

Keywords: Cytokinesis, RhoGEF, GTPase Rho1, S2 cells, *Drosophila* neural stem cells

*Speaker

Metabolic regulation of blood progenitor homeostasis and heterogeneity by TCA cycle in development and immune response in *Drosophila* larvae

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Immunity as well as metabolism are quite old, extensively focused and sort after fields, but their inter-dependency, as Immune-metabolism, is being advocated very recently. This crosstalk infers how under immune compromised state, metabolic shift occurs and metabolites (a-KG, Succinate, Fumarate, etc.) takes on the tasks of proliferation, differentiation and activation of the concerned immune progenitors/cells. *Drosophila* in recent past has also being established as a powerful model system for immune studies, due to the presence of only myeloid lineage, comprising three cell types (plasmacytes, crystal cells and lamellocytes). Recent work from our lab highlighted GABA (from brain) can elicit distinct immune cell population, under wasp infestation, which is nowhere to be seen in homeostasis (Madhwal et. al., 2020). Through our work we would like to switch the conventional paradigm of looking at TCA cycle as a "unique cycle and an intermediary step in glucose metabolism" to the "congress of different pathways/cycles and reservoir of cardinal immune metabolites". Metabolites are well known to get exchanged between the cellular compartments, which brands them competent for being signalling molecules. And where could be the better place for studying metabolites than TCA cycle, which is source as well as sink for all the three macromolecules of life. Each of the metabolites generated/exchanged here can be an independent signalling molecules and directs the heterogeneity of different systems. Here through our thorough analysis of TCA cycle across the development; with the help of different developmental stage markers-GAL4 (Tep4, Dome, ChIZ, and Hml), established the fact that TCA cycle does not exist as a complete cycle from the beginning. Rather it build up as a cycle with time during the development, with a few steps (Citrate to Succinyl CoA) are prioritized during the development of the lymph gland which are further joined in by the remaining steps for the maintenance and differentiation of blood cells in the lymph gland.

Keywords: Metabolism, Immunity, Growth, Development and TCA cycle

*Speaker

Mitochondrial dysfunction in immune cells leads to a distinct transcriptome profile and improved immune competence in *Drosophila*

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The efficiency of immune responses is affected by many endogenous and exogenous factors. As central metabolic and signaling hubs, mitochondria play an important role in immune response modulation. However, the mitochondrial involvement in immune responses is complex, cell type specific and not fully understood. The aim of our study is to understand the role of mitochondria in innate immunity using *Drosophila melanogaster* as a model. Our results show that silencing mitochondrial oxidative phosphorylation (OXPHOS) genes specifically in *Drosophila* immune cells leads to immune activation prior to infection and enhances immune responses against parasitoid wasps, without being harmful to the organism. It appears, however, that defects in mitochondrial function below a certain threshold can be beneficial in the context of infection. To identify the genes, pathways and the mechanism involved in this immune activation, we performed RNA sequencing of *Drosophila* immune cells with OXPHOS complex III (cIII) knockdown. Our data indicates that disrupting mitochondrial respiration triggers specific changes in cell metabolism, leading to immune cell activation and improved immune responses. Even though the cell-mediated immune competence was enhanced in the cIII knockdown immune cells, the nuclear factor- κ B (Nf- κ B) target genes were downregulated in these cells in comparison to the controls, suggesting a possible mechanism to reduce inflammation and increase disease tolerance. In addition, the transcriptome profiles of the cIII knockdown immune cells showed that they are utilizing aerobic glycolysis as the primary energy source, indicated by the upregulation of multiple genes involved in aerobic glycolysis. We are currently conducting experiments to follow up on these findings to gain more insight into the effects of mitochondrial function on innate immunity.

Keywords: hemocyte, innate immunity, OXPHOS, membrane potential, parasitoid infection

*Speaker

Mitochondrial redox protein quality control as a key determinant in ageing

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Mitochondrial biogenesis relies heavily on protein import from the cytosol as 98% of mitochondrial proteins are synthesized outside the mitochondria. The main determinant in the retention of proteins in the mitochondrial intermembrane space (IMS) is correct oxidative folding, which is mediated by the MIA import pathway and strongly aided by the oxidizing environment of the IMS. In order to sustain proper protein folding while preventing oxidative damage, the IMS must maintain a healthy redox balance. However, our current understanding of the interplay between oxidative and reductive pathways in the IMS is very limited and while an increase in mitochondrial protein oxidation is a known hallmark of ageing, very little is known about the activity levels of the oxidant and reductive pathways in aged cells.

Our lab has recently shown that the thioredoxin machinery is dually localized to the cytosol and the IMS where it may govern redox protein quality control by reducing improperly or overly oxidized proteins. Our aim is to further dissect the reductive machinery operating in the IMS and improve our understanding of the role of the redox balance in ageing. To build on the insights gained from work in yeast and mammalian cell models, we are analyzing how a disruption of the redox balance in the IMS affects lifespan in flies. In knocking down the main sulfhydryl oxidoreductase of the MIA import pathway, Mia40/CHCHD4, we aim to increase the reduction state of the IMS, whereas a knockdown of the reductive thioredoxin machinery is expected to have the opposite effect.

Our findings suggest that impairing the reductive machinery via knockdown of the main thioredoxin, Trx2, does indeed decrease fly lifespan. Interestingly, the same holds true for a downregulation of the oxidoreductase Mia40. Decreasing the levels of Mia40 renders flies more susceptible to starvation and oxidative stress but has no effect on mitochondrial oxygen consumption suggesting that the mitochondria of *MIA40* knockdown flies remain functional. Activity analysis of such flies has shown that they move significantly more and sleep far less than controls. Importantly, this difference in activity and sleep levels is maintained throughout their lifespan hinting at a so far unknown role of the mitochondrial IMS in the regulation of sleep.

Keywords: ageing, mitochondria, oxidative stress, reductive systems

*Speaker

Modelling senescence-linked disease onset and progression in *Drosophila* adult male accessory gland

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Senescence—an age-associated decline in cellular architecture and function—displays two opposing, context-dependent associations with diseases such as cancers. Deciphering these senescence-linked disease promotions or inhibition requires examination of the causally associated genetic circuitries in a genetically highly tractable model organism like *Drosophila*. Toward these goals, we are exploring *Drosophila* organs that are likely ideal for probing these questions. Here we show that the adult male accessory organ (MAG) represents one such organ. The squamous epithelial lining of the fluid-filled lumen of *Drosophila* adult MAG displays age-related changes in its tissue architecture, a loss of its tricellular junctional integrity being one such. We also find that starved or over-fed adults alter the pace of senescence, which is recapitulated by genetic perturbation of the nutrient-sensitive TOR signaling pathway. Likewise, genetic perturbations in FoxO signaling, a regulator of cellular senescence, hastens the loss of the tricellular junctional architecture of the MAG squamous epithelium. Finally, the gain of a human oncoprotein, the ERG transcription factor, in *Drosophila* adult MAG induces the hallmarks of oncogene-induced senescence. We will present evidence of the utility of the adult MAG in modeling an entire gamut of senescence-linked human diseases in *Drosophila* and uncovering their mechanistic underpinnings.

Keywords: Cellular senescence, Male Accessory Gland, Tricellular junctions, FoxO, ERG oncoprotein

^{*}Speaker

Mthl10 regulates changes in cellular biomechanics around epithelial wounds

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The repair of epithelial wounds requires patterned coordination of cell behaviors and cellular biomechanics. Previous investigations of wound repair in the *Drosophila* pupal notum have shown that a G-protein-coupled receptor, Methuselah-like 10 (Mthl10), mediates wound-induced calcium signaling. Further, knockdowns of Mthl10 reduce survival rates after wounding. Here we show that a key role for Mthl10-mediated calcium signaling is the regulation of cortical tension. We used a laser-ablation assay to measure tension in the *Drosophila* notum both before and after wounding, and with Mthl10 or the IP3 Receptor (IP3R) knocked down on just one side of the wound. We find that wounding leads to a widespread loss of cortical tension – extending out to 210 μm from the wound center. When wound-induced calcium signaling through Mthl10 is active, the lost tension is restored in a wave that moves from distal locations back toward the wound. The wave of restored tension coincides with a visible contractile wave and reaches the wound margin in ~ 10 minutes. When either Mthl10 or IP3R are knocked down, the contractile wave is still observed, but the restoration of tension is prevented. These studies thus provide a link from biochemical signaling around the wound to spatially and temporally patterned changes in cellular biomechanics needed for optimal wound repair.

Keywords: biomechanics, wound healing, cortical tension, calcium signaling

*Speaker

Non-cell-autonomous circadian regulation of brain function in *Drosophila melanogaster*

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Nearly all aspects of behavioral, cognitive, and emotional states and physiological processes exhibit circadian (near 24-h) rhythms in most organisms including *Drosophila melanogaster*, in which, only approximately 150 neurons and 1800 glia cells contain circadian clocks. Therefore, clock neurons presumably input the day-of-time information to non-clock cells in other regions of the brain to modulate their function and output in a circadian fashion. However, the underlying mechanisms are still poorly understood.

Here, we addressed this question by focusing on the mushroom body (MB), non-clock cells contained region of the *Drosophila* brain that constitutes the center of associative learning and sleep regulation. By conducting a circadian RNA-seq analysis of the mushroom body neurons, we identified a large number of genes rhythmically expressed, including *neurofibromin 1 (Nf1)* tumor suppressor gene and cAMP-dependent *Protein kinase a catalytic subunit 1 (Pka-c1)* gene. Their rhythmic expression is controlled by circadian clocks since it was abolished in *period* null mutants. Subsequently, taking advantage of calcium and cAMP imaging as well as behavioral analysis, we showed that circadian clocks drive the rhythms of the excitability of MB neurons via NF1-cAMP/PKA signaling, eliciting higher mushroom body activity during the day than at night and thereby, promoting daytime wakefulness.

Furthermore, we develop a promising luciferase *in vivo* reporter that will allow us to figure out the molecular pathway behind the circadian modulation of the gene expression in the MB and what pre-synaptic inputs are playing a role in the maintenance of the rhythmic gene expression pattern previously observed.

Altogether, our work demonstrates the widespread non-cell-autonomous control of rhythmic brain function by circadian clocks and its implication in sleep.

Keywords: Circadian rhythms, Mushroom body, PkaC1, luciferase reporter, sleep

*Speaker

Oenocytes orchestrate lipoprotein trafficking and systemic lipid metabolism during prolonged starvation in a Desat1-dependent manner

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Liver steatosis induced by starvation or malnutrition is characterized by the accumulation of lipids in hepatocytes. Moreover, the human liver plays important roles in regulating systemic lipid homeostasis under prolonged starvation or fasting and re-feeding cycles. Here, we use *Drosophila* as a model to investigate the role of hepatocyte-like cells, oenocytes, in lipid metabolism. By using a modified starvation holidic medium that allows adult *Drosophila* survival for up to 12 days in starvation, we show that oenocytes take up lipids from the surrounding fat body in the first days of fasting before releasing them back at later stages. We also show that the knockdown of Desat1 specifically in oenocytes leads to higher lipolysis in the fat body as well as higher saturation, shorter carbon length and lower amount of DAG in hemolymph lipids, indicating an intimate crosstalk between fat body and oenocytes in starvation. Mechanistically, we identify a strong sequestering of Apolpp- and Apoltp-containing lipoproteins by the Desat1-deficient oenocytes. Lipoproteins were found in intracellular vesicles that are surrounded by a dense actin cytoskeleton, preventing lipid storage and release by the oenocytes. Additional cell-autonomous changes suggest that membrane fluidity is strongly perturbed in Desat1-deficient oenocytes. we are also doing single nuclear RNA sequencing in adult fly at different days of starvation to have a better understanding of oenocytes' function. Together, our findings reveal a Desat1-mediated role of oenocytes in controlling lipoprotein trafficking, fat body lipolysis and systemic lipid metabolism in prolonged starvation.

Keywords: oenocytes, lipid metabolism, lipoproteins, desat1

*Speaker

Pathogen infection drives tumorigenesis by abrogating cell competition via insulin signaling regulation

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Cell competition is cell elimination evoked by direct cell-cell interactions. For instance, oncogenic polarity-deficient cells are eliminated by cell competition, suggesting that cell competition acts as an intrinsic tumor-suppressive machinery. Epithelial cells bearing mutations in the apico-basal cell polarity gene *scribble* (*scrib*) overgrow and form tumors when occupying the tissue homogenously, but such cells are eliminated from the tissue when surrounded by wild-type cells via cell competition. We have previously shown that cellular insulin signaling plays a crucial role in determining the fate of polarity-deficient cells, whether *scrib* cells are eliminated or not eliminated by cell competition. Indeed, upregulation of insulin signaling in polarity-deficient cells is sufficient to abrogate cell competition and cause tumorigenesis. Here, we found that Toll signaling, *Drosophila* innate immune signaling activated by systemic pathogen exposure, disrupts cell competition by elevating insulin signaling in polarity-deficient cells. Mechanistically, Toll signaling upregulates the transcription of *Insulin-like Receptor* (*InR*) via *Drosophila* NFkB Dorsal. Elevated insulin signaling in polarity-deficient cells leads to activation of Yorkie, resulting in tumorigenesis. Our findings provide a novel insight into how tumor-suppressive cell competition is regulated by environmental or animal status.

Keywords: Tumorigenesis, Cell competition, Immunity

Probing the role of mechanosensory pathways in regulating intestinal stem cell niche homeostasis

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Intestinal epithelium exhibits remarkable robustness against external and internal insults despite uncertain needs; a feature that is maintained by the adaptive proliferation and turnover of resident intestinal stem cells (ISCs). Stringent instruction on such proliferative responses is essential to maintain the gut barrier function; left unchecked can lead to hyperplasia. Extensive research in the recent past has led to the identification of several macromolecular factors (autocrine, paracrine, juxtacrine) regulating ISC homeostasis. While the biochemical nature of ISC homeostasis signaling has gained much attention, a question that is less resolved is how the tissue-scale forces are transduced to biochemical cues to regulate ISC-niche homeostasis. The gut is constantly exposed to stretch and strain and there is mounting evidence that tissue mechanics also play a key role in regulating SC activity in adult tissues. Understanding the crosstalk between mechanotransduction and cell fate specification during gut renewal is critical as it can provide a mechanistic framework for disease modeling and regenerative medicine. We use the adult midgut epithelium from *Drosophila melanogaster* as a model system to identify biomechanical signals related to stem cell homeostasis. The adult *Drosophila* midgut encompasses a pseudostratified monolayer epithelium, without any crypt/villi, and hosts stem cells that reside basally, attached to the basement membrane. We performed an unbiased genetic screen to identify factors coupling environmental cues with ISC-dependent adaptive growth by using RNAi-mediated knockdown of ISC niche-expressed cellular receptors and membrane proteins. Strikingly, we found enrichment for receptors, which mediate mechanotransductive signaling, indispensable for preserving gut barrier function. The aim of this project is to investigate the role of mechanosensory inputs in maintaining adult ISC niche homeostasis. To achieve these goals, we employ bioinformatic-, and genetic tools, and new quantitative bioimaging techniques coupled with mechanical manipulation of guts using state-of-the-art live imaging tools.

Keywords: Intestinal stem cells, Mechanotransduction, Stem cell maintenance, Cell fate specification, Regeneration

^{*}Speaker

RASSF8 is a novel WAVE interaction partner controlling border cell cohesion through E-Cadherin and Echinoid

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The WASP family verprolin homologous (WAVE) protein is a key activator of the Arp2/3 complex and coordinates actin nucleation in many cellular processes including cell shape, cell adhesion and cell migration. A yeast-2-hybrid screen was performed to find novel interaction partners of the *Drosophila* WAVE WHD domain, required for the formation of the WAVE Regulatory Complex (WRC). One interesting candidate we found is RASSF8, the Ras association domain-containing protein 8. Originally identified as a tumor suppressor, it is involved in the stability of adherens junctions, controlling cellular proliferation, cell adhesion and actin organization. A RASSF8 construct of 156 aa is necessary for efficient binding to WAVE WHD. Coimmunoprecipitations further confirm a specific interaction between RASSF8 and the first 96 amino acids of WAVE. To functionally study this interaction *in vivo*, we analyzed single and trans-heterozygous *rassf8*, *scar/wave* mutants. Remarkably, *rassf8* loss of function mutant females are semi-sterile. Reduced fertility of *rassf8* mutant females correlates with a loss of cohesion of the outer border cell cluster, a phenotype that is partially rescued by removal of one copy of *scar/wave*. Co-localization studies in mutant clones further suggest that RASSF8 acts on cell adhesion molecules such as E-cadherin and Echinoid at contact sites between outer border cells. Thus, we propose a model, in which RASSF8 is important for the localization of Echinoid and E-cadherin to control proper cohesion in the migrating border cell cluster.

Keywords: RASSF8, E, cadherin, Echinoid, Border cell migration, Cell, cell adhesion

*Speaker

Regulation of F-actin flows during epithelial folding in *Drosophila* wing imaginal discs

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During animal development, epithelial tissues undergo various transformations to attain their final form. Bending or folding are common transformations of epithelia. They can be described as a two-step process: upstream cues define the position of the fold, followed by cell shape changes in the defined region bringing about folding. Besides the well-defined process of apical constriction, changes in the basal or lateral sides of cells contribute to epithelial folding. In the *Drosophila* wing imaginal disc, the fold formed at the border of the prospective hinge and pouch regions (H/P fold) forms by lateral cell constriction. We previously showed that this involves pulsatile flows of F-actin along the lateral cell edges. The F-actin flows correlate with increased mechanical tension along these edges, indicating that they drive fold formation. However, how the F-actin flows, and thus the H/P fold, is positioned within the tissue remains unclear. Moreover, the molecular mechanisms that regulate the F-actin flows are unknown. Here, we show that F-actin flows are present at the intersection between the expression domain of the T-box transcription factors Doc 1, 2, 3 and the region of Hedgehog signal transduction. Moreover, we reveal that Hedgehog signal transduction is necessary and (within the Doc 1, 2, 3 expression domain) sufficient for inducing ectopic F-actin flows and fold formation. Furthermore, we show by pharmacological perturbations that Rho kinase activity and F-actin turnover are required for F-actin flows while microtubules are not. Our work provides insights into how F-actin flows are generated and positioned during fold formation in epithelial tissues.

Keywords: epithelial folding, actin flows, wing imaginal disc

*Speaker

Restoring the NAD⁺/NADH balance is essential to support proliferation of neural stem cells with mitochondrial dysfunction

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Mitochondrial dysfunction is a major cause of neurodegenerative disease progression. Previous studies have revealed that defective oxidative phosphorylation (OxPhos) adversely impacts neural stem cell (NSC) proliferation, causing a decrease in postmitotic neurons and reduced brain size in *Drosophila*. However, how OxPhos contributes to cell metabolism to support NSC proliferation in vivo is not yet fully understood. ATP depletion is often considered a major consequence of OxPhos dysfunction. However, redox imbalance, in particular a decrease in the NAD⁺/NADH ratio, has been proposed to be equally or perhaps more responsible for phenotypes related to OxPhos dysfunction, and mitochondrial disease progression. Our investigation revealed that the depletion of Complex I of OxPhos negatively affects NAD⁺/NADH balance in NSCs of the developing *Drosophila* brain. Recycling of NAD⁺ by the ectopic expression of bacterial derived NADH oxidase (LbNox) was found to significantly rescue NSC proliferation. In addition, we observed increased levels of the redox-dependent glycolytic enzyme, lactate dehydrogenase (Ldh), in response to OxPhos dysfunction. Surprisingly, co-depletion of Ldh failed to rescue proliferation defects, suggesting that the increased Ldh expression is likely an NSC response to compensate for the reduced NAD⁺ levels caused by OxPhos dysfunction. In addition, we found nuclear localization of Ldh and further explored whether nuclear Ldh plays a role in the maintenance of the nuclear redox balance and gene expression. Targeted DamID analysis showed Ldh associated to specific gene loci on the nuclear chromatin. Further research is needed to fully understand the role of Ldh in metabolic rewiring and nuclear gene expression under both normal and mitochondrial disease conditions.

Keywords: Neural stem cells, NAD⁺/NADH metabolism, Mitochondrial dysfunction

*Speaker

Robustness of the Canonical Mitochondrial Fusion Machinery Promotes Nebenkern Formation in *Drosophila* Spermatids

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Mitochondria are the bioenergetics powerhouses and biosynthetic centers of the cell. The mitochondria are constantly changing shape and subcellular distribution according to function, energy and metabolic demands of the cell. Mitochondrial morphology usually ranges from small spheres and short tubules to elongated tubules and reticular networks. These changes are mainly controlled by the balance between two opposing mechanisms of membrane dynamics, fusion and fission, which when perturbed, can lead to severe pathologies.

Perhaps the most dramatic morphological changes and structural organizations of the mitochondria occur during spermatogenesis. In *Drosophila* spermatids, individual mitochondria aggregate near the newly formed haploid nucleus and subsequently coalesce and fuse into a giant sphere called Nebenkern. The Nebenkern is composed of two giant mitochondria wrapped around each other and arranged in an onion-like spherical segments of layers upon layers. During subsequent spermatid elongation stages, the Nebenkern is transformed from a 6.7 μm sphere to two, 1.8 mm long, cylindrical mitochondrial derivatives extending alongside the axoneme. Although detailed ultrastructural description of Nebenkern formation was already reported five decades ago, the molecular mechanisms underlying the formation of this extraordinary organelle remains largely obscure.

To further characterize the genetic components, involved in Nebenkern formation, we utilized several advanced microscopy techniques such as live imaging, electron microscopy and expansion microscopy. We show that already during the second meiosis division, mitochondria start fusing to elongated tubular organelles, which continue fusing and collapsing to form the spherical Nebenkern, achieved by the robust and lasting action of the canonical fusion machinery. We demonstrate that the testis-specific mitochondrial fusion protein, Fzo, and the more generally expressed mitochondrial fusion protein, Marf, function similarly in promoting spherical mitochondrial fusion.

Finally, using a candidate screen through a compiled list of mitochondrial and cytoskeletal genes, we identified additional components involved in Nebenkern formation.

Keywords: mitochondria, microscopy, gametogenesis, testis, organelle

^{*}Speaker

Role of Cyclase-associated protein and actin disassembly in epithelial cells

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During developmental and regenerative processes, the spatial integration of cellular behaviours results in the dynamic remodelling of epithelial cells. The cellular forces that drive these morphogenetic changes are regulated by actin networks that are constantly remodelled and turned over. Our objective is to gain deeper understanding of the regulation of actin turnover and its functional consequences during epithelial morphogenesis. Actin turnover is regulated by actin depolymerizing factor (ADF)/cofilin. Other actin binding proteins, such as Cyclase associated protein (Capulet, CAPt), aid in the disassembly and recycling of actin filaments for new rounds of assembly. We employ *Drosophila* genetics and confocal microscopy for studying morphogenetic processes *in vivo*. As CAPt-loss of function is known to result in apical accumulation of filamentous actin in the *Drosophila* follicular epithelium, we wanted to investigate the consequences of this accumulation. We further aim to understand CAPt mechanistically control cellular processes, such as spatially distinct actin arrays and actomyosin contractility during epithelial morphogenesis.

Keywords: Actin, follicular epithelium

*Speaker

Role of N-glycans for the function of E-cadherin in tissue morphogenesis

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E-cadherin (*shotgun*, *shg* in *Drosophila*) is the core component of adhesion in epithelial cells and a key player in the dynamics of epithelial tissues. Besides adhesion, E-cadherin mediates force transmission from the pulling cell to the cadherin-catenin complex and associated actomyosin cortex inside the plasma membrane. The forces are sensed by conformational changes of a-catenin, which reinforces the cortical link, for example. We previously found that mutations in the N-glycosylation pathway, *wol*, *gny*, and *xit* lead to hypoglycosylation of E-cadherin and impaired cell intercalation in germband extension. As these mutations affect the N-glycosylation of many proteins passing through the ER, we introduced single or multiple Asn-> Gln (N/Q) point mutations at predicted and biochemically determined N-glycosylation sites into the *shotgun* locus by attB/phiC mediated genome engineering. We analyzed the phenotypes of the E-cadherin-NQ mutants in comparison to *xit* mutants and wild type with respect to clustering as seen by confocal microscopy, mobility by FRAP, the stoichiometry of E-cadherin and a-catenin, mechanical properties by junction ablation. We tested the relation of E-cadherin, a-catenin and cortical link by introducing an E-cadherin-a-catenin fusion protein into the *shotgun* locus, in this way fixing the stoichiometry to 1:1. As these mutants show a similar phenotype to the E-cadherin-NQ and *xit* mutants, we propose a balance between supermolecular clustering of E-cadherin on the one side and tight link to the cortex on the other side. We hypothesize that large and stable clusters are involved in efficient mechanotransduction in tissue morphogenesis. We now investigate the phenotypes of E-cadherin-NQ mutants in cell-cell coordination during germband extension and in the amnioserosa.

Keywords: E, cadherin, clustering, N, glycans, cadherin, catenin complex

*Speaker

Role of the Ensconsin/Kinesin-1 complex in the oocyte microtubule network reorganisation

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The building of cell-type specific microtubule (MT) arrays are key features that regulate cell shape, intra-cellular transport and cell division. Although it is established that these processes are regulated by the MT-Associated Proteins (MAPs) including motors such as kinesin and dynein, it is not clear how these players fulfil their function to reorganize MT cytoskeletons in space and time. In this study, we have used *Drosophila* oocyte as a model system to investigate how a polarized array of short MTs is shaped into long MTs required for cytoplasmic advection and oocyte polarization. Unlike Kinesin-1, that exhibit homogenous localisation in nurse cells and oocyte chamber, its activator Ensconsin (Ens/MAP7), is strongly enriched in the oocyte suggesting the presence of a specific mechanism that target this MAP from nurse cells to the oocyte through ring canals. Interestingly, an Ens variant with low affinity for MT is not enriched in the oocyte. Moreover, loss of Dynein severely impairs Ensconsin enrichment in the oocyte in agreement that Ensconsin-decorated MTs are transported from nurse cells in the oocyte. We also show that MT elongation is strongly compromised in *ens*mutant, in agreement with the MT polymerising ability of Ensconsin. Finally, we show that Ens-Kinesin-1 interaction is strictly required to fully activate Kinesin1 that generate MT streaming. Altogether our work suggests that dynein dependant transport of Ensconsin-labeled MTs may promotes targeted enrichment of MTs with enhanced polymerising properties, in the oocyte chamber. We propose that this step could be a prerequisite for MT reorganisation by Kinesin-1 into MT streams required for advection. Altogether, our work may imply that specific MT cytoskeletons may be assembled locally by motor-dependant transport of MTs harbouring enhanced polymerisation potential associated with Kinesin-1 activating properties.

Keywords: Microtubules, Oocyte, Kinesin, Ensconsin

*Speaker

Role of the Hmgcr pathway and Wunens in promoting *Drosophila* germ cell migration

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During development many cell types migrate along stereotyped routes determined through deployment of cell surface or secreted guidance molecules. Whilst we know the identity of many of these molecules, how gradients of these cues are fine-tuned and maintained remain relatively poorly understood. The migration of *Drosophila* embryonic germ cells is affected by expression of genes in the 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (Hmgcr) pathway and by expression of the lipid phosphate phosphatase encoding genes, *wunen* and *wunen2*. We will present our results using co-misexpression studies and live imaging to investigate the mechanistic link between these two pathways and the posited germ cell chemoattractant Hedgehog.

Keywords: wunen, wunen2, PGC, germ cell, migration, hmgcr

*Speaker

Santa-maria receptor acts with SIMU in recognition and engulfment of apoptotic neurons by embryonic glia

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Apoptosis plays a crucial role in embryonic development and tissue homeostasis. Phagocytosis of apoptotic cells is the last critical step of apoptosis, which proceeds in four steps: (1) *recruitment* of phagocytes to the apoptotic cell, (2) *recognition* of the cell as a target for phagocytosis and (3) *engulfment*, followed by (4) *phagosome maturation* and degradation of the apoptotic debris. In *Drosophila*, there are three main phagocytic receptors for apoptotic cells: Six – Microns – Under (SIMU), Draper (Drpr) and Croquemort (Crq). SIMU and Drpr are expressed on glial membranes and recognize phosphatidylserine exposed on apoptotic neurons in the developing Central Nervous System (CNS).

Santa-maria (CG12789) was first identified in a screen for mutations affecting the rhodopsin biosynthesis in *Drosophila*. It belongs to class B scavenger receptors (around 30% identity with human SR-BI, mouse CD36, *Drosophila* NINAD and Crq) and is required in adult brain neurons and glia for retinoid (vitamin A and its derivatives) formation. Santa-maria is specifically expressed in embryonic glia, but its role during embryogenesis remains obscure. According to protein domain prediction software and previous studies, Santa-maria has no signaling domains, suggesting that it may function, similarly to CD36, as a tethering receptor in the recognition step of phagocytosis.

We discovered a new role of Santa-maria in glial phagocytosis of apoptotic neurons during embryogenesis. CRISPR-generated *santa-maria* mutant embryos show unengulfed apoptotic neurons in the embryonic CNS suggesting Santa-maria's involvement in the recognition and engulfment steps of phagocytosis. In addition, we found that Santa-maria and SIMU genetically and physically interact. To corroborate these data on the cellular level, *in vivo* in the embryo, we generated transgenic flies carrying an inducible secreted version of Santa-maria by removing two predicted transmembrane domains from its sequence. When expressed in embryonic macrophages outside the CNS, the GFP-tagged secreted version of Santa-maria was found on apoptotic neurons and on glial cells. These results demonstrate that Santa-maria binds to apoptotic cells and interacts with SIMU on glial membranes, further validating its role in recognition and engulfment of apoptotic neurons.

Keywords: Apoptosis, Phagocytosis, Santa maria, glia cells

*Speaker

Specific tissue features govern two distinct pathways driving cytokinesis

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Cytokinesis is essential to partition the cellular content into two daughter cells. This fundamental process occurs after sister chromatid segregation and relies on the assembly of an acto-myosin contractile ring at the cell equator. The constriction of the ring drives the ingression of the cleavage furrow. The small GTPase Rho1 and its activating RhoGEF (called Pbl in *Drosophila*) are essential for this process by driving ring assembly and constriction. The team has discovered that two Pbl isoforms, Pbl-A and Pbl-B act concurrently to control Rho1 activity during the asymmetric division of the *Drosophila* neuroblast. Furrow-enriched Pbl-A focuses Rho1 activity at the furrow to sustain efficient ingression, while Pbl-B pan-plasma membrane localisation broadens the zone of Rho1 activity, which is critical to adjust furrow position, thereby preserving correct daughter cell size asymmetry. These findings highlight how the use of two isoforms with distinct localisation make asymmetric division of the neural stem cell more robust. However, how these two distinct pathways for Rho1 activation participate in making cytokinesis more robust in other tissues is not known. We found that flies lacking Pbl-B are viable but the adults have a reduced lifespan, cognitive impairment and are male sterile. In contrast, flies lacking Pbl-A are fertile but exhibit severe embryonic and pupal lethality rate. These findings indicate that, while Pbl-A and B have redundant activities, the level of their contribution to cytokinesis is tissue-dependent. Using genetics and live imaging we will present how the Pbl-A vs Pbl-B pathway is favoured depending on specific tissue features.

Keywords: Cytokinesis Mitose RhoGTPase Myosin cell division RhoGEF Pebble

^{*}Speaker

Superresolution imaging uncovers the Maturation of E-Cadherin Nanostructure In Vivo

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E-cadherin is the central component of adherens junctions and is required for epithelial cell adhesion. E-cadherin forms *trans* homodimers in which a molecule from one cell binds to an E-cadherin molecule from the adjacent cell, thus creating a mechanically stable link. Beside *trans* complexes, up to several hundred E-cadherin molecules laterally cluster within the same membrane, forming super molecular *cis*-complexes. Due to finite optical resolution, it has remained unclear to which degree *cis*-clusters engage in *trans* interaction. Adherens junctions morphology was first defined in electron micrographs by their electron-dense material and characteristic size of about 40–50 nm distance between the two sides. In contrast to electron microscopy, despite the nanoscopic size of adherens junctions, the two sides of E-cadherin *trans* homodimers have not yet been resolved by superresolution fluorescence microscopy. Resolution of E-cadherin clusters is essential to differentiate the distribution and respective relation of *cis* clusters and *trans* interactions. We applied single-molecule localization microscopy via DNA-PAINT (DNA- and peptide-point accumulation for imaging in nanoscale topography) to visualize the distribution of E-Cadherin at the nanoscale in the lateral epidermis of *Drosophila* embryos during gastrulation. E-cadherin was tagged at the intracellular C-terminus by GFP, inserted at the genetic locus and detected by a GFP nanobody linked with oligonucleotides. We achieved a lateral localization precision of ~ 10 nm. Reconstructed images resolved the two sides of E-cadherin *trans* complexes. We detected paired clusters across junctions and unpaired clusters without a corresponding E-cadherin cluster on the other side of the junction. Cross-correlation of the signal intensities along the two sides of junctions reveals a typical distance of ≈ 50 nm, consistent with EM analysis. The pattern of paired and unpaired clusters changed during junction maturation. Furthermore, the distribution pattern was changed in hypoglycosylated E-cadherin mutants without leading to a loss of adhesion. Cluster analysis revealed changes in cluster size distribution during maturation and the impact of mutants on the clustering statistics. Thus, we present direct evidence for two types of E-cadherin clusters: paired and unpaired, along adherens junctions. We provide an assay for a quantitative understanding of developmental and genetic control of the E-cadherin dynamics on the nanoscale *in vivo*.

*Speaker

Keywords: Ecadherin, Nanostructure, Superresolution, DNA PAINT

Synthetic lethal screening identifies existing drugs with selective viability effects on Neurofibromatosis type-1 model systems

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Neurofibromatosis type 1 (NF1) is a genetic disorder associated with a variety of symptoms including the formation of benign tumours. Drug treatments are currently limited, with selumetinib, a MEK inhibitor, being the only drug currently available to treat these tumours. However, selumetinib has significant drawbacks and is not approved for all patients. Therefore, there is a clear need to discover new drugs to target *NF1*-deficient tumour cells. One approach to identify candidate drug targets for tumorigenic diseases is to use synthetic lethal interaction screens. Synthetic lethal interactions are a type of genetic interaction in which inhibition of either of two genes alone is viable, but the combined inhibition of both genes is inviable. When one of these genes is mutated in tumour cells, such interactions can be exploited to kill those cells exclusively by targeting the synthetic lethal partner gene using a drug. We applied this method to identify candidate drug targets to treat *NF1*-deficient tumours in a *dNf1* null mutant *Drosophila* cell line. Thus, synthetic lethal screening was used to identify candidate drug targets to specifically kill *dNf1*-mutant tumour cells. Using the *dNf1*-mutant *Drosophila* cell model, we identified 72 candidate targets, of which 19 could be inhibited with existing drugs. The most promising targets were 1) inhibition of autophagy using chloroquine (CQ) or bafilomycin A1, and 2) inhibition of hTERT with azidothymidine (AZT), each of which resulted in a selective reduction in cell viability in *dNf1* null *Drosophila* cells, a panel of human *NF1*-mutant cell lines, including **NF1**-mutant malignant peripheral nerve sheath tumour (MPNST) cell lines, a *Drosophila in vivo* model (CQ only), and in MPNST xenografts in mice. Finally, we found that combined treatment with either CQ and selumetinib or AZT and selumetinib resulted in a further reduction in *NF1*-mutant cell viability. The results of this study highlight two key points: 1) the use of *Drosophila* cells as a model to screen for drugs specifically targeting *dNf1*-mutant cells was highly successful as the candidate interactions were conserved across a panel of human *NF1*-mutant cells and in *NF1* mutant models *in vivo*, demonstrating the robustness of our combinatorial screening method. 2) *NF1*-deficient cells have vulnerability to disruption of the autophagy pathway and inhibition of telomerase activity. Not only do these pathways

*Speaker

represent promising targets for the treatment of NF1-associated tumours, but we identified CQ, bafilomycin A1, and AZT as candidate drugs for the treatment of NF1 tumours.

Keywords: Neurofibromatosis, *Drosophila*, NF1, tumour, drug discovery

The LIM domain protein Smallish regulates actomyosin contractility during epithelial morphogenesis in *Drosophila*

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The cortical actomyosin network generates the mechanical force that drives highly dynamic processes occurring during epithelial morphogenesis, such as cell division, cell rearrangements or cell shape changes. Proper apical-basal and planar cell polarity are required to regulate the spatiotemporal behavior of the actomyosin network during morphogenesis. In *Drosophila*, Bazooka/Par3 (Baz) is one of the key regulators controlling both apical-basal and planar cell polarity.

In a protein interaction screen, we identified the LIM domain protein Smallish (Smash) as a binding partner of Baz. Mutation of *smash* affects the planar polarized localization of several actomyosin-associated proteins. *smash* null allele mutants show reduced membrane tension, whereas overexpression of Smash leads to apical constriction in epithelial cells, pointing to a function of Smash in regulation of actomyosin contractility. *smash* null mutant embryos show strong epithelial morphogenesis defects, consistent with a general reduction in cortical tension.

Although we have several lines of evidence demonstrating that Smash is functioning in a large multi-protein complex to control membrane tension via the actomyosin network, the precise mechanism of its function remains to be elucidated. To this aim different methods were combined. Smash interaction partners were identified by a proximity labeling technique. The results of these *in vivo* experiments were complemented by *in vitro* interaction studies in tissue culture cells. The biological relevance of these interactions was investigated by testing whether loss of Smash influences the subcellular localization of the identified binding partners and vice versa. In addition, a GFP knock-in into the *smash* locus via CRISPR/Cas9 was performed. The newly generated fly strain allows us to investigate the dynamics of endogenous GFP-Smash localization during epithelial morphogenesis *in vivo*.

Keywords: polarity, morphogenesis, actomyosin, bazooka, smallish

*Speaker

The PECAn image and statistical analysis pipeline identifies Minute cell competition genes and features

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Investigating organ biology often requires methodologies to induce genetically distinct clones within a living tissue. However, the 3D nature of clones makes sample image analysis challenging and slow, limiting the amount of information that can be extracted manually. Here we develop PECAn, a pipeline for image processing and statistical data analysis of complex multi-genotype 3D images. PECAn includes data handling, machine-learning-enabled segmentation, multivariant statistical analysis, and graph generation. This enables researchers to perform rigorous analyses rapidly and at scale, without requiring programming skills. We demonstrate the power of this pipeline by applying it to the study of Minute cell competition. We find an unappreciated sexual dimorphism in Minute cell growth in competing wing discs and identify, by statistical regression analysis, tissue parameters that model and correlate with competitive death. Furthermore, using PECAn, we identify several genes with a role in cell competition by conducting an RNAi-based screen.

Keywords: Cell competition, minute, image analysis, screen

^{*}Speaker

The adult *Drosophila* salivary gland exhibits an unusual mode of cell division

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Larval salivary glands of *Drosophila* are well known to exhibit polytene salivary glands formed via endoreplication and have been long used to study this process. In contrast, little is known of the development of adult *Drosophila* salivary glands except that they consist of a single layer, tubular epithelium that originates from a population of diploid cells found as an imaginal ring near larval salivary gland ducts. We have shown that the adult salivary glands contain three distinct epithelial domains, two of which are comprised of cuboidal epithelial cells and one of squamous epithelial cells. These cell types develop during the pupal period and after eclosion secretory cells develop extensive apical membrane invaginations. The junctional polarity of the epithelial cells exhibits an unusual change soon after eclosion as E-cadherin localisation migrates from a position apical to the septate junction to a more basal position. We have discovered that polyploid adult epithelial cells are not regenerated via mitosis, yet total cell numbers increase within 2 days of eclosion. By using genetic tools designed for the MARCM lineage tracing technique we have shown that the polyploid cells lose chromosomes during the division period and appear to be using amitosis as a mechanism to increase cell number. We have identified the first evidence for amitosis involvement in primary formation of a tissue and the adult *Drosophila* salivary gland will serve as a model for genetic analysis of this mode of division.

Keywords: salivary glands, amitosis, epithelium

*Speaker

The role of Asp during transit amplification of male germline stem cell progeny

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Tissue development and regeneration require cell proliferation. Several tissues achieve this by using a hierarchical system where resident stem cells divide asymmetrically to form a population of transit amplifying progenitor cells, which will ultimately differentiate. During *Drosophila* male gametogenesis, germline stem cells divide asymmetrically so that one of the daughter cells, the gonialblast, exits the stem cell niche and progresses through four rounds of transit amplification prior to ultimately undergoing meiosis. The transit amplifying cells complete mitosis but not cytokinesis, remaining as a synchronously dividing cyst from the 2- to 16-cell stage with cells interconnected by a fusome structure. Mitosis requires the formation of a mitotic spindle, which in *Drosophila* requires the function of a microtubule-associated protein called Abnormal spindle (Asp). Interrupted expression of *asp* or the human orthologue *ASPM* leads to microcephaly (small brains), but is also associated with reduced fertility. Our data show that *asp* point mutation leading to the abolishment of two neighbouring proline-directed Cyclin-dependent kinase 1 (Cdk1) phosphorylation sites results in fertility issues ranging to complete male sterility accompanied by reduced transit amplifying cell numbers and structural disorganization of the transit amplifying cysts. The *asp* mutant transgene was expressed ubiquitously in an *asp* CRISPR knockout background; however, no other defects such as microcephaly were observed. Cdk1 is best known as an important cell cycle kinase, although recent reports have expanded Cdk1 function to include direct roles in both microtubule regulation during spindle assembly and spermatogonial transit amplification. Our work aims to understand the mechanism by which Cdk1 could influence Asp function during transit amplifying mitoses of male spermatogonial cysts. In doing so this work will further our understanding of phospho-regulation of Asp during cell proliferation.

Keywords: Transit amplifying cells, Mitosis, Asp, Cdk1, spermatogonia

*Speaker

The role of nuclear factor NF-YA in maintaining intestinal homeostasis

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The intestine possesses high plasticity due to changes in environmental conditions. *Drosophila* midgut resizes in response to nutrient availability. The tissue size control is achieved through genetically determined processes like cell death, autophagy, cell rearrangements, and cell division. The role of Intestinal Stem Cells (ISCs) is known to be crucial to sustain cell composition due to dietary changes.

In this study, the role of nuclear factor Y α sub-unit (NF-YA) in ISC and tissue size regulation is investigated. NF-YA was found from a genetic screen to identify potential tissue and cell size regulators. Several recent studies have revealed NF-YA acting on vital cellular processes including cell division, apoptosis, and stemness maintenance. However, the role of NF-YA in ISCs and tissue resizing remains unclear.

Our results show that silencing of NF-YA leads to the accumulation of GFP marked clonal cells. Also, we observed increased cell number, as well as increased tissue size in the *Drosophila* midgut. Moreover, NF-YA downregulation influences ISC differentiation. Driving the NF-YA silencing in enterocytes (ECs) and enteroendocrine cells (EEs) leads to bigger cell size and the formation of progenitor cell clusters, suggesting also a cell non-autonomous regulation.

Taken together, these results suggest that NF-YA acts on cell turnover and ISC differentiation, thus affecting intestinal resizing. In the future research we aim to identify biological processes NF-YA is involved, and downstream targets mediating tissue and cell-specific effects.

Finding the role of NF-YA in ISCs will uncover novel molecular mechanisms regarding ISC regulation and their role in tissue size control.

Keywords: intestinal homeostasis, intestinal stem cells, tissue size

*Speaker

The role of the integrin complex in the coordination of tracheal and epidermal remodeling during *Drosophila* embryogenesis.

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During development, the rearrangement of different tissues depends on adequate communication between each other and on their coordination to form functional structures. Halfway during *Drosophila* embryogenesis, two lateral epidermal sheets stretch to fuse at the dorsal mid-line; concomitant with this, the main tubes of the respiratory system also shift towards the dorsal side of the embryo. What mechanisms coordinate these processes have not been studied but given that the tracheal system lies directly below the epidermis, it is possible that the behavior of both tissues is mechanically coupled. In this work, we study this using genetics and microscopy. We show that the main tubes of the tracheal system are attached to the epidermis through a layer of ECM. Also, we show that tracheal tube repositioning is highly coordinated with epidermal dorsal closure and production of extracellular matrix components. Perturbing integrin complexes in the tracheal system results in tracheal defects that persist until larval development but without affecting the coordination of both tissues during embryogenesis.

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Keywords: adhesion, tissue coordination, integrins, respiratory system, dorsal closure

*Speaker

The role of the microtubule network and the nuclear positioning in fat body cell migration

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Cell migration is involved in normal developmental and pathological processes. Migrating cells employ various modes of motility which require the different cytoskeletal components in a different manner. Adhesion-dependent migration is well-known to depend on actin polymerization and traction forces, while microtubules (MTs) transport secretory vesicles containing integrins and other membrane components to facilitate cell adhesion and protrusion formation (Bergert et al., 2015; Paluch et al., 2016). Other cells migrate in an adhesion-independent manner often termed "amoeboid-like migration" which in some cell types involves formation of uropods, a MT rich membrane protrusion at the rear of the cell (Serrador, 2009). MTs transport mitochondria into the uropod to promote migration (Campello et al., 2006). Additionally, in some migrating cells, MTs are necessary for cell polarization by positioning the nucleus at the rear (Calero-Cuenca et al., 2018; Friedl et al., 2011). Here, we use *Drosophila* pupal adipocytes, called fat body cells, as a model to study adhesion-independent, swimming migration by live-imaging. We find that in migrating fat body cells the nucleus along with its associated perinuclear microtubule organising centre, is positioned at the front of the cell. We focus further on the role of the microtubule network and the significance of the nuclear positioning during this mode of migration.

Keywords: cell migration, microtubules, nuclear positioning, fat body cells

*Speaker

The role of the novel genes *kkz* and *clw* in *Drosophila* tracheal system organogenesis

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The embryonic tracheal system of *Drosophila melanogaster* is formed by a network of interconnected epithelial tubes that branch inside the body to allow the distribution of oxygen to all tissues. Aimed at finding novel genes involved in subcellular lumen formation and branching, we focused on the tracheal terminal cells (TCs), which are specialized cells that form unicellular branches with a cytoplasmatic seamless tube called subcellular lumen. This lumen is produced by an extensive reorganization of the cytoskeleton and the growth of a new membrane which invaginates from the adjacent stalk cell while the TC is elongating. We analysed mutants from an EMS screen with mutant phenotypes in tracheal system development. From this, we selected one mutant that displayed a phenotype in tracheal TCs. This mutant showed extra TC branching at embryonic stages. We mapped the mutation to a region of chromosome 2 using the Bloomington Deficiency Kit. We confirmed the position of the mutation with complementation tests and identified a previously unidentified gene in *Drosophila*, which we named *kid kazoom* (*kkz*). The human ortholog of *kkz* encodes for a kinase. This kinase associates with a nuclease to form a complex involved in ribosomal RNA (rRNA) processing. In *Drosophila*, this nuclease is encoded by another novel gene which we termed *clowny* (*clw*). Here we characterize the role of *kkz* and *clw* during tracheal organogenesis. We establish that *kkz* and *clw* have a limited function during the embryonic tracheal formation, but they are crucial in larvae tracheal development. In *kkz* and *clw* tracheal knockdown experiments we observe severe defects in third instar trachea larvae which cause the death of the animal at this stage. Knockdown larvae also show signs of drastic hypoxia. Together, these results provide the first characterization of novel genes *kkz* and *clw* in *Drosophila*; as well as the first evidence of a direct link between *kkz*, *clw* and the development and growth of the *Drosophila* tracheal system.

Keywords: Tracheal system, ribosomal RNA processing, organogenesis, development, hypoxia

*Speaker

Tissue mechanics support intestinal regeneration in response to injury via the tracheal tissue

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The vasculature is an important component of adult stem cell niches. Stem cell crosstalk to the vascular endothelium is well characterised in many tissues. However, it remains largely understudied in the context of the intestinal stem cell niche. Our lab has recently uncovered a novel crosstalk between the *Drosophila* vasculature-like tracheal system and the intestinal epithelium whereby tracheal remodelling was necessary to drive intestinal stem cell regeneration following damage. It is well known that the mammalian vascular endothelium is a highly mechanosensitive tissue which responds to tissue intrinsic forces such as shear stress and matrix stiffness to drive angiogenesis in both developmental and adult contexts. Yet, mechanisms by which mechanical stimuli in the surrounding vascular microenvironment are sensed and transduced by the endothelium remains largely unknown. To further understand the role of intestine-trachea interaction in our system, we are studying the role and impact of intestinal tissue mechanics on its microenvironment where the gut is subject to significant mechanical stress. Using *in vivo* live imaging, automated image analysis and computational modelling, we have characterised the mechanical forces experienced by the intestinal epithelium in the contexts of regeneration and tumorigenesis and its impact on tracheogenesis. Moreover, we have observed upregulation and activation of the mechanosensitive ion channel, Piezo, in the tracheal tissue upon damage which is necessary to induce tracheal remodelling and intestinal regeneration. This biophysical approach allows us to better understand the importance of mechanical forces in controlling tissue regeneration and how this type of signal can be sensed and integrated by neighbouring tissues such as the trachea.

Keywords: piezo, mechanobiology, cancer, regeneration, stem cells, trachea, angiogenesis, intestine, ion channel, inter, organ communication, calcium

Tools for the live study of protein trafficking in *Drosophila* tissues

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Wnt pathway is one of the main signaling pathways required for development and homeostasis. Wnt proteins must be secreted into the extracellular space in order to trigger the signaling cascade. Regulation of Wnt protein secretion is therefore crucial for Wnt pathway activity. In order to study Wnt secretion in living tissue, we developed novel *Drosophila* tools. We endogenously tagged the Wg carrier protein Evi/Wls with the fluorescent proteins mScarlet and EGFP, as well as generated a Wg:mScarlet fly line based on the pre-existing Wg:EGFP fly line. Using these tools, we characterized the different fluorescence localization of proteins tagged with EGFP and mScarlet. We confirmed that the fluorescent tags do not affect Wg secretion, but that the difference in signal localization is due to the intrinsic properties of the fluorescent proteins. Additionally, we also adapted the LAMA (ligand-modulated antibody fragments) system, an acute protein trap-and-release system previously published in human cell culture, for use in *Drosophila*. The system was functional in wing discs and salivary gland cells, as well as allowed the trapping of both overexpressed and endogenous GFP-tagged proteins on the mitochondrial membrane and the endoplasmic reticulum (ER). The addition of the release molecule TMP triggered the relocation of the tagged proteins from the mitochondrial membrane into the cytoplasm. Using the LAMA system, we were able to show that the Wg accumulation induced by Rab7 overexpression appeared within sixty minutes of the Rab7 release.

Keywords: protein trap, and, release system, endogenous protein tagging, Wnt secretion, wing imaginal disc

*Speaker

Turning Off Autophagy: Unveiling the Final Players

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Cells in living organisms are dynamic compartments continuously responding to changes in their environment to maintain physiological homeostasis. While basal autophagy exists in cells to aid in the regular turnover of cellular debris, starvation-induced autophagy is a critical cellular response to nutritional stress. However, to prevent excessive cell damage and cell death, it is of utmost importance that cells also terminate autophagy, the knowledge of which is still scarce. Autophagy deregulation has been linked to several diseases such as neurodegeneration and cancer, which underscores the need for more research in this field. Hereby, we performed a high-content RNAi-mediated screen in *Drosophila* S2 cells using the DRSC FDA library of *Drosophila* orthologs of human genes encoding targets of FDA-approved drugs to unravel novel regulators of autophagy termination. Live cell imaging of tandem tagged RFP-GFP-atg8a, as an autophagosome marker, was used to monitor the autophagic flux in S2 cells during starvation and refeeding. Starvation induces the formation of RFP+ spots due to the fusion of autophagosomes with lysosomes for degradation and recycling of cargo; while refeeding results in GFP+ and RFP+ spots not participating in fusion events with lysosomes and hinting to a termination of the autophagic process. Monitoring atg8a flux over time in RNAi-treated cells uncovered potential hits such as an E3 ligase, Cullin3, as a regulator of autophagy termination. Results from S2 cells are validated in vivo using *Drosophila* fat body as a key sensor of the organism's nutritional status. Collectively, unraveling novel regulators of autophagy termination grants researchers better control over targeting the autophagic process for therapeutic purposes.

Keywords: Autophagy termination, RNAi, S2 cells screen

*Speaker

Understanding nuclear mechanics during cell migration in vivo

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Cell migration is an essential process in many biological events and migrating cells must navigate a complex and confined extracellular environment which is dependent on the ability of the cell to adapt to its surroundings. Nuclear deformation (ND) is a crucial step in cell migration as cells adapt to pore sizes smaller than themselves. Since the nucleus is the largest and most rigid structure of the cell it becomes a rate limiting factor in migration. Mechanisms behind nuclear deformation are still poorly understood, and many studies have utilised *in vitro* methods of compression and force application to replicate the *in vivo* environment. To unravel the molecular regulators of ND during confined migration this project aims to identify key nuclear envelope proteins regulating nuclear stiffness as well as the influence of force generation produced by the cytoskeleton using macrophage (hemocyte) migration during *Drosophila melanogaster* embryogenesis as a model. Our data shows that hemocyte migration through well-defined pathways occur under confinement and has captured ND in live time lapse imaging. Preliminary data shows distinctive differences in ND depending on the tissues surrounding the migration route. Additionally, using computational tools to quantify deformation by segmentation analysis, our data shows a transient change in expression of lamin Dm0, *Drosophila* lamin B homolog, under confinement suggesting a role for Lamin B in mediating nuclear deformation. To further confirm the role of lamin and other key LINC complex and cytoskeleton proteins involved in ND and translocation, genetic and biophysical tools will be utilised to disrupt expression of key proteins, estimate intracellular tension, and quantify effects on ND and migration. The combination of these techniques will elucidate the specific involvement of nuclear envelope and cytoskeleton proteins in the translocation and deformation of the nucleus during cell migration, potentially facilitating the understanding of migration, invasion and metastatic related diseases and identification potential therapeutic targets.

Keywords: Cell migration, nuclear deformation, mechanosensing

*Speaker

Unraveling cellular mechanisms of tumor-induced tissue wasting

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Approximately 80% of advanced cancer patients suffer from cancer cachexia syndrome which is characterized by systemic inflammation, metabolic reprogramming, and organ wasting of muscle and adipose tissue. Studies conducted using larval and adult *Drosophila* models of cancer cachexia have revealed multiple tumor-derived regulators of muscle and ovary atrophy, including Bnl (FGF), Pvf1 (VEGF/PDGF), Gbb (BMP) and IMPL2 (dILP antagonist). We previously demonstrated that systemic autophagy execute organ wasting and nutrient mobilization of amino acids and sugar to the circulation and that nutrients derived from host organs constitute the main building blocks for tumor growth. The objective of this study is to further investigate how cachectic tumors induce host organ response and wasting that reciprocally affect tumor growth. To this end, we have developed a QF/QUAS-RasV12, scribIR tumor model that allows for organ-specific genetic screening using tissue specific GAL4 drivers. Based on the RNAseq analysis of dissected muscle and adipose tissues during cachectic wasting, we identified differentially expressed genes in the muscle or adipose tissue that were selected for our targeted RNAi screening. Through our fat body-specific RNAi screen we have uncovered several hits that suppress fat body wasting and/or tumor growth. Among these hits are previously known factors such as autophagy genes (atg1 and atg5), and components of the JAK-STAT signaling pathway, as well as several novel factors. These preliminary data allow us to further dissect the complex tumor-host reciprocal interactions during cancer cachexia.

Keywords: Cancer cachexia, organ wasting, two component genetic tool, RasV12 scribble, RNAi screening

*Speaker

Unravelling the Morphodynamics of the Mesenchymal-Epithelial Transition in Embryonic Midgut Development Using Single-Cell Transcriptomics

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Loss and gain of the epithelial character, including polarity, adhesion and barrier function is the feature of many tissues during development, remodeling, and regeneration as well as pathologies such as metastasis formation in cancer. In contrast to the epithelial-mesenchymal transition (EMT), when polarity and adhesiveness is lost, the gain of epithelial features during a mesenchymal-epithelial transition (MET) has received comparatively little attention and is not well understood. We analyze the MET during embryonic midgut development in *Drosophila*, when a signal from the underlying visceral mesoderm triggers MET in the endodermal cells, involving Laminin and Integrin. The complete molecular mechanism of cell polarization, remodeling of the cytoskeleton and junction formation in midgut development is still unclear. To understand this mechanism, we have been focusing on the transcriptional program of MET using single-cell RNA sequencing (scRNA-seq) to capture the heterogeneity of cell states. By analyzing nearly 800 midgut precursor cells, we have generated a full-term transcriptome atlas of MET. Employing trajectory inference methods, we ordered cells along distinct paths and established time courses of development (termed pseudotime). Directionality was established by RNA velocity analysis which considers the ratio of spliced and unspliced transcripts. We established a population of cells at the root of the trajectory expressing high levels of *Notch*, *Delta*, *forkhead*, and *DnaseII* and likely representing the primordial mesenchymal precursors. The cell population at the end of the trajectory are characterized by expression of smooth septate junction components, including *Ssk*, *mesh*, *Tsp2A*, and *hoka*, as well as typical gut enzymes such as trypsin and maltase. Most interesting for us are the cells in the medial part of the trajectory, named as transient cells. These transient cells are characterized by downregulated stem cell markers and yet low levels of differentiation markers. We detected several signaling molecules upregulated like *fra*, *Ama* and *drl* whose counterparts are expressed in the visceral mesoderm. In conclusion by inferring gene expression profiles, we revealed new insights into the transcriptional dynamics underlying a MET, which could have implications for understanding MET in other organisms and, more importantly, in cancer.

Keywords: single cell RNA sequencing, mesenchymal epithelial transition, midgut development

*Speaker

Versatile ncMTOC organization during spermatogenesis

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Gamma Tubulin Ring Complex (γ -TuRC) is known as the main player for microtubule nucleation in MTOC. Proteins involved in the formation and development of MTOC in *Drosophila* are conserved among eukaryotes. Mutations of many MTOC proteins are known to cause diseases in humans. Centrosomes undergo massive changes during spermatogenesis, as they turn into basal bodies, which nucleate and stabilize microtubules of the axoneme.

We aimed to study the γ -TuRC distribution and function during the late stages of *Drosophila* spermatogenesis. γ -tubulin exists in two complexes in *Drosophila*: γ -Tubulin Small Complex (γ -TuSC) and γ -TuRC. We conducted a phylogenetic analysis and identified three testis-specific γ -TuRC proteins. γ -TuSC is represented by t-Grip84 and t-Grip91 a paralogue of Grip84 and Grip91 respectively, the third one is t-Grip128 a paralogue of Grip128. This suggests the existence of testis-specific γ -TuRC (t- γ -TuRC).

Analyzing the phenotype of t- γ -TuRC mutants we found that *t-Grip84* mutant is male sterile, *t-Grip91* mutant is male semi-sterile and *t-Grip128* mutant fertility is normal. We made transgenic lines and checked the localization of the t- γ -TuRC proteins. They localize to the centriole adjunct after meiosis, and the nuclear tip during nuclear elongation, then to the surface of the mitochondria during cyst elongation. We proved the binding of the t- γ -TuRC to γ -Tubulin, Mzt1 and each other biochemically. We describe the interaction of several basal body components with different γ -TuRC proteins and also prove the presence of ubiquitous γ -TuRC in the alternative MTOCs of the post-meiotic spermatids. Our results can lead us to understand better the molecular composition of the different MTOCs during the late stages of spermatogenesis and in the matured sperm.

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Keywords: MTOC, spermatogenesis, γ , TuRC, testis

*Speaker

Xport-A functions as a chaperone by stabilizing the first five transmembrane domains of Rhodopsin-1

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Rhodopsin-1 (Rh1), the main photo-sensitive protein of Drosophila, is a seven-transmembrane domain protein, which is inserted co-translationally in the endoplasmic reticulum (ER) membrane. Biogenesis of Rh1 occurs in the ER, where various chaperones interact with Rh1 to aid in its folding and subsequent transport from the ER to the rhabdomere, the light-sensing organelle of the photoreceptors. Xport-A has been proposed as a chaperone/transport factor for Rh1, but the exact molecular mechanism for Xport-A activity upon Rh1 is unknown. Here, we propose a model where Xport-A functions as a chaperone during the biogenesis of Rh1 in the ER by stabilizing the first five transmembrane domains (TMDs) of Rh1.

Keywords: Rhodopsin, 1, transmembrane domain protein, Drosophila, Xport

*Speaker

Yki is a tumor suppressor in the squamous epithelium of adult *Drosophila* male accessory gland

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The differentiated squamous cells of disparate lineages in *Drosophila* and mammals display permanent nuclear localization of YAP/Yki-the transcription co-factor of the Hippo signaling pathway. While abnormal activation of YAP often culminates in most epithelial cancers, why only select squamous cell carcinogenesis (SCC), such as mammalian lung SCC, exhibited loss of YAP-related carcinogenesis, remains unresolved. Here we show that nuclear Yki in the squamous epithelium of the male accessory gland (MAG) represses growth and functions as a tumor suppressor. Loss of Yki, instead of gain, promotes cell growth, subsequently resulting in SCC of the adult MAG. Moreover, the emergence of Yki loss-driven MAG SCC can profoundly initiate cachexia-associated early death of affected host adults. Our findings reveal a paradoxical role of nuclear Yki that negatively regulates the TOR pathway in the adult MAG, in contrast to the positive co-relation of Yki-TOR axis seen in most epithelial tissues. Parallely, loss of Yki signaling in adult MAG also aggravates non-apoptotic caspases, contributing to abnormal cell growth and subsequent SCC. Inhibiting the activation of PI3K-Akt-Tor signaling or caspases delays the onset and progression of MAG SCC induced by Yki loss. Overall, our results not only elucidate the tumor suppressor role of YAP in specific types of squamous cells but also provide insights into the distinct regulation of cell growth in these squamous epithelia.

Keywords: Yorkie, Squamous Cell Carcinogenesis, Tumor suppressor

*Speaker

eIF4F activity is specifically required to maintain stem cell self-renewal in the *Drosophila* testis downstream of JAK/STAT

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Stem cells maintain tissue homeostasis by balancing self-renewal and differentiation. Research to date has focused mostly on transcriptional and epigenetic regulation of stem cell fate. However, recent work has identified a discrepancy between transcriptomes and translomes in both stem cells and their daughter cells, indicating that there is post-transcriptional control of stem cell identity. We use the *Drosophila* testis as a model to study how the regulation of mRNA translation can influence stem cell fate. The testis niche, called the hub, secretes the Upd ligand to activate JAK/STAT signaling in surrounding stem cells and support self-renewal. Two stem cell populations reside at the niche: Germline stem cells (GSCs) which give rise to sperm and somatic cyst stem cells (CySCs), which generates somatic cyst cells that encapsulate germ cells and support their development. Here we focus on the mechanisms controlling CySC self-renewal, asking whether the control of mRNA translation plays a role or not.

mRNA translation is regulated mostly at the point of initiation, which is controlled by several eukaryotic initiation factor (eIF) complexes. Eukaryotic initiation factor 4F complex (eIF4F) can bind to the mRNA 5'cap and recruits the 43s ribosome, which is comprised of the small ribosome subunit and other initiation factors, including eIF3, eIF5 and others. The recruitment of the 43s ribosome to mRNA by eIF4F is mediated by eIF4F-eIF3 interaction. We found that eIF4F is specifically required for CySC self-renewal but not differentiation. Loss-of-function of any eIF4F subunit in CySCs leads to rapid differentiation. In contrast, loss-of-function of other eIFs results in a block in differentiation, demonstrating that specific initiation factor complexes have distinct roles in maintaining CySC identity. Epistasis experiments suggest that eIF4F acts downstream of JAK/STAT pathway, the main signaling pathway regulating CySC self-renewal.

Interestingly, loss-of-function of a subunit of eIF3, eIF3d1, leads to a similar self-renewal defect as eIF4F loss instead of phenocopying other loss of eIF3 subunits and other eIFs. Previous studies found that eIF3d1 can promote eIF4F-eIF3 interaction, thus promoting cap-dependent translation initiation, depending on its phosphorylation state, suggesting that eIF3d1 could be modulated to change translation initiation during stem cell differentiation. We are actively testing this hypothesis, and exploring whether JAK/STAT regulates eIF4F, through the regulation of eIF3d phosphorylation, to maintain stem cell self-renewal.

In sum, our results indicate that translation initiation directly influences stem cell fate in the testis and demonstrate that translation initiation can be modulated to induce differentiation.

*Speaker

Keywords: stem cell, mRNA translation, JAK/STAT

Methods

A simple MiMIC based approach for tagging endogenous genes to visualize live transcription in vivo

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The precise temporal and spatial control of gene transcription is essential for the correct development of an organism. Transcription is a dynamic process which occurs in bursts of activity. Over the last 10 years the study of transcriptional bursting has rapidly advanced due to the development of techniques to visualise and measure this process in both fixed and live cells. Excitingly these techniques have allowed us to watch and measure transcription live in a developing organism. By inserting bacteriophage derived repetitive stem-loop sequences (MS2 or PP7) into the endogenous locus of a gene, the nascent transcription of that gene can be visualised with high temporal resolution at a single cell level. As *Drosophila* is a highly tractable model system, many gene tagging strategies already exist to assess protein expression and localisation. However a method for visualising nascent transcription at scale is lacking. The Minos-mediated integration cassette (MiMIC) transposon based tagging system, developed by the Bellen lab, can be utilised to tag endogenous genes. Here we present a new set of fly stocks that we have developed that can be used with the existing MiMIC library of insertions that target thousands of genes. With an easy crossing scheme, recombination mediated cassette exchange allows insertion of repetitive stem-loop sequences into any MiMIC containing gene locus in order to study the dynamic transcription of that gene in its endogenous context *in vivo*. We have made a variety of loop donor lines, including 24xMS2, 128xMS2, 24xPP7 and 24xMS2V6, and new complementary fluorescently tagged coat protein stocks to provide versatility dependent on the experimental context. I will present preliminary data using this gene tagging system to study dynamic transcription during development in the early embryo and in the ovary.

Keywords: Transcription, live imaging, MiMIC

*Speaker

Bright fly: model system to study somatic mobilization of mariner transposable element

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Transposable elements (TEs) are mobile DNA sequences. TE mobilization in somatic cells is associated with cancer and other aging-related diseases. Monitoring TE mobilization in somatic cells remains, however, a challenge due to their repetitive nature, and only a few systems and methods are available for this, each with several limitations. Our aim is to investigate somatic mobilization with spatial and temporal detail during animal development and aging. For this, we are engineering a system, the "Bright fly" model, which consists of a transgenic *Drosophila melanogaster* that reports somatic mobilization of an active and widespread TE, *mariner*, at the cellular level by activating watermelon (a cistron of plasmamembrane-targeted green fluorescent protein and nuclear-targeted mCherry). This should allow detection of tissue-specific and age-dependent excision events by fluorescence microscopy. For this, we have used two different *mariner* elements: *Mos1*, an autonomous element that can mobilize itself, and *peach*, a non-autonomous element that requires the *Mos1* element to mobilize. To monitor *Mos1* excision, we generated a reporter by placing the *peach* element in between an Actin 5c (Act5c) promoter and the watermelon coding sequence. In contrast to a control uninterrupted Act5c-watermelon construct, the construct carrying the *peach* element completely blocks watermelon expression in transfected *Drosophila* DL2 cells. This also shows that *peach* is not able to self-excise itself at detectable levels in cell culture. To test if *peach* is mobilized when a *mariner* transposase source is provided in *trans*, as has been reported, we generated a plasmid where the transposase-positive *Mos1* element is driven under its own promoter sequences. *peach* mobilization would allow watermelon expression from the Act5c promoter. However, *Mos1* and *Act5c-peach-watermelon* co-transfection experiments did not reveal detectable watermelon expression. This could be due to insufficient *Mos1* mRNA expression or disruption of the Act5c-watermelon cassette upon *peach* excision (e.g., by mutation or inefficient repair of the *peach* plasmid). To start testing these possibilities, we are generating new plasmids and testing, for instance, if increased *peach* mobilization levels can be achieved by the overexpression of transposase induced either by the GAL4-UAS system or by heat shock, as *mariner* is activated by higher temperatures. Following the demonstration of a successful in vitro system, we will generate transgenic flies to verify the mobilization of *mariner* in different tissues and ages. As *mariner*-like elements (aka Tc1/mariner class elements) are found in many animals, including humans (where they are known as Hsmar1/2 subfamilies), a better understanding of the timing and tissue-specificities

*Speaker

of *mariner* mobilization events may contribute to our understanding of age-related diseases associated with TE mobilization, such as cancer and neurodegenerative diseases.

Keywords: mariner, transposable elements, somatic mobilization, *Drosophila*

DIGITtally – An Unbiased Tool for Systematic Specificity Meta-Analyses

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Drosophila melanogaster has one of the deepest research bases within the life sciences, with a wealth of high-quality tissue- and cell type-specific transcriptomic data available. Intuitively, this lays an excellent foundation for interrogating the transcriptional programs underpinning cell-type specific biological functions. However, integrating large datasets derived from disparate sources is not trivial. We have designed a broadly applicable solution to this problem in the form of the **D**rosophila **I**nteresting **G**enes in **I**ndividual **T**issues-tally, or DIGITtally, system. Freely available on the web (www.digittally.org), DIGITtally is highly customisable and completely hypothesis-free, allowing meta-analysis of gene expression patterns across the *Drosophila* research space, along with analysis of expression pattern conservation in other species. We have applied DIGITtally to a pertinent question within entomology – that is, whether a specific pattern of gene expression underlies the transporting activity of specific epithelial tissues (an "epitheliome"). By using DIGITtally to survey gene expression throughout the tissues comprising the *D. melanogaster* alimentary canal (salivary gland, midgut, tubules, and hindgut), we identified a set of genes which may comprise this epitheliome. This gene list supports the existence of an evolutionarily conserved system for epithelial proton transport. Furthermore, functional analyses of several of the genes identifies suggests a crucial role in structure and function across the studied tissues.

Keywords: Gene Expression, Tools, Online, MetaAnalysis, FlyCellAtlas, FlyAtlas

*Speaker

Eight principal chromatin states functionally segregate the fly genome into developmental and housekeeping roles

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Different chromatin forms, or states, represent a fundamental means of controlling developmental gene regulation. Chromatin states have been studied through either the distribution of histone modifications or, more rarely, via the occupancy of chromatin proteins. However, these two approaches fundamentally disagree on the nature and composition of active chromatin states, and modelling chromatin via both histone marks and chromatin proteins has been lacking.

In order to resolve this, we used Targeted DamID to profile up to 15 separate chromatin modifying proteins cell-type specifically in 4 different developmental cell types. We developed a new technique, ChromaTaDa, to profile two histone modification marks cell-type-specifically; and also took advantage of existing DamID and ChIP-seq profiling of chromatin proteins and histone marks in 3 additional cell types. In total, our dataset combines chromatin proteins and histone marks from seven separate cell types.

Here, combining protein and histone mark binding data with new chromatin state modelling routines, we show that chromatin in *Drosophila melanogaster* is organised into eight principle chromatin states that have consistent forms and constituents across cell types. These states form through the association of the Swi/Snf chromatin remodelling complex, Polycomb Group (PcG)/H3K27me3, HP1a/H3K9me3 or H3K36me3 complexes with either active complexes (RNA Pol/COMPASS/H3K4me3/NuRF) or repressive marks (histone H1 and nuclear lamin occupancy).

Importantly, these eight chromatin states explain the biology of downstream regulatory and developmental processes. Enhancers, core promoters, transcription factor motifs, and gene bodies all show distinct chromatin state preferences that are separated by developmental and housekeeping/metabolic gene ontology. Within the 3D genome, chromatin states add an additional level of compartmentalisation through self-association of topologically associated domains (TADs) of the same state.

Our results suggest that the epigenetic landscape is organised by the binding of chromatin remodellers and repressive complexes, and that through chromatin states the genome is fundamentally segregated into developmental and housekeeping/metabolic roles.

*Speaker

Keywords: chromatin, epigenetics, development, transcription, systems biology, gene regulation, transcription factors, genome biology

Expanded and Improved *Drosophila* Modular Binary Gene Expression Tools

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The ability to reproducibly target expression of transgenes to small, defined subsets of cells is a critical experimental tool for understanding many biological processes. Here, we have developed a new *Drosophila* vector series - pBID2 - with significant enhancements over prior constructs designed to improve gene expression levels and ease the production and analysis of transgenic manipulations. pBID2 is designed for phiC31 targeted transgene integration and features insulator sequences to ensure specific and uniform expression in addition to a DSCP (*Drosophila* Core Synthetic Promoter) to reduce unwanted ‘leaky’ expression. To add versatility, pBID2 constructs are available for three binary systems - Gal4/UAS, LexA/LexOp and QF/QUAS. Thanks to these new vectors, we created new genetic tools, such as nucleus-membrane markers, strong activators for motor neuron expression and most importantly, a novel hybrid transcription factor, QF:G4, which enables regulation of QF dependent gene expression under temporal or spatial regulation by GAL80, allowing simultaneous coordinated regulation of both UAS and QUAS transgenes

Keywords: cloning vectors, phiC31, Gateway, motor neurons, binary system, Gal4, Lex, QF, Gal80

*Speaker

Identification and characterization of GAL4 drivers that mark distinct cell types and regions in the *Drosophila* adult gut

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The adult *Drosophila* gastrointestinal tract provides an excellent model system for studying various mechanisms, including digestion, absorption, excretion, stem cell plasticity, and inter-organ communication, particularly through the gut-brain axis. It is also valuable for investigating cellular and adaptive responses to dietary changes, alterations in microbiota and immunity, as well as systematic and endocrine signals. However, limited tools are currently available to target and manipulate specific cell types and regions within the gastrointestinal tract and modulate their gene expression.

We reported a collection of 353 GAL4 lines, along with several split-GAL4 lines, that exhibit expression in enteric neurons (ENs), progenitors (ISCs and EBs), enterocytes (ECs), enteroendocrine cells (EEs), and potentially other unidentified cell types in distinct regions of the gut. To begin, we initially identified approximately 600 GAL4 lines with potential gut expression based on RNA sequencing data. Subsequently, we performed immunohistochemistry by crossing these lines with UAS-GFP to selectively identify those expressed in the gut. Currently, we have annotated 3,267 images from 992 GAL4 and split-GAL4 lines.

This comprehensive dataset, accessible through the Korea *Drosophila* Resource Center (KDRC) website at <http://kdrc.kr/index.php>, provides information on cell types and regional expression patterns associated with the entire set of GAL4 drivers and split-GAL4 combinations. By utilizing this GAL4 resource, researchers can precisely target specific populations of distinct cell types within the *Drosophila* gut, enabling a more accurate investigation of the gut cells involved in regulating important biological processes.

Keywords: Kgut, KDRC, Gut Gal4

*Speaker

Improved CRISPR systems for the generation of highly penetrant loss-of-function phenotypes

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CRISPR nucleases generate a broad spectrum of mutations, including undesired editing outcomes that attenuate phenotypes and complicate experimental analysis. To address this issue, we have developed two novel CRISPR strategies for inducing loss-of-function phenotypes with high penetrance in vivo. First, we set up a cytosine base editing system for gene inactivation through predictable C-to-T editing. We find that the deaminase domain, temperature, expression level and DNA repair background are critical parameters for the efficiency, tolerance and precision of base editing. Using an evolved CDA1 domain we achieve near homogenous biallelic gene inactivation, due to its predictable editing outcome, high efficiency, and minimal byproduct formation. In a second approach, we developed a system that allows for higher-order sgRNA multiplexing using the RNA processing activity of Cas12a. This leads to the frequent generation of larger deletions that are more likely to disrupt gene function. By quantitatively comparing CRISPR-induced phenotypes across many targets, we demonstrate that this system is superior to a previously generated Cas9-based system in revealing loss-of-function phenotypes. Our work significantly improves the ability to generate precise loss-of-function alleles in *Drosophila*.

Keywords: CRISPR, gene editing, functional genomics

*Speaker

LarvaTagger: manual and automatic tagging of *Drosophila* larval behavior

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As more behavioral assays are carried out in large-scale experiments on *Drosophila* larvae, definitions of the stereotypical actions of a larva are regularly refined. In addition, the video recording and tracking technologies constantly evolve. As a consequence, automatic tagging tools for *Drosophila* larval behavior need to be retrained to learn new representations from new data. Existing tools lack the ability to transfer knowledge from the large amounts of previously accumulated data.

We introduce LarvaTagger, a piece of software that combines a pretrained deep neural network for stereotypical behavior identification, with a graphical user interface to manually tag the behavior and train new automatic taggers with the updated ground truth.

We reproduced qualitative results from a popular tagger with high accuracy. We demonstrate that pretraining on large databases accelerates the training of a new tagger, achieving similar prediction accuracy using fewer data.

All the code is free and open source. Docker images are also available. See <https://gitlab.pasteur.fr/nyx/LarvaTagger>

Keywords: larva, crawl, roll, bend, hunch, deep neural network

*Speaker

Linking spatial transcriptomics and tissue morphogenesis

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During development, the formation of functional tissues and organs entails tissue shaping or morphogenesis. Morphogenesis is modulated by genetic expression profiles and signaling pathways activities. However, tools that allow a systematic exploration of genetic factors controlling morphogenesis are lacking. To explore the role of gene expression in morphogenesis, we present a novel spatial transcriptome reconstruction method. We apply our method to the development of *Drosophila* dorsal thorax (notum) at the onset of its morphogenesis. We validate that the method allows for spatial transcriptome characterization and the identification of novel gene expression patterns. Using non-negative matrix factorization as dimensionality reduction and clustering approach, we decompose the spatial transcriptome in meta-regions. We then correlate these meta-regions with signaling pathways activity. This analysis, with the quantitative description of the morphogenetic properties at the scale of the tissue (e.g. cell area, rate of cellular proliferation, tissue deformation) allows us to study how gene expression controls cellular properties and morphogenesis.

Keywords: Spatial transcriptomics, Epithelial Morphogenesis, Machine learning

^{*}Speaker

Probing the fragile X syndrome in *Drosophila* using a new platform for circadian rhythm and sleep research with videography

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Circadian rhythms (CR) are impaired in many diseases in humans, and these impairments can be monitored and used to study disease prognosis, treatment, and help develop therapeutic approaches. We aimed to model the effects of fragile X syndrome on CRs in *Drosophila melanogaster*. Fruit flies with *dfmr1* mutation (mimicking the fragile X syndrome) have impaired CRs. CRs are studied in *Drosophila* majorly with infrared-based beam-crossing methods, which may lead to moderate to severe under-estimation of the total activity of flies throughout the day, which may, in turn affect estimation of critical CR and sleep statistics, whereas continuous video monitoring methods provide higher resolution spatial timeseries data. We have integrated a commercial video acquisition system (Zantiks MWP) with an open-source, highly customized version of VANESSA for CR and sleep data acquisition, analysis, and visualization. In our preliminary studies, we have used *w1118* (wt) and *dfmr1B55* mutant flies to accurately measure their locomotor activity rhythms measured as distance traveled every 30 seconds, under light-dark cycles of 12 hours (LD12:12) for 3 days, and under constant darkness (DD) for 3 days. We analyzed DD periodicity in these flies using autocorrelation (AC) and continuous wavelet transformation (CWT) methods as employed in VANESSA. We successfully detected significant rhythmicity using both methods in wild-type and mutant flies, albeit the powers of the rhythms detected using both methods were low, and we hope to improve this significantly in next iterations of experiments with > 5 days DD data, and more methods (chi-square and lomb-scargle periodograms). Overall, we observed a trend of lower periodicity, lower power of the rhythm, and higher percentage of arrhythmicity in the mutant flies, but the differences were not statistically significant, which can be attributed to the lower amount of data under DD. With our system, we can also analyze sleep patterns in these flies, and interestingly, we detect

*Speaker

considerably more daytime sleep in the mutant flies than wt flies under LD12:12. We believe that our system provides an improved method for CR and sleep studies using videography. The incorporation of an already available and affordable commercial unit for data acquisition and an open-source software for CR and sleep studies in disease models will ensure that the entry barrier for biologists from various facets of science to CR and sleep research is minimal, and hopefully, be of broad appeal to the clock and sleep community.

Keywords: circadian rhythm, fragile X syndrome, dfmr1 mutant, videography

Proteome-wide mutational landscape prediction in *Drosophila melanogaster*

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Motivated by the large-scale assessment of protein sequence variation's impact on ageing in *Drosophila melanogaster*, we have developed a high-throughput workflow for the accurate proteome-wide mutational landscape prediction. Our pipeline relies on an evolution-based model (GEMME, E.Laine et al. MBE 2019) leveraging information extracted from multiple sequence alignments generated by the newly developed ColabFold protocol (M.Mirdivta et al. NatMet 2022).

To validate the approach, we compared our predictions against a large set of Deep Mutational Scanning assays, which involved the analysis of over 1.5 million missense variants across 72 protein families (ProteinGym P.Notin et al. PMLR 2022). We demonstrate the quality and the efficiency of the pipeline in our paper (Abakarova et al. GBE 2023, in revision).

Furthermore, we applied this protocol to the entire *D.melanogaster* proteome, comprised of approximately 30 000 protein isoforms, and generated its proteome-wide mutational landscapes in just 1.5 days using MeSU-supercalculator (hpcave.upmc.fr/index.php/resources/mesu-supercomputer/).

We validated the accuracy of our predictions on a thousand EMS-induced single nucleotide polymorphisms (SNPs) annotated on FlyBase as associated with developmental lethality. We observe an overall agreement, 80% of mutations are classified as deleterious using a Gaussian Mixture Model.

Our intention is to establish a public resource that will provide the *Drosophila* community with access to these valuable mutational landscapes, allowing for facilitated identification of genome-edition targets. We hope to facilitate further research, and promote a deeper understanding of the genotype-phenotype relationship for *Drosophila* as well as for other model organisms.

Keywords: genomics, protein mutations, genotype, phenotype relationship

*Speaker

Quantitative analysis of dynamic cellular structure Interplanar Amida Network during pupal wing development

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To generate proper tissues and complex 3D organs, cell shape must be adapted to sustain overall tissue morphogenesis. However, current knowledge is limited to understand how successive changes of cellular structures are coupled with dynamic tissue morphogenesis. The emerging cellular structure Interplanar Amida Network (IPAN) provides a framework for exchanging signaling information and coordinating mitosis among the two layers as the 3D pupal wing forms. Our observations reveal the following: the IPAN consists of vertical protrusions composed of microtubules (MTs) and microfilaments (MFs) and lateral filopodia-like structures; the vertical protrusions derive from dorsal and ventral epithelial cells, meeting midway between the two epithelia; the IPAN is dynamic, undergoing both programmed disassembly and bundling; mitotic cells are observed after disassembly of the IPAN; and dynamics of the IPAN involve changes in MT organization. This presentation introduces a quantitative analysis of the IPAN dynamics to understand the molecular mechanisms behind the cellular dynamics of the IPAN. By employing a 5D imaging protocol (the x, y, and z dimensions, as well as the t (time) and λ (wavelength) dimensions) for pupal wings, we carry out in vivo live imaging by using *Drosophila* pupal wing expressing α Tubulin:GFP and pericentriolar material (PCM) marker Centrosomin (Cnn):mCherry to track dynamics of the IPAN and mitotic cells in dorsal and ventral epithelia. α Tubulin is useful for capturing MT protrusions of the IPAN. Cnn accumulates at the centrosome only during mitosis and serves as a tool to observe mitotic cells. Furthermore, by combining conditional manipulation of gene expression in wing compartment specific manner with in vivo live imaging, we can investigate the physiological significance of the IPAN-mediated morphogenesis.

Keywords: epithelia morphogenesis, live imaging, cellular protrusion, three dimensional architecture, microtubule dynamics, non centrosomal microtubule organizing center

*Speaker

Revealing the 3D ultra-structure of the yolk and the connections to the blastoderm in fruit fly embryos by Expansion Microscopy

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During early *Drosophila* development, the embryo is a moving layer of cells (the blastoderm), sandwiched between a rigid vitelline envelope and the yolk. Time series imaging with light sheet microscopy suggests that there is a connection between these three layers as structures in close proximity move together or against each other. This observation corroborates previous studies relating the role of actomyosin constriction rings during cellularization. In order to understand the exact nature of these connections, we need both high resolution imaging of ultra-structure as well as the ability to identify specific constituents. Electron microscopy (EM) offers the possibility for high resolution characterization of tissues and organism scale reconstructions have recently been demonstrated. However, in the context of imaging whole *Drosophila* embryos at multiple stages, EM is a low throughput technique and the staining is unspecific. Expansion Microscopy (ExM), in combination with NHS-Ester staining and immunolabelling, offers the unspecific EM-like contrast required to highlight arbitrary structures of interest in a volume as well as the correlative specific staining. Using our methods, we are able to acquire 3D isotropic volumetric data at organelle resolution over entire embryos, using confocal and light sheet microscopy, without the need for sectioning. This enables us to study the structure of the yolk, which generally has a low complexity in terms of protein composition. We show that the organization of the yolk changes during embryogenesis. We further demonstrate that the yolk vesicles and glycogen granules are connected to the cells of the blastoderm by the syncytium before gastrulation and by a complex network spanning the entire yolk at gastrulation, hence there is a dynamic structured interface between the yolk sac and the blastoderm.

Keywords: Expansion Microscopy, Electron Microscopy, Light Sheet Microscopy, Yolk, Embryo

*Speaker

Revealing the ultra-structure of the muscle sarcomere by combining DNA-PAINT super-resolution with nanobodies

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Skeletal muscles, responsible for movement and force generation in animals, are packed with contractile sarcomeres. Sarcomeres are the repeating structural and functional units of the muscles and are composed of a super assembly of proteins and protein filaments. One of the main sarcomeric proteins is titin, the largest protein in humans and spans half the sarcomere length from its Z-disc to the M-band. Other proteins include Zasp52 and α -actinin at the Z-disc and obscurin at the M-band. While muscle contraction is rather well understood, the ultra-structure of sarcomeres and in particular how the sarcomere is built during development is still relatively unknown. Here, we used the indirect flight muscles of *Drosophila melanogaster* as a model. We combined the super-resolution technique DNA-Point Accumulation in Nanoscale Topology (DNA-PAINT) with oligo-labelled nanobodies specific against sarcomere protein domains to study how different proteins and their domains are arranged in the sarcomere with nanometer precision. We focused on the two titin homologs called Sallimus and Projectin and the Z-disc proteins Zasp52 and α -actinin in mature sarcomeres. We found that Sallimus and Projectin are linearly arranged in the sarcomere in a staggered organization: Sallimus N-terminus begins at the Z-disc and spans across the entire I-band reaching the myosin filament at a distance of about 90 nm from Z-disc; Projectin covers the first 260 nm of the A-band and its N-terminus overlaps with the C-terminus of Sallimus over a small 10 nm region at the end of the I-band/beginning the A-band. We find that Zasp52 and α -actinin are both located in a region of about 40 nm centered on the Z-disc, defining the area at which actin filaments are cross-linked and likely the Sallimus N-terminus is anchored. These results confirm that we can achieve super-resolution below 5 nm precision in a dense tissue as the muscle. Our future aim is to compile a complete molecular map for the mature sarcomere and investigate earlier developmental stages to understand how sarcomere proteins assemble to form the periodic ordered structure.

Keywords: Muscle development, sarcomere, titin, Super resolution, DNA PAINT

*Speaker

Spatial lipidomics in the larval brain using OrbiSIMS imaging

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Environmental stresses such as nutrient restriction or hypoxia can lead to fetal growth restriction. It is well established, however, that growth decreases less in the CNS than in other organs, an effect known as brain sparing (Gruenwald, 1963 PMID: 14081642). The molecular mechanisms underlying brain sparing are not yet fully understood. We previously demonstrated that the *Drosophila* larval brain recapitulates many of the features of mammalian brain sparing (Cheng et al., 2011 PMID: 21816278; Bailey et al., 2015 PMID: 26451484; Lanet et al., 2013 PMID:23478023). To investigate how brain sparing changes metabolism in the larval brain, we have been developing ambient temperature and cryogenic workflows for mass spectrometry imaging using our recently developed OrbiSIMS instrument (Parsarelli et al., 2017 PMID: 29131162; Newell et al., 2020 PMID: 32603009). OrbiSIMS provides high lateral and mass resolution simultaneously, enabling metabolite imaging at a near-cellular level. In this study, we use OrbiSIMS to map the localizations of more than 100 polar and apolar metabolites in the larval CNS. We also conduct a spatial lipidomics survey of the effects of environmental stresses upon larval CNS metabolism.

Keywords: *Drosophila* larval brain, metabolite imaging, OrbiSIMS, SIMS, lipidomics

*Speaker

Towards Tissue-specific Labelling of Glycoproteins in *Drosophila melanogaster*

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Intercellular communication is vital to all multicellular organisms because it regulates and coordinates the behaviour of individual cells. However, methods to study intercellular communication in a cell-type specific manner are limited and mainly rely on *ex vivo* studies which lack the complexity of these interactions in whole organisms. The Schumann laboratory has developed a method termed Bio-orthogonal Cell-specific Tagging of Glycoproteins (BOCTAG) which labels proteins via one of the most abundant post-translational modifications: glycosylation. Cells are equipped with an artificial biosynthetic pathway to activate and incorporate chemically modified sugars onto proteins. These can then be chemically tagged using click chemistry, allowing for the detection by imaging *ex* or *in vivo* or by proteome analysis. A wide range of proteins can be tagged using this method, including intracellular and cell-membrane proteins as well as secreted proteins. Only cells that express the artificial biosynthetic pathway are able to glycosylate proteins using the chemically modified sugars. Therefore, secreted proteins can be traced back to the cells that produced them. Herein, we outline the use of BOCTAG to tag glycoproteins in cell culture as well as ongoing work to implement BOCTAG *in vivo* using *Drosophila melanogaster* as a model system. Extensive knowledge of the *Drosophila* genome combined with numerous well-established methods for targeted transgene expression with temporal tissue- or cell-specificity make *Drosophila* an ideal model system. We hope to use BOCTAG in combination with the UAS/GAL4 system to map the secretome of a subset of cells of interest.

Keywords: Glycosylation, Bioorthogonal Cell specific Tagging of Glycoproteins (BOCTAG)

*Speaker

Using a combination of novel research tools to understand social interaction in fruit flies

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Fragile X syndrome (FXS) is caused by a full mutation in the *FMR1* gene and is the most common monogenic cause of inherited intellectual disability and autism spectrum disorder. Disruption in social behaviors and impaired social interactions (SIs) are distinctive characteristics of FXS. Deficits in SI are also typical for the FXS model *Drosophila*, where the only ortholog of human *FMR1* (*dFMR1*) is mutated. The aim of this study was to investigate and compare SI in the two different *Drosophila* strains wild type *wt118* and mutant *dFMR1B55* using (i) the new SI chamber (SIC) and (ii) the novel open-source library in Python (NetworkX) for analysis of SI in *Drosophila*. All experimental groups (30 flies and both sexes) were recorded for 15 min. The new design of SIC (Maze Engineers) restricted the flies to a shallow volume of space, forcing all behavioral interactions to take place within a monolayer of individuals. Complex network-based analysis of extracted data was performed using NetworkX library in Python. Our results demonstrate that: (i) locomotor activity is significantly reduced in *dFMR1B55* mutants compared to *wt118* ($p < 0.001$); (ii) *dFMR1B55* flies achieve a significantly lower total number of interactions than *wt118* ($p < 0.001$); (iii) the average number of interactions per fly is lower in *dFMR1B55*; (iv) the average duration of SI is shorter in *dFMR1B55* than in *wt118*. Based on the heatmap generated using the Matplotlib library in Python, the highest frequency of *wt118* interactions took place centrally in the chamber, while *dFMR1B55* flies tended to interact closer to the edge of the chamber. In conclusion, using a combination of a new SIC and a new new data processing pipeline built with Python, we confirmed that impaired SI is present in *dFMR1B55* mutants. We also demonstrated that this combination of tools can be useful in assessing potential drug effects on SI in fruit flies.

Keywords: FragileX syndrome, dFMR1, FXS model, social interaction

*Speaker

sgRNA structural constraints and genetic limitations for efficient Cas9 genome editing to generate knock-outs

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Single guide RNA (sgRNA) directs Cas9 nuclease for gene-specific scission of double-stranded DNA. High Cas9 activity is essential for efficient gene editing to generate gene deletions and gene replacements by homologous recombination. Here we describe constraints in sgRNA design originating from maintaining the secondary structure of the sgRNA. Correct folding is essential for high-efficiency DNA scission of Cas9, but aberrant with about 50% of sequences adjacent to PAM sites in *Drosophila*. We developed an sgRNA design tool (PlatinumCRISPR) to evaluate base-pairing for optimal design of highly efficient sgRNAs for Cas9 genome editing. Applied to generate gene deletions in *Drosophila Ythdc1* and *Ythdf*, that bind to *N*6 methylated adenosines (m6A) in mRNA we further discovered, that generating small deletions with sgRNAs and Cas9 leads to ectopic reinsertion of the deleted DNA fragment elsewhere in the genome. These insertions, however, can be removed by standard genetic recombination and chromosome exchange.

Keywords: sgRNA design CRISPR/Cas9 genome editing

*Speaker

Disease models

A *Drosophila* model for TMEM43-induced cardiomyopathy (ARVC type 5)

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Dysfunction of the heart is one of the most common causes of death in the Western world. An inherited form of heart malfunction is arrhythmogenic right ventricular cardiomyopathy (ARVC). A highly aggressive form of ARVC is subtype 5, caused by a p.S358L mutation in the ER/SR transmembrane protein TMEM43/CG8111. Male carriers of the mutation have an extremely high risk of dying from sudden cardiac death between the ages of 20-40. Together with human geneticists, we decipher the molecular basis of this cardiomyopathy, using *Drosophila* and human cells as models in a translational approach. We will provide evidence, that TMEM43/CG8111 regulates energy homeostasis in mitochondria via ER-mitochondrial contact sites. TMEM43 may interact directly or indirectly with one of the major ion channels of mitochondria, VDAC, and controls membrane potential and accessibility of stored energy. Reference: Klinke, N., Meyer, H., Ratnavadivel, S., Reinhardt, M., Heinisch, J.J., Malmendal, A., Milting, H. and Paululat, A. (2022) A *Drosophila melanogaster* model for TMEM43 related Arrhythmogenic right ventricular cardiomyopathy type 5. Cellular and Molecular Life Sciences (Cell Mol Life Sci), 79(8):444. DOI: 10.1007/s00018-022-04458-0

Keywords: cardiac, heart, cardiomyopathy, contact sites

*Speaker

A monocarboxylate transporter rescues frontotemporal dementia and Alzheimer's disease models

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Brains are highly metabolically active organs, consuming 20% of an organisms' energy at resting state. A decline in glucose metabolism is a common feature across a number of neurodegenerative diseases. Another common feature is the progressive accumulation of insoluble protein deposits, it's unclear if the two are linked.

Glucose metabolism in the brain is highly coupled between neurons and glia, with glucose taken up by glia and metabolised to lactate, which is then shuttled via transporters to neurons, where it is converted back to pyruvate and fed into the TCA cycle for ATP production. Monocarboxylates are also involved in signalling, and play broad ranging roles in brain homeostasis and metabolic reprogramming. However, the role of monocarboxylates in dementia has not been tested.

Here, we find that increasing pyruvate import in *Drosophila* neurons by over-expression of the transporter *bumpel*, leads to a rescue of lifespan and behavioural phenotypes in fly models of both frontotemporal dementia and Alzheimer's disease.

The rescue is linked to a clearance of late stage autolysosomes, leading to degradation of toxic peptides associated with disease. We propose upregulation of pyruvate import into neurons as potentially a broad-scope therapeutic approach to increase neuronal autophagy, which could be beneficial for multiple dementias.

Keywords: pyruvate, FTD, C9orf72, transporter

*Speaker

A novel in vivo tool mimicking Fabry disease in *Drosophila melanogaster*

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Fabry disease is a X-linked lysosomal storage disease caused by a defect in the enzyme α -galactosidase A (aGAL). This defect results in an aberrant hydrolysis of terminal α -galactosyl moieties from glycolipids and glycoproteins and subsequently in the intracellular and progressive accumulation of globotriaosylceramide (GB3 and other glycosphingolipids), which leads to a multi-systemic disease phenotype. Affected tissues and cells are various, including kidneys, heart, vessels and the nervous system, as almost all cells present with an accumulation of GB3. Symptoms are as manifold as the affected tissues and cells and comprise nephropathy, cardiomyopathy, depression, pain crises as well as phenotypes observed in eyes and ears, which can manifest early during childhood and progress during adulthood. Although treatments successfully reduce GB3 accumulation, existing organ and cellular damage cannot be reversed. Recent studies suggest that additional mechanisms are involved in Fabry disease and explain the inefficiency of therapies. A limitation in the identification and characterization of novel pathways is the lack of suitable in vivo tools to mimic Fabry disease. The model organism *Drosophila* could prove as a novel and advantageous tool for Fabry research. Here, we performed a comprehensive morphological and functional phenotyping of Fabry flies, analysing several organs and tissues. We confirmed expression of CG7997 (a-GAL) in different fly organs. Using CG7997 KO flies, we were able to show a kidney phenotype, including a severe nephrocyte filtration defect and formation of zebrafly bodies. CG7997 KO flies also presented with a neurological phenotype as stereotaxis and nociception were significantly altered. We also observed impaired development and fertility in CG7997 KO flies. Assessment of the heart function in the KO flies confirmed a mild phenotype, reflected by arrhythmia. Here, we describe a novel in vivo model to mimic Fabry disease, which phenocopies the majority of symptoms observed in patients.

Keywords: Fabry disease, nephrocyte, heart

*Speaker

A quantitative model of sporadic axonal degeneration in the *Drosophila* visual system

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In human neurodegenerative diseases, neurons undergo axonal degeneration before they die. Therefore, for potential intervention and to better understand early phases of neurodegeneration, defining the initiation of axon damage is of great importance. Invertebrate models, have significantly contributed to our understanding of neurodegenerative disorders. However, these models mainly rely on manipulation of genes identified in familial cases of neurodegenerative diseases. Nonetheless, the vast majority of cases of neurodegenerative diseases are thought to be sporadic. We developed a system modelling early degenerative events in *Drosophila* adult photoreceptor cells, in which mild constant light stimulation for several days overcame the intrinsic resilience of R7 photoreceptors and led to progressive axonal degeneration in the absence of cell death. Aged flies displayed an accelerated and increased vulnerability in this system and loss of synaptic integrity between R7 and its postsynaptic partner preceded axonal degeneration, thus recapitulating important features of human neurodegenerative diseases. Furthermore, we defined precisely the time window in which the axonal damage becomes irreversible. We will present our ongoing work towards a dissection of the cellular circuit mechanisms involved in the early events of axonal degeneration, allowing for a better understanding of how neurons cope with stress and lose their resilience capacities.

Keywords: Sporadic axon degeneration age

APP-Like Cleavage and β -Amyloid-Sensitive Protein Aggregation Drive Dense-Core Granule Biogenesis in *Drosophila* Secondary Cells

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APP-like (APPL) is the *Drosophila* homologue of human Amyloid Precursor Protein (APP), the parent molecule of β -amyloid ($A\beta$). Proteolytic cleavages in and around the transmembrane portion of APP, release peptides of varying lengths, including $A\beta$, which can oligomerise and then aggregate into amyloid plaques in Alzheimer's Disease (AD). Almost 400 known autosomal dominant mutations in APP affect $A\beta$ expression or structure, resulting in Familial AD (FAD). Plaque formation can occur years after the onset of cognitive impairments, suggesting that other $A\beta$ -mediated events might initiate disease. APP has physiological roles in secretion, but the molecular mechanisms involved are poorly understood. Here we show that *Appl* is expressed in *Drosophila* secondary cells (SCs), prostate-like secretory cells of the male accessory gland that contain large secretory compartments harbouring dense-core granules (DCGs), which store aggregated proteins prior to secretion. This aggregation event is dependent on Midline Fasciclin (MFAS), the homologue of human amyloidogenic protein TGF- β -induced (TGFBI). The process involves rapid fusion of many highly motile, MFAS-containing mini-cores formed at the limiting membrane of secretory compartments. Knockdown of *Appl* prevents normal DCG biogenesis by suppressing mini-core motility, a phenotype that can be rescued by human APP expression. We show that in SCs, APPL protein is trafficked to the limiting membrane of DCG compartments and the intraluminal vesicles (ILVs) inside them, from where APPL's extracellular domain (ECD) is released and packaged into DCGs. Knocking down β -secretase, which cleaves off the ECD and is implicated in $A\beta$ processing, suppresses normal DCG formation and targets some compartments for degradation. Furthermore, overexpression in SCs of $A\beta$ -peptides involved in AD, also suppresses the motility of mini-cores, blocking normal DCG formation. Finally, by expressing different APPL deletion mutants in which the ECD cannot be released from membranes, we show that APPL can act as a primer for MFAS mini-core aggregation at the limiting membrane of compartments, but its cleavage is required to form a mature DCG and prevent large-scale lysosomal degradation of compartments. We conclude that in SCs, DCG biogenesis is controlled by an APPL-, and membrane-dependent protein aggregation event, which involves the cross-talk between homologues of two human amyloidogenic proteins. This process is disrupted by several genetic manipulations associated with AD and can lead to both abnormal secretion

*Speaker

and lysosomal degradation defects. We propose that this cleavage-associated physiological role for APPL/APP might be affected during the earliest stages of AD.

Keywords: Male Accessory Gland, Secondary Cells, DenseCore Granule(DCG), Tumour Growth Factor Beta Induced(TGFBI), Midline Fasciclin(MFAS), Amyloid Precursor Protein(APP), Amyloid Precursor Protein Like(APPL), β Amyloid, Protein Aggregation, Alzheimer's Disease(AD)

Activity Based Protein Profiling (ABPP) as a tool to map orphan Serine Hydrolases involved in the *Drosophila* innate immune response

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The serine hydrolase (SH) superfamily is the largest functional enzyme-class in all forms of life, and consists of proteases/peptidases, lipases, and carboxylesterases and involve many pathophysiological processes. We have earlier (*Kumar et. al., Biochemistry* 60:p1312) demonstrated that *Drosophila* encodes 354 SH genes and have mapped an SH developmental-activity atlas, in the fly life-cycle, using a mass spectrometry-based chemical proteomics (ABPP, activity-based protein profiling) platform. In this study, we use ABPP to identify novel SHs that are enzymatically active during *Drosophila* host defence, in response to septic injury. Further, using the well-characterized loss-of-function mutant lines available in the canonical Toll/NFkB and Imd/NFkB pathways, we uncover several novel immune-responsive SHs that have previously not been implicated in these signal transduction pathways. In my poster, I will describe immune-responsive SHs, highlight their sexually dimorphic behaviour and show mechanistic data for two SH genes in the immune response, based on gain-of and loss-of-function genetics. Since many genes/proteins involved in the immune response are conserved from flies to mammals, orthologous SH genes are expected to have similar roles in human host defence.

Keywords: Serine Hydrolase, Activity Based Protein Profiling, Immune response, Sexual Dimorphism, Toll Signalling, *Drosophila melanogaster*

*Speaker

Age Dependant Dynamics of Neuronal VAPB(P58S) Inclusions in the adult brain in a *Drosophila* model of Amyotrophic Lateral Sclerosis 8

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Amyotrophic Lateral Sclerosis (ALS) is a relentlessly progressive and fatal disease, caused by the degeneration of upper and lower motor neurons within the brain and spinal cord in the ageing human. The dying neurons contain cytoplasmic inclusions, linked to the onset and progression of the disease.

In this study, we use a *Drosophila* model of ALS8 (*VAPP58S*) to understand the modulation of these inclusions in the ageing adult brain. The adult *VAPP58S* animal shows progressive deterioration in motor function till its demise by 25 days post-eclosion. In line with the motor dysfunction, the density of VAPP58S-positive brain inclusions increased by ~20% between 5 to 15 days of age. In contrast, with the addition of a single copy of *VAPWT* in the *VAPP58S* animal, the inclusions decreased by ~50% in the same period. The presence of *VAPWT* clears inclusions and suppresses all phenotypes associated with the disease. Concomitantly, unlike in the *VAPP58S* animal, where ER stress, a major contributing factor in disease, increases with age, ER stress decreases with the age-dependant decrease in VAP aggregate density.

In a previous study (Chaplot et. al., 2019), we uncovered a suppression of proteasome-mediated degradation as the primary mechanism for the retention of inclusions in the larval brain, which in turn could be relieved by increasing ROS levels, either by reducing SOD1 activity or by suppressing TOR signaling. In the adult brain, we find that the proteasomal clearance of VAPP58S is not the dominant mechanism and that clearance of inclusions is instead regulated by autophagy. Intriguingly, TER94 in neurons appears to play a major role in the clearance of VAPP58S inclusions with Caspar (Tendulkar et. al., 2022), a TER94 interactor and a suppressor of glial-mediated inflammation not having a significant neuronal role.

Our study sheds light on the complex regulatory events involved in the neuronal clearance of ALS8 aggregates, suggesting a context-dependent switch between proteasomal and autophagy-based mechanisms. A deeper understanding of the nucleation and clearance of the inclusions, which in turn modulate ER stress, is essential for understanding the initiation and progression of ALS.

Keywords: Amyotrophic Lateral Sclerosis, VAPB, TER94, Autophagy, Proteosome, Inclusion,

*Speaker

Clearance

An exploration of cellular dysfunctions induced by SARS-CoV-2 nonstructural proteins in *Drosophila*

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Like cell stress, cellular senescence is a pathologically critical fallout of SARS-CoV-2 infection. However, its causal origin from the viral gene(s) remains elusive. Here, we aimed to decipher the candidate SARS-CoV-2 genes in a reductionist approach by using *Drosophila* as a model system. We particularly focused on SARS CoV-2 nonstructural gene that induces a host of cellular stress and senescence. We found that expression of SARS-CoV-2 gene in *Drosophila* larval imaginal disc epithelia induces a diverse range of cell stress: oxidative stress, DNA damage, autophagy, and pro-inflammatory cytokines. Crucially, SARS-CoV-2 gene expressing epithelia also displayed senescence-associated β -galactosidase, a hallmark of cellular senescence, while the squamous cells of adult male accessories gland (MAG) showed a premature loss of tricellular junctions and epithelial barrier-telltale signatures of aging epithelia. In both cell stressed and senescent epithelia, we noticed a gain of signaling pathways: its knockdown reversed both these cellular pathologies. Our results reveal a causal underpinning of a hitherto intractable pathology of COVID-19 besides revealing SARS-CoV-2 gene and signaling pathways as potential therapeutic targets for arresting cell stress and senescence of SARS-CoV2 infected cells.

Keywords: SARS, CoV, 2, cellular stress, Senescence, ROS, DNA damage, *Drosophila*

*Speaker

Analysing the regulation of the *Drosophila* MAST kinase homologue Drop out in *Drosophila melanogaster*

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The *drop out* (*dop*) gene encodes the single homologue of the MAST kinase family in *Drosophila melanogaster*. The human MAST kinases are associated in intracellular transports and in a variety of diseases like breast cancer, rabies infections and cystic fibrosis (Robinson *et al.*, 2011; Terrien *et al.*, 2012; Ren *et al.*, 2013), but their regulatory mechanisms are unknown yet. Besides the *Drosophila* MAST homologue Dop is associated in intracellular trafficking and is expressed during the whole embryogenesis. However, *dop* mutants start to show a phenotype during cellularisation, where the midblastula transition peaks (Hain, 2010). Concerning the ubiquitous presence of Dop during the development and the delayed appearance of a phenotype, the Dop kinase has to be activated at a specific time during embryogenesis. This time dependent activation of Dop gives rise to the question regarding the way of its regulation and considering Dop as the single MAST homologue in *Drosophila*, regulation studies of Dop may give a further insight into the regulation of the human MAST kinases.

Keywords: Cellularisation, Protein domain analysis, Kinase, Live imagings

*Speaker

Between benefit and harm – the effect of antibiotics-induced mitochondrial stress on innate immune responses

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Because mitochondria fuel and supply resource-intensive immune pathways and moreover contribute to immune signaling, immune competence hinges on mitochondrial performance. In accordance with the hormetic principle low-dose mitochondrial stress seems to stimulate immune competence, whereas high-dose mitochondrial stress evidently undermines it. It is assumed that artificial environmental conditions are the most rapidly developing source of mitochondrial stress, which is exemplified by the ongoing global increase in the use of antibacterial drugs. Antibiotics are canonically used to support host immune resistance, and therefore immediately relevant to immunometabolism. But antibiotics may moreover exert strictly host-dependent effects by directly impairing the function of bacteria-derived mitochondria as off-targets in addition to the intended bacterial targets. These effects are implicated in ambivalent health outcomes and have been linked to immunomodulation. In this project we investigate the impact of antibiotic drugs on *Drosophila* mitochondrial immunometabolism. The aims of this project are to a) characterize the relation between drug-induced mitochondrial stress and immune competence, b) determine the influence of mitochondrial gene x gene x environment interactions on immune competence and drug sensitivity. Flies are reared on food medium supplemented with different doses of focal antibiotic drugs to investigate the impact of drug exposure on egg-to-adult development and larval and adult phenotypes indicative of systemic mitochondrial dysfunction. Moreover, young adult flies are infected with bacterial pathogens to determine the impact of drug-induced stress during development on infection outcomes. By performing experiments on a panel of flies with different mitochondrial-nuclear genotypes we probe the impact of certain nuclear- and mitochondrial-encoded genetic variants. Preliminary results suggest that antibiotics may induce dose-dependent developmental delay, influence body size and the composition of circulating hemocytes.

Keywords: Antibiotics, Mitohormesis, Stress, Infection, Genetic Variation

*Speaker

Brain Inflammation Triggers Macrophage Invasion Across the Blood-Brain Barrier in *Drosophila*

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The brain is well protected by the blood-brain barrier (BBB) from the external environment. However, inflammation, infection as well as neurodegeneration can cause the invasion of blood or hemolymph resident macrophages over the BBB into the CNS by yet unknown mechanisms. Recently, it was shown that *Drosophila* glial cells and not neurons are able to mount an inflammatory response to infection of the CNS which triggers the invasion of macrophages. This invasion was shown to be mediated by an upregulation of the PDGF/ VEGF related factor 2 (Pvf2). Here, we further elucidate how the signaling of macrophage infiltration is mediated and how Pvf2 is able to mediate this invasion. For this, we followed Pvf2 upregulation in response to immunity induction by different genetic approaches. We found that Pvf2 is mainly located at glial cells of the BBB upon an immunity induction. However, downregulation of the only known corresponding receptor for Pvf2 within glial cells and macrophages did not impact the infiltration. In addition to that, we were able to show that integrity of the lamella of the CNS plays an important role during the infiltration, as the knockdown of matrix metallo proteinases altered the infiltration process significantly. Taken together, we could show that Pvf2 must act externally of the brain to attract macrophages, while the glial cells of the BBB play an important role in the infiltration of macrophages by manipulating the integrity of the lamella.

Keywords: Blood Brain Barrier, Immune response of CNS, Hemocytes, Immune Deficiency Pathway

*Speaker

Cigarette Smoke Exposure influences airway morphology, transcriptome and fitness in *Drosophila melanogaster* with *ORMDL* and *Scca1* dysregulation

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Background: The development of asthma is influenced by an interaction of genetic and environmental factors. *ORMDL3* and *Scca1* are genes involved in asthma pathology and cigarette smoke (CS) exposure is a risk factor for wheeze and asthma. We therefore asked how fitness, the airway morphology, and transcriptome of *D. melanogaster* larvae with airway specific over-expression (OE) or knock-down (KD) of the fly homologs *dORMDL* and *Spn43Aa* are modified by CS exposure.

Methods: Fly lines with KD or OE of *Spn43Aa* and *dORMDL* were generated using the Gal4-UAS system. Gene-modified larvae and genetic controls were exposed to CS or air on 3 consecutive days (3 puffs/min for 1 h). Fitness parameters like the survival of adult flies and the crawling distance of larvae were observed. The morphology of airways, including epithelial thickness and total number of terminal cells was analyzed by microscopy. Data were analyzed by 2-way ANOVA. One day after CS, airways were isolated for RNASeq (Illumina, NextSeq500) and analysed using *DESeq2*(1.36.0).

Results: The lifespan of adult males was significantly reduced by OE of *Spn43Aa* and *dORMDL* and further decreased by CS ($p < 0.001$ - < 0.0001). KD of *dORMDL* resulted in thickening of the epithelium after CS, but only in females ($p < 0,0095$). Larvae with *Spn43Aa* OE had more terminal cells than genetic controls, which was further increased by CS. Larvae with *dORMDL* OE had a reduced crawling distance after CS exposure ($p=0,0102$). RNASeq data showed no effect of CS exposure in WT flies, but additional gene dysregulation of *dORMDL* flies altered regulation in genes related to immunology metabolism and proteolytic processes. The response to CS was stronger in males than in females.

Conclusion: We propose that *Spn43Aa* and *dORMDL* are important in regulating the morphology of airways, while fitness depends on both CS exposure and genetic factors. To further

*Speaker

characterize the effect of *Spn43Aa* and *dORMDL* on airway development we will now investigate the airway epithelial cell proliferation and cell integrity.

Keywords: Asthma model, Smoking, *Scca1*, *ORMDL*, RNASeq

Circadian Dysfunction in a *Drosophila* Model of Amyotrophic Lateral Sclerosis

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Behavioural changes like circadian rhythm (CR) dysfunction and disruption in the sleep-wake cycle are common features of most neurodegenerative diseases. Whether these behavioural changes are a cause, or an effect of neurodegeneration and their underlying molecular mechanisms remain to be understood. Amyotrophic Lateral Sclerosis (ALS) which causes progressive loss of motor neurons is one such human neurodegenerative disease with more than 30 causative gene loci being identified. Human vesicle-associated membrane protein-associated protein B (hVAPB); an endoplasmic reticulum (ER) membrane protein is coded by the 8th familial ALS locus identified. We have developed a CRISPR/Cas9-engineered *Drosophila* model of ALS with a proline to serine mutation in the hVAPB fly homolog, *dVAP33A/dVAPB*. Our *Drosophila* model phenocopies the disease showing reduced lifespan and progressive age-related motor deficits in the mutant (*dVAPBP58S*) flies. Our study shows that *dVAPBP58S* flies have a disrupted sleep-wake cycle and become arrhythmic with age. Introduction of a single copy of wildtype VAPB can rescue motor defects, the sleep-wake cycle as well as CR. Interestingly, RNAi mediated knock-down or overexpression of *dVAPB* or *dVAPBP58S* in molecular clock neurons can modulate CR. In this poster we further describe roles for the dVAPB: Caspar: TER94 (Tendulkar et. al., 2022) complex in regulating CR function and dysfunction. The CRISPR-generated *dVAPBP58S* disease model appears to be beneficial for a better understanding of the molecular mechanisms connecting neurodegeneration and behaviour.

Keywords: Circadian rhythm, Amyotrophic Lateral Sclerosis, Vesicle associated membrane protein associated protein B, CRISPR/Cas9, *Drosophila*

*Speaker

Circadian clock disruption promotes the degeneration of dopaminergic neurons in *Drosophila melanogaster*

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Circadian clocks are endogenous time-keeping mechanisms with the near-24h period, which underlie cellular, physiological and behavioral rhythms of the organism. Sleep and circadian rhythm disruptions are frequent comorbidities of Parkinson's disease (PD), a disorder characterized by the progressive loss of dopaminergic (DA) neurons in the substantia nigra. Although sleep/circadian disturbances can be observed years before diagnosing PD, it remains unclear whether circadian clocks have a causal role in the degenerative process. We demonstrated here that circadian clocks regulate the rhythmicity and magnitude of the vulnerability of DA neurons to oxidative stress in *Drosophila melanogaster*, especially in the protocerebral anterior medial (PAM) cluster. Since PAM neurons do not contain circadian clocks, these results indicate that clock neurons directly or indirectly modulate the vulnerability of PAM neurons. Indeed, circadian pacemaker neurons are presynaptic to a subset of DA neurons and rhythmically modulate their susceptibility to degeneration. The arrhythmic *period* (*per*) gene null mutation exacerbates the age-dependent loss of PAM neurons. Additionally, this neuronal death is reversed by the rescue of *per* in different clock neurons clusters. These findings suggest that circadian clock disruption promotes dopaminergic neurodegeneration in *Drosophila melanogaster*.

Keywords: Circadian rhythm, Parkinson's Disease

Connecting Cachexia, Host Physiology, and Microbiota

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Cachexia is a complex metabolic disorder characterized by progressive organ wasting that is strongly associated with a bad prognosis in cancer patients. Traditionally, cachexia studies focused on tumor-autonomous effects such as inflammation and nutrient exploitation. However, using an adult *Drosophila* cancer model, we found that cachexia is also regulated systemically by gut microbiota. Specifically, tumor-injected flies reared in conventional (microbiota-associated) condition exhibited serious degeneration of the muscle and adipose tissue, both hallmarks of a cachectic metabolic switch. Strikingly, this cachexia phenotype was restored by antibiotics treatment. The analysis of microbiota over time after injection showed a significant dysbiosis associated with cancer progression, distinguished by an overabundance of *Escherichia*. By inoculating various non-pathogenic microbiota strains to cancer flies, we found specific strains displaying a systemic interaction with the tumor for cachectic induction. In cachectic flies, lipid allocation was compromised with accumulating lipid droplets in the gut and depleted from the fatbody. Gene expression analysis suggested that cholesterol, a core component for lipid transport, is compromised in conventional cancer flies. Taken together, our data suggest that microbiota contributes to the pathogenesis of cachexia by impairing lipid allocation.

Keywords: Cancer cachexia, Microbiota, Lipid allocation, Systemic effect, Metabolism

*Speaker

Death by Ferroptosis: A key element and a therapeutic avenue in *Drosophila* models of Friedreich Ataxia

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Friedreich's Ataxia (FRDA) is the most prevalent autosomal recessive ataxia in the European population (1:50000). The disease is caused by the reduced expression of frataxin, a mitochondrial protein involved in iron-sulfur cluster biogenesis. Deficiency of frataxin leads to a drastic reduction in the cellular energy production. Remarkably, the characterization of the type of cell death that affects the cells deficient for frataxin still remains unsolved. Several studies in cell culture models point towards apoptosis. However, no marker of apoptotic cell death has been observed in *in vivo* models. A very attractive possibility is a new type of cell death named as ferroptosis. Deregulation of iron metabolism, depletion of glutathione and accumulation of lipid peroxides are the major hallmarks of ferroptosis. Remarkably, these three molecular signatures have been detected in disease models including *Drosophila melanogaster*, suggesting that loss of frataxin recapitulates ferroptotic cell death.

In agreement, we failed to detect any evidence of apoptosis in our frataxin deficient flies and observed increased levels of ferroptosis markers such as iron-metabolism genes, lipid regulators.... Moreover, frataxin-deficient flies are more sensitive to ferroptosis induction. Indeed, we have observed worsening of longevity, a drastic reduction of locomotion and enhancements of neurodegeneration and cardiac features along with an increased generation of lipoperoxides and a further impairment of mitochondrial function upon pharmacological (erastin, buthionine sulfoximine (BSO) or genetic (downregulation of GPTx1, the fly ortholog of Glutathione Peroxidase 4) induction of this type of cell death. Remarkably, inhibition of ferroptosis either by increasing cysteine levels by means of N-acetylcysteine-derived compounds or reducing lipid peroxidation by means of overexpression of fly or mouse Glutathione Peroxidases greatly improved fly locomotion, brain degeneration or mitochondrial energy production, among others. Similarly, downregulation of lipid biosynthesis also showed beneficial effects.

Our results might suggest ferroptosis inhibition as a therapeutic possibility for Friedreich's Ataxia.

*Speaker

Keywords: Disease model, Ataxia, Neurodegeneration, mitochondria, treatment, ferroptosis

Decoding the molecular and cellular mechanisms mediating tumor/stroma crosstalk.

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Normal mesenchymal cells undergo reprogramming by aberrant cancerous cells, acquiring tumorigenic properties. Consequently, these mesenchymal cells contribute to tumor growth and metastasis through reciprocal signaling. While extensive research has focused on identifying the mutations driving cancer initiation, the understanding of the reciprocal signaling orchestrated by mesenchymal cells remains limited. To address this knowledge gap, we employed a *Drosophila* tumor model that relies on the crosstalk between genetically modified epithelial cells and normal host mesenchymal cells. Specifically, we overexpressed the EGFR pathway in the epithelial compartment (Ap-Gal4) of *Drosophila* wing imaginal discs within an epigenetically compromised context, known as the EGFR-psq model 1,2. By utilizing this model, we aimed to unravel the reciprocal signaling events orchestrated by mesenchymal cells and their impact on tumor development.

We investigated the involvement of Secreted Protein, Acidic, Rich in Cysteine (SPARC) in the interplay between mesenchymal and epithelial tumors. Our findings demonstrate a high expression of SPARC protein in mesenchymal cells, with residual levels detected in neighboring epithelial cells. Utilizing Single molecule FISH (smFISH) against *sparc*, we exclusively identified its transcription in mesenchymal cells. These observations suggest that in EGFR-psq tumors, SPARC is predominantly expressed by mesenchymal cells and potentially secreted towards adjacent cancerous epithelial cells.

To explore the functional role of SPARC in tumor growth, we specifically depleted it in the mesenchyme using the LexA system. Strikingly, we observed a prevention of growth in both epithelial and mesenchymal cells upon SPARC depletion, highlighting its dual role in tumor development. Subsequently, we investigated the diffusion dynamics of SPARC through FRAP analysis. Our data revealed a complete turnover of SPARC within an epithelial cell group after photobleaching ($t_{1/2}$: approximately 20 minutes). Collectively, these results indicate that SPARC functions as a feedback mesenchymal signal that can be exploited to drive growth in both tissue types. Currently, we are actively investigating the mechanistic aspects of SPARC-mediated tumors/stroma crosstalk

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*Speaker

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Keywords: Drosophila, SPARC, cell, cell signaling, inter, tissue signaling, tumor, tumor, stroma interaction

Development of Drosophila model system for studying mutation Arg109Ter in human gene MED11 and Glu133Del in human gene MED22.

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Recently, a homozygous MED11 C-terminal variant, causing a lethal neurodegenerative disease, was described (Cali E. et al., 2022, Genet Med).

The mediator (MED) is a multi-subunit protein complex evolutionarily conserved in the animal kingdom. Human MED11 and MED22 have clear single orthologs with a high rank in Drosophila. The binding partners of MED11 and MED22, MED28 and MED30, whose interactions with MED11 and MED22 might be affected by the MED11(Arg109Ter) and MED22(Glu133Del) mutations, also have clear single Drosophila orthologs. According to the protein sequence homology between the human and Drosophila proteins, introducing the terminal codon in position 105 (Glu105Ter) in Drosophila MED11 and deletion of the codon in position 136 (Glu136Del) are most appropriate to model the human mutation in these genes.

Gene editing was performed using the CRISPR/Cas9 technology. The resultant mutant is under investigation now.

Keywords: dGao, CRISPR/Cas9, neurological disorders, epilepsy

*Speaker

Dietary protein intake influences cell proliferation and survival in *Drosophila* intestinal tumor model

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Intestinal tumors are among the most common cancers worldwide, and like many other tumor entities, show an unsatisfactory prognosis necessitating the development of safe and highly effective therapies. Different nutritional regimens have been found to have a strong impact on tumor cells. Therapeutically relevant nutritional interventions include caloric restriction and dietary restriction. Caloric restriction is characterized by a moderate reduction in daily caloric intake, while dietary restriction usually refers to a specific reduction in the protein content of the diet. Both interventions can increase lifespan in various organisms. In the present study, we established an intestinal tumor model by targeting ectopic expression of an oncogene to intestinal stem cells (ISC) of the fruit fly *Drosophila melanogaster*. In this project, we used this intestinal tumor model to assess the effects of different concentrations of dietary protein on cell proliferation, gut phenotype, and, most importantly, on lifespan. Here, this intervention resulted in a substantially reduced lifespan and structural changes in the midgut. Reducing dietary protein intake in different concentrations reduced tumor proliferation dose- dependently. Another effect of reduced protein intake was a mostly restored gut structure and functionality, as well as a normalized ISC phenotype. Reducing the dietary protein intake was also able to increase the lifespan of animals but depended on the amount of protein intake. In conclusion, reduced protein administration reduces the growth of intestinal tumors effectively. Dietary protein restriction can therefore be a very promising form of adjuvant cancer therapy.

Keywords: diet, dietary protein, intestine, tumor, proliferation, intestinal tumor model

*Speaker

Drosophila Melanogaster Model for the study of Fragile X Syndrome

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Fragile X syndrome (FXS) is the most common known cause of inherited intellectual disability (ID), and a monogenic cause of autism spectrum disorder (ASD), with incidence of approximately 1 in 5.000 in male and 1 in 8.000 in female. In affected individuals, it is caused by full mutation (> 200 CGG triplets) in the 5 untranslated region (UTR) of Fragile X Messenger Ribonucleoprotein 1 (*FMR1*). These expanded CGG triplet repeats are hypermethylated with consequent transcriptional gene silencing, halting gene expression, thereby resulting in a reduction or absence of gene's product, FMRP. A few animal models of FXS were described. The *FMR1* homolog in *Drosophila* was identified in 2000 and designated *dfmr1*. The aim of this research is to present *dfmr1* mutants as a model for the study of FXS. Various *Drosophila* mutant strains (e.g., *dfmr1B55* - *FBal0141745*, *Fmr1Δ50*- *FBal0131033*) were created by turning off the *dfmr1*. There is overlapping between the phenotypes in humans with FXS (for example, intellectual disability, sleep problems, autistic features, and attention deficit/hyperactivity disorder-ADHD) and the loss-of-function phenotypes in *dfmr1* mutants. Thus, the *dfmr1* mutants are capable to learn during training in young age, but this ability is diminished by their age. Also, their short and long-term memories are impaired. In fly FXS model sleep abnormalities are represented in altered circadian rhythm and sleep pattern. *dfmr1* mutants cannot sustain that rhythmicity in total darkness. Their sleep is prolonged and deeper, but with decreased ability for adequate recover after sleep deprivation. Changes in grooming, courtship, and other types of social behavior are typical in *dfmr1* mutants and associated with autistic features present in individuals with FXS. Grooming in fly mutants is excessive and with notable presence of repetitive patterns. This alteration mimics stereotypic and repetitive behavior in persons with ASD. Also, *dfmr1* mutant's males spend less time in courting the female. High activity of *dfmr1* mutants is related to ADHD in individuals with FXS. Further, *dfmr1* mutants' poor results in climbing and fight essays are corresponding to delayed motor development in patients suffering from FXS. In conclusion, *dfmr1* mutant is an excellent model for investigation of behavioral changes associated with FXS and drugs candidates' screening that might be helpful in FXS.

*Speaker

Keywords: fragile X syndrome, dfmr1 mutant, FMR1 gene, human model disease, behavioral research

Drosophila system for modelling neurological disorders caused by GNAO1 mutations

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Drosophila Gao (dGao) protein is highly conserved in the animal kingdom and shares many features with its mammalian counterpart, such as the predominant expression in the CNS. We modified endogenous *dGao* using the CRISPR/Cas9 system producing diverse genetic lines, such as the null mutant, the gene with humanized sequence, dGao fused with GFP, the mutant with the pathogenic G45E amino acid substitution, and the progenitor stock for facilitated generation of more substitutions inside exons 4-7 of *dGao* by the Recombinase-Mediated Cassette Exchange (RMCE) technique. Using RMCE, we further introduced the pathogenic mutations *G203R*, *C209R*, *C215Y*, *E246K*, and *insPQ*. All mutant lines are assessed by the parameters such as viability/ stage lethality in the homo- and heterozygous states, longevity, and by behavioural tests, including the negative geotaxis assay (NGS, to detect motor dysfunctions in adult flies). *G203R/+* flies were raised with the ZnCl₂ food supplementation, demonstrating ameliorating motor abilities in NGS compared to the mutant line raised on standard food. Our model system can thus be used to discover and validate drug candidates for potential therapeutic applications. Interestingly, none of the modelled mutations causes epileptic seizures in heterozygous mutant adult flies. Aiming at escalating the epileptic phenotype in the mutant flies, we used RNA-interference mediated conditional gene knockdown of the wild-type allele (targeting the dGao-GFP fusion) under the heterozygous mutant background. Using different Gal4 driver lines, we can achieve such conditional gene knockdown in the desired cell types, for example, neurons or glial cells, to determine their role in the development and progression of epilepsy.

Keywords: dGao, CRISPR/Cas9, neurological disorders, epilepsy

*Speaker

Enhancing autophagy by redox regulation extends lifespan in *Drosophila*

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Dysregulation of redox homeostasis has been implicated in the ageing process and in the pathophysiology of age-related diseases. To study redox signalling *in vivo* and to explore its involvement in metabolic health and longevity at an organismal level, we established a redox-shifted model by manipulating levels of the H₂O₂-degrading enzyme catalase in *Drosophila*. We found that ubiquitous overexpression of catalase robustly extends lifespan in flies. As anticipated, these flies were strongly resistant to a range of oxidative stress challenges, but interestingly were acutely sensitive to starvation stress, which could not be explained by differences in levels of energy reserves. This led us to explore the contribution of proteostasis, specifically autophagy, which is an important mechanism for cellular survival in response to starvation. We show that autophagy is enhanced in the catalase flies and essential for their increased lifespan, which was completely abolished upon global autophagy down-regulation. Furthermore, using a specific redox-inactive knock-in mutant, we highlight the role of a key regulatory cysteine residue in Atg4a, which is required for the lifespan extension. Altogether, these findings emphasise the redox regulation of autophagy *in vivo* as an important modulator of longevity.

Keywords: Autophagy, redox signalling, catalase, ageing, longevity, oxidative stress

*Speaker

Exploration of CYP27A1 function in retinal neuron-glia cholesterol signaling in *Drosophila*

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*Cholesterol is essential to maintain the integrity of cell membranes and plays important roles in neuron viability and transmission of nervous signals. In mammalian brain, a fine regulation of its metabolism is associated with the communication between glial cells and neurons. The dysregulation of cholesterol homeostasis is associated with many degenerative disorders (1)(2). In particular, cholesterol accumulation in the retina is involved in age-related macular degeneration, the leading cause of vision loss in western countries (3). Under physiologic conditions, cholesterol elimination from the retina partly relies on its conversion into oxysterols via the action of several cholesterol hydroxylases, including the MmCYP27A1 enzyme (4)(5). The aim of the present study was to determine the consequence of inhibiting cholesterol elimination via oxysterol on neuron-glia interaction and retinal integrity. We have used the *Drosophila* model as a powerful genetic tool allowing the exploration of neuronal signaling. Mmcyp27a1 orthologs were screened for their retinal expression in *Drosophila* and three genes were identified, namely Dmcyp12c1, Dmcyp49a1 and Dmcyp12a5. Their potential roles in cholesterol elimination and the maintenance of retinal structure in ageing is being studied. For that purpose, we first used the global retina driver, gmr-Gal4, to inactivate the targeted genes using RNA interference. Interestingly, inactivation of Dmcyp12c1 in retinal cells was associated with a developmental retinal disorganization. However, cholesterol levels measured by gas chromatography coupled with mass spectrometry on drosophila whole heads did not reveal any difference between control flies and mutant flies. The next step is to determine whether down-regulation of Mmcyp27a1 orthologs are associated with cholesterol accumulation and neurodegeneration in the aging eye. Since Dmcyp12c1 invalidation during development is linked to structural abnormalities in the eye, we decided to down-regulate this gene only during the adulthood. Interestingly, preliminary immunostainings show an age-related ommatidia disorganization in mutant adult flies.*

A better understanding of the role of retinal cell types in retinal cholesterol homeostasis should help for developing new therapies to prevent retinal degeneration.

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*Speaker

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Keywords: cholesterol, glia, neuron interactions, retina, Mmcyp27a1, Dmcyp12c1

Exploring how *Drosophila* gut tumours respond to sex and reproduction

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The risk and progression of disease are influenced by both sex and reproductive status, yet these factors are often overlooked in experimental disease models. Here we use the fruit fly *Drosophila melanogaster* as a model system to investigate how sex and reproduction affect tumour progression.

The fly midgut is a highly sexually dimorphic organ. As well as differences in the metabolism, size and shape of the midgut, the intestinal stem cells divide more in females than in males. In addition, female guts undergo dramatic remodelling after mating, to sustain energy-intensive egg laying. This post-reproductive remodelling of the gut can have a trade-off, in altering the risk of tumorigenesis. It has been previously reported that females produce more proliferative midgut tumours than males in several genetic models perturbing the *RTK/Ras*, *Wnt* and *Notch* pathways. The proliferation of these tumours increases in mated females.

Unexpectedly, we have found that the *Hippo*-pathway midgut tumours, induced by overexpression of a constitutively active oncogene *Yorkie* (*Yki3SA*) or downregulation of tumour suppressor *Warts* (*WtsRNAi*), behave differently in response to sex and reproduction. *Hippo*-pathway tumours exhibited unusually high proliferation in virgin females and have lost most of their transcriptional post-mating differences. Furthermore, we have observed that male-biased metabolism is suppressed in highly proliferative *Yki3SA* male tumours, but not in *WtsRNAi* male tumours. Our current work focuses on linking these transcriptional changes with tumour proliferation. We demonstrate that the tumour response to sex and reproductive status is dependent on genetic model, with different *Hippo*-pathway tumours losing dependency on sex and reproductive cues. These findings highlight the importance of considering tumour genotype, sex and reproductive status when studying cancer.

Keywords: tumour, cancer, metabolism, sexual dimorphism, sex, reproduction, hippo

*Speaker

Expression of the Alzheimer susceptibility gene BIN1 in the presynaptic compartment leads to isoform-specific synaptotoxicity

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BIN1 is a major susceptibility gene for Alzheimer Disease (AD). However, the contribution of *BIN1* and its isoforms to AD pathogenesis remains unclear. We recently showed a functional evolutionary conservation of human *BIN1* isoforms in *Drosophila* as expression of human *BIN1* isoform8 (BIN1iso8) was able to rescue the locomotor defects of *Amph* null flies, the *Drosophila* *BIN1* ortholog. In addition, we observed that human BIN1iso1 induces an accumulation of early endosome vesicles leading to neurodegeneration in *Drosophila* retina photoreceptor neurons and that the early endosome size regulation was conserved in human induced neurons. This role was specific to BIN1iso1, as compared to BIN1iso8 and BIN1iso9.

Because endosomal trafficking is essential for synapse, we further analyzed *BIN1* isoforms neurotoxicity at the synaptic level. Using electrophysiology, we observed an early loss of synaptic transmission upon BIN1iso1 expression in *Drosophila* retina photoreceptor neurons. This was characterized by a strong accumulation of abnormally large vesicles in the presynaptic compartment. In addition, expression of BIN1iso1 in motoneurons of the larval neuromuscular junction altered the morphology of synaptic boutons, with an increase in their number and a decrease in their size, and the appearance of satellite boutons. BIN1iso1 synaptotoxicity could be partially rescued by endosomal regulator modulation suggesting a similar endosomal defects as in the cell body. Finally, we tested a functional conservation in rat primary neurons using tricompartiment microfluidic devices and assessing a presynaptic vs post-synaptic role. We observed a loss of synaptic connectivity only when expressing BIN1iso1 in the presynaptic compartment.

In conclusion, our results suggest that *BIN1* has an isoform-specific, deleterious effect on synaptic integrity when expressed in the presynaptic terminal. Therefore, we propose a role for *BIN1* in the synaptic loss observed early in AD.

Keywords: Synapse, neurodegeneration, Alzheimer, BIN1

*Speaker

FUBP1/Psi function in the niche is essential to prevent neural stem cell overproliferation

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As a transcriptional activator of *MYC*, the single-stranded DNA binding protein FUBP1 functions as an oncogene. Somewhat surprisingly, FUBP1 loss-of-function is predicted to drive the primary brain cancer, oligodendroglioma. Here we aim to take advantage of the conservation of FUBP1 (Psi) in *Drosophila* to elucidate the molecular basis of FUBP1's context-dependent tumour suppressor function in the brain. We further address the heterogeneous nature of glioma by dissecting lineage-specific FUBP1/Psi function in the cortex glial niche, which provides stem cells with the structural support and secreted signals required for stemness and differentiation. Our exciting data demonstrate FUBP1/Psi function in the cortex glia niche is essential for preventing neural stem cell overproliferation. To determine the molecular basis of FUBP1/Psi's capacity to control neural stem cell renewal and differentiation cell non-autonomously from the supporting glial niche, we used Targeted DamID (TaDa) to identify direct, genome-wide targets specifically in the cortex glia. We further identified differentially expressed (DE) targets via RNA-seq of FACS-isolated FUBP1/Psi-depleted cortex glia (compared with control). Intersection of RNA-seq and TaDa identified direct DE target genes including secreted factors (e.g. EGFR and FGFR ligands) and major developmental signals (e.g. Notch and Hippo). Together, our data demonstrate FUBP1/Psi functions cell non-autonomously in the glial niche to regulate structural support and signaling networks required to prevent excessive neural stem cell renewal and promote differentiation. Thus, given the high degree of functional homology with Psi, we predict FUBP1 loss-of-function drives tumourigenesis, at least in part, by dysregulating intra-tumour interactions between the glial niche and glioma stem cells.

Keywords: stem cell, cortex glia, brain, glioma, Psi, DamID

*Speaker

Factors affecting a Notch-induced malignant transformation of larval neuroblast lineages

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Factors affecting a Notch-induced malignant transformation of larval neuroblast lineages
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Persistent Notch signalling in larval neuroblasts (NBs) and their progeny is known to generate ectopic aberrant NBs by preventing differentiation of ganglion mother cells (GMCs) and early neurons. We will present an overview of what we have recently learned about how this NB hyperplasia becomes malignant after allografting to an adult host. Allografts rely on Hes transcription factors, the Myc oncogene and the Insulin receptor (InR) for rapid growth. In fact, Hes overexpression (dpm + E(spl)mγ) closely phenocopy the malignant phenotype of high-Notch tumours. During the transplantation procedure, the CNS becomes fragmented and sheds its basement membrane, facilitating host haemocyte recruitment. These haemocytes resist tumour growth by phagocytosing it, employing, among others, the NimC1 phagoreceptor. At the same time, reactive oxygen species (ROS) produced by haemocytes seem to promote tumour growth. We are currently using genetic tools to address both tumour intrinsic and host factors that affect the progression of these neural stem cell tumours.

Keywords: Neuroblast, tumour, Notch, haemocyte

*Speaker

Fatty acid binding protein reduces A β 42-induced neuronal damage in *Drosophila* model of Alzheimer disease

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Fatty acid binding proteins (FABPs) are small cytoplasmic lipid binding proteins that bind to free fatty acids, cholesterol, and retinoids, and are involved in intracellular lipid transport. There are nine FABPs that are expressed in different tissues in mammals, of which, FABP3, 5, and 7 are known to be expressed in the central nervous system. Although expression levels of FABP3, the major neuronal FABP in the adult brain, are known to be reduced in the brains of Alzheimer disease (AD) patients, the role of neuronal FABPs in the pathogenesis of AD is not clear. Here, we investigated the role of *fabp*, the *Drosophila* ortholog of *FABP3* and *7*, in the pathogenesis of AD using a *Drosophila* model. Knockdown of *fabp* in adult neurons reduced the lifespan of flies with or without A β 42 expression, while overexpression of *Fabp* increased the lifespan of flies. Furthermore, *fabp* knockdown in neurons of AD model flies increased the accumulation of A β as well as Rel(2)P(p62) and polyubiquitinated proteins and promoted A β 42-induced cell death and neurodegeneration. In contrast, *fabp* overexpression suppressed the A β 42-induced phenotype. In addition, overexpression of *fabp* in neurons or fat body increased autophagy, which was inhibited by knockdown of *Eip75B*, the *Drosophila* orthologue of the peroxisome proliferator-activated receptor (PPAR). Together, these results suggest that *Drosophila fabp* protects neurons from A β 42-induced cell death by upregulating autophagy through the PPAR pathway, and that the function of *FABP* in neurons may be important in the progression of AD pathology.

Keywords: Alzheimer disease, autophagy, Eip75B, fatty acid binding protein, neurodegeneration

*Speaker

Fear Me Not: Adaptive Metabolic and Behavioural Responses to Predator-Induced Stress in *Drosophila melanogaster*

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Predators are integral to ecosystem functioning, exerting both lethal and non-lethal influences that alter prey population dynamics and shape biological communities. Sustained predation risk can cause chronic stress in prey animals, altering their behaviour, physiology, body composition, and life history. Chronic stress and its associated mental disorders have also been demonstrated to play a role in the development of metabolic disorders in humans, such as obesity, cardiovascular disease, and type 2 diabetes. This suggests that predator-induced stress can be utilized in animal model systems for metabolic disorders. In this study, we subjected larvae of *Drosophila melanogaster* to predator-induced stress by rearing them together with spiders. After recording the movement of developed imago flies, we observed that flies reared with spiders were moving less frequently, and covered less distance, but in the meantime showed higher acceleration and greater speeds during these shorter movement bouts, compared to control flies. We also observed that flies exposed to predation risk during larval development had greater survival rates when exposed to predation by spider. In addition, we demonstrate that predation risk induced stress during larval development impairs carbohydrate metabolism by systemic inhibition of Akt protein kinase, a central regulator of glucose uptake. Impaired ability of flies to use carbohydrates as an energy source can explain the unusual movement patterns of these flies, potentially showing an adaptive value of this diabetes-like metabolic disorder. Our study also provides a new animal model system to explore mechanisms of the onset of metabolic disorders prevalent in human population.

Keywords: predator induced stress, metabolic disorders, akt protein kinase, adaptive response, glucose uptake

*Speaker

From A β peptides to animal behaviour: A neural circuit model of Alzheimer's Disease

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The pathogenesis and progression of neurodegenerative diseases are poorly understood at the neural circuit level. How cellular hallmarks affect communication between neurons and translate to behavioural changes remains elusive. To bridge the gap between circuit mechanisms and behaviour during neurodegenerative processes, we built a comprehensive circuit model for Alzheimer's Disease (AD) in *Drosophila* larvae as a proof-of-concept. Previously, we mapped a three-layer mechanosensory circuit with complex mechanisms. We showed that silencing synaptic transmission of each neuron-type had specific effects on larval behaviour in response to an external stimulus. Here, we modeled AD by expressing human A β 1-40 or 1-42 peptides in a neuron-specific manner. We assayed neural function on a single-neuron level with live imaging and quantified whole-animal behaviour using machine-learning classification. We found that neurons within the circuit were differentially impacted by A β expression. Some neurons showed increased activity; some were not impacted; and others show decreased activity. The mechanosensory chordotonal (Cho) neurons, for example, demonstrated significantly impaired Ca²⁺ flux and neurotransmitter release, which consistently translated into decreased behavioural response. From a behaviour screen combining DGRP wild-type lines and the Cho-specific AD model line, we further identified genetic candidates that modified the behavioural defect. Based on these results, we are currently developing computational methods to infer neuron type-specific changes within the AD-like circuit from high-throughput behavioural quantification. Together, the methods involved can be expanded to study other brain disorders and used for large-scale genetic and compound studies to rapidly dissect functional impacts within specific neuron types.

Keywords: amyloid beta, functional imaging, machine learning, high, throughput screen

*Speaker

Identification of nutrition-dependent cardiokines in adult flies

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Diabetic cardiomyopathies are cardiac diseases resulting from diabetes. They are characterized by contractile dysfunctions and tissue remodeling without vascular disorders. In recent years, the heart has been suggested to be a secretory organ, producing cytokines, called cardiokines, which act in an autocrine/paracrine manner in response to cardiomyocyte stresses and fibrotic processes, but also systemically to impede on metabolic homeostasis. *Drosophila* has proven to be a valuable model for the study of metabolic disorders and associated cardiomyopathies. The fly heart tube is constituted of cardiomyocytes with molecular, physiological and mechanical properties similar to mammalian cardiomyocytes. It is the simplest physiological equivalent of the mammalian heart. Adult flies raised on High-Fat (HF) or High-Sugar (HS) diets (D) become obese and develop cardiac dysfunctions with characteristics of human diabetic cardiomyopathies, including heart rhythm abnormalities and cardiac remodeling. To better understand the molecular perturbations leading to the onset of diabetic cardiomyopathies, we performed a transcriptome analysis on isolated hearts of young females fed HSD or HFD. Candidate genes were selected for each rich diet. Sequence analysis of the candidate genes predicts that third of these genes encode secreted proteins, suggesting that nutritional stresses impede dramatically on the secretory function of the cardiac tube by modulating the expression of putative cardiokines. Paracrine and systemic effects are evaluated by knockdown of the candidates in the different cardiac cell types. By high-speed video imaging on whole flies, we are analyzing the role of these new molecular partners on both the structure of the heart and its contractile function. We show that the previously identified satiety hormone Fit is expressed by cardiac cells. Our results show that Fit has a paracrine function and also suggest a systemic function.

Keywords: cardiomyopathies, diabetes, cardiokines

*Speaker

Impact of chronic exposure to nanopolystyrene on *Drosophila* development- and cancer-related traits

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Plastic pollution is becoming a global environmental emergency, with micro- and nanofragments accumulating in fatty tissues of marine and land-based organisms or absorbed by plants. Their ingestion or inhalation is increasingly being associated with metabolic changes, decreased survival and genotoxicity in several experimental systems, whose application greatly helps assess the expected harmfulness of plastic pollution to human beings. *Drosophila* is an excellent model for investigating the genotoxic and tumourigenic potential of nanoplastics, and here we present our data concerning the biological effects and the carcinogenic potential of 100 μm polystyrene nanoparticles (PSNPs), focusing on the *Drosophila* intestine and imaginal epithelia. First, we characterised PSNPs by scanning electron microscope (SEM), both in an organic-free milieu and in the flies' excrements. Second, their presence/accumulation in the intestine and the fat body of wild-type flies regularly fed with fluorescent PSNPs-additioned food was analysed by fluorescence microscopy. The effects of a number of development-related traits were investigated, such as developmental time, eclosion rate, larval crawling, adult weight and climbing in young and old individuals, finding a significant reduction in weight and developmental time, as well as a decline in the ability to recover from starvation. In addition, a generic PSNPs-mediated DNA damage observed in the intestinal tissue prompted us to analyse the expression of apoptosis-related factors in the digestive tract, and their genotoxicity was also investigated in *DNAIlg4*, *rad50* and *mei-41* DNA repair mutants. Finally, to assess the carcinogenic potential of PSNPs, ongoing experiments in dedicated cancer models will define their contribution to central cancer-related traits such as loss of tissue architecture, mass expansion and invasiveness. Results will be presented either in a development- or a cancer-related perspective, so as to stimulate an integrate discussion.

Keywords: Nanoplastics, Genotoxicity, Cancer, Gut, Imaginal Disks

*Speaker

Improved *Drosophila melanogaster* models of Rett Syndrome

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Rett syndrome (RTT) is a rare neurodevelopmental disorder characterized by mental and motor disabilities with a prevalence of 1:10,000 people. 95% of patients harbor loss-of-function (LOF) mutations in the gene encoding for *MECP2*, a transcription factor that modulates chromatin plasticity. Although neurological defects are the hallmarks of RTT, most patients manifest gastrointestinal (GI) symptoms such as constipation and dysbiosis. Nonetheless, the correlation between neurological and gastrointestinal defects has not been fully elucidated.

Our work aims to develop a *Drosophila melanogaster* model of RTT to unravel the complex mechanisms that link neurological and GI defects by overexpressing *wild-type* or mutated human *MECP2* in different fly tissues. Interestingly, by selectively driving the overexpression of *MECP2* in visceral muscles, we observed complete lethality at pupal stage. We are now investigating the origin of such phenotype.

Recently, it has been reported that PHF14, a histone-binding protein, physically interacts with MECP2. A *PHF14* point mutation within the MECP2 interacting domain has been found in a subject manifesting RTT-like symptoms. However, the function of *Drosophila PHF14* (annotated as *CG15439*) has not been studied, and to date, mutant alleles of *CG15439* are not available. By using the imprecise excision technique, we are currently generating *CG15439* LOF mutant lines in the effort to dissect the mechanism behind the interaction between endogenous PHF14 and ectopically expressed MECP2. Also, we will explore the consequences of *CG15439* downregulation in the nervous system, visceral muscles and other tissues with available RNA interference lines. Our goal is to use *Drosophila melanogaster* to generate improved models of RTT that recapitulate the reported GI phenotypes of patients. With such models, we hope in the future to identify novel therapeutic approaches that might include microbiota and nutritional-based interventions.

Keywords: Rett syndrome, MECP2, gastrointestinal system, monogenic disease, disease model

*Speaker

Integrins can act as metastasis suppressor in *Drosophila* models.

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Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells leading to invasion of normal tissues, being the second leading cause of death worldwide. Over the last years have been revealed that cancer is a genetically complex and heterogenous disease, where each single tumour carries mutations not in a single gene but in several genes. Moreover, the crosstalk between cancer cells and their microenvironment plays a key role in cancer initiation and progression.

The extracellular matrix (ECM) is a major component of the tumour microenvironment and is involved in almost every step of cancer progression, that's why we decided to focus on proteins present in this matrix, specifically in Integrins. Integrins are heterodimeric molecules containing an α and a β subunit which are the main cell adhesion receptors for components of the ECM. Their biological roles in cancer, as signaling molecules, mechanotransducers and essential components of the cell migration machinery, are quite complex and highly dependent on the type and developmental stage of the tumor.

For our purpose we are using as model the primordium of the *Drosophila* wing, the larval wing imaginal disc, which has been successfully used to study epithelial tumour progression and oncogenic cooperation. Using the Gal4 system we can express the Rasv12 human oncogene alone or in combination with other genes in the apterous domain of this tissue, producing an overgrowth and ectopic folds. We chose the human oncogene Ras because it's mutated in almost 30% of cancers.

Here, we demonstrated that the reduction of integrin levels in tumour cells by expressing a *mys* specific RNAi, a method that was shown to cause a strong reduction in bPS protein levels, enhances the Rasv12 hyperplastic phenotype. This increase in phenotype is due to an increase in cellular changes and growth associated with Rasv12 cells, inducing a columnar to cuboidal cell shape change, showing reduced height and increased apical and basal surfaces compared to control cells, what could be explain by an increase in the number of cells in G2 phase quantified by flyfucci. Moreover, previous studies have shown that activated Ras promotes the death of nearby wild-type cells. In agreement with this, we detected an increase of apoptosis in wild-type ventral cells together with an increase in the JNK activity. All together, we could suggest that in this epithelial tumour the integrins could act as suppressor in the progression of the disease.

*Speaker

Keywords: Cancer, Integrins, wing disc

Investigating the cellular and systems physiology of obesity with *Drosophila*

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Obesity levels are rising rapidly around the world and are a major risk factor for diseases such as Type 2 diabetes, cancers and recently, COVID-19. New strategies and innovations are thus needed to prevent and treat obesity and associated disease. This requires an in-depth understanding of the genetic, cellular, and physiological mechanisms underlying obesity. Obesity is a systemic disorder that affects multiple organs and perturbs inter-organ communication. A comprehensive understanding of obesity thus benefits from studies in simpler model organisms where this integrative network can be examined *in vivo*. With highly conserved signalling pathways and effective genetic and biochemical tools, the model system *Drosophila melanogaster* offers many advantages to study obesity and associated disorders. A characteristic feature of obesity is the excessive expansion and dysfunction of adipose tissue. We are leveraging the strength of the *Drosophila* model to investigate fundamental molecular and cellular mechanisms regulating adipose tissue plasticity and its dysfunction in obesity. We utilize a powerful interdisciplinary approach by combining genetic data from human obesity studies to establish specific *Drosophila* obesity models. Investigation of adipose tissue dynamics in these models will provide new insights into how adipose tissue dysfunction contributes to obesity and other metabolic disorders. Complex interactions between genetic and environmental factors that are known to influence obesity will also be assessed. This research will thus provide novel insights into the cellular processes within adipose tissue that regulate metabolic homeostasis as well as pathogenic mechanisms underlying obesity.

Keywords: Obesity, adipose, fat body, genetics of obesity, inter, organ, physiology

*Speaker

Investigating the effect of neurodevelopmental disorder missense variants on cognitive function with *Drosophila*

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Over 200 million people in the world are affected by Intellectual Disability (ID) and/or Autism Spectrum Disorder (ASD), debilitating and often co-occurring neurodevelopmental disorders. They have problems with cognitive and adaptive functioning, including learning, communication, and social skills. ID/ASD have mostly monogenic causes. Majority of the mutations (non-sense, frameshift or splice-site) are likely gene disrupting (LGD). However, there is a growing number of *de novo* missense genetic variants with uncertain significance (VUS) that increase the disease risk to similar or even greater degree than LGD. It is challenging to comprehend how VUS affects ID/ASD symptoms; hence it is important to develop an efficient model. We are introducing 40 conserved recurrent VUS in *Drosophila* orthologs of ID/ASD genes with CRISPR/Cas9-based prime editing. We will investigate their effect on cognitive function with habituation, a conserved form of learning based on suppressing a response to a repetitive but meaningless stimulus. Habituation is a prerequisite for higher cognitive functions and, as we have shown previously, is affected in LGD models of ID/ASD. Thus, habituation is suitable for investigating the effect of VUS on cognitive function in ID/ASD.

We use a high-throughput light-off jump habituation platform that is based on the suppression of a jump response to a light-off stimulus. After assessment of habituation, we determine whether habituation deficits correspond with altered molecular function, predicted from protein structure analysis and *in vitro/in vivo* functional assays. This study should shed light on the effect of VUS on ID/ASD pathology and, most importantly, on cognitive function that cannot be easily assessed with simpler cell-based models.

Keywords: habituation, neurodevelopmental disorders, CRISPR/Cas9 based prime editing, missense mutations

*Speaker

Is circadian disruption an effect or a cause of neurodegeneration? *Drosophila melanogaster* as a model to investigate the link between circadian clock and neurodegenerative disorders

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Between 60 and 90% of Parkinson's disease (PD) patients display circadian and sleep disturbances, as REM sleep behavior disorder (RBD), excessive daytime sleepiness (EDS) or insomnia, that occur early in the pathology progression and profoundly impact on the quality of life. Moreover, diurnal fluctuations in symptoms and signs associated with PD, as motor symptoms, visual performance and responsiveness to dopaminergic treatments, have also been described, leading to the hypothesis of a circadian influence on the expression of clinical features of PD. Nevertheless, it is still unclear whether circadian disruption can influence the pathogenesis of this neurodegenerative disorder and therefore be a risk factor for developing the disease. In the context of neurodegenerative diseases, *Drosophila melanogaster* is a very useful model. Other than its most known advantages, *Drosophila* is a well-established model because the great majority of human disease-associated-genes are conserved. Regarding Parkinson disease, *pink1/parkin* mutants and flies overexpressing *pre-fibrillar human alpha-synuclein* (SNCA), recapitulates the human conditions in the dopaminergic loss, lifespan reduction and both motor and non-motor symptoms. In the latter case, flies display circadian-related alterations like sleep fragmentation and defects in the anticipation of dawn. We are therefore exploiting this model to investigate the link between circadian clock and neurodegenerative disorders, trying to understand whether the circadian disruption impacts on the pathogenesis of PD. The identification of circadian disruption as an environmental factor that influence the pathogenesis and progression of Parkinson could be very important in the modern society, where the life expectancy is increasing and the disruption of individual circadian rhythms is very common due to the presence of light pollution and in night-shift workers.

Keywords: parkinson, drosophila, circadian, neurodegeneration

*Speaker

Metabolism and lifespan of *Drosophila melanogaster* after smoke exposure regulation following Wnt pathway activation

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Chronic obstructive pulmonary disease (COPD) is the leading cause of mortality and morbidity worldwide and is characterized by incomplete reversibility of expiratory airflow limitation, dysregulated chronic inflammation, and emphysematous destruction. The lack of a mechanistic understanding of the underlying mechanisms is certainly one of the main reasons for the lack of causal therapeutic options. Recent studies pointed to a prominent role of the WNT signaling pathway in COPD and the possibility that manipulating the WNT pathway might enable regenerative processes in the damaged lung. For this, we used a simple cigarette smoke-induced *Drosophila* model of COPD based on chronic cigarette smoke exposure that encapsulates the main pathological features of the disease and can therefore be used to investigate new therapeutic strategies. We used this model to examine the role of WNT signaling in COPD and showed that overexpression of WNT/ β -catenin exclusively in the airway epithelium rescued most of the phenotypes associated with chronic cigarette smoke exposure. Besides reduced physical activity, reduced body fat and protein, increased metabolic rate, and reduced tolerance to hypoxia the most important effect was the increased lifespan in chronic cigarette smoke-exposed animals. This showed that targeted activation of the canonical arm of the WNT signaling exclusively in the airway epithelium might be a valid way to treat COPD. Additionally, we observed that ectopic expression of β -catenin in the airway also increased lifespan under control conditions, which implies that aging-associated death might be caused by premature functional decline of the airways.

Keywords: COPD, WNT/ β , catenin, drosophila

*Speaker

Mitochondrial Complex IV activity during development determines lifespan

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Due to the advances in healthcare, life expectancy is increasing as is the incidence of age-related diseases. Mitochondria play a very important role in determining the length and quality of life, however as ageing progresses there is an accumulation of damaged mitochondria, with less capacity to produce ATP, and an increase of Reactive Oxygen Species (ROS) levels. Since the main role of mitochondria is the production of ATP through oxidative phosphorylation, it is not surprising that mitochondrial dysfunction is a hallmark of ageing. In this work, we reduce the activity of complex IV (CIV) using the GeneSwitch-GAL4/UAS system that allows temporal control of gene expression. Using this system we knock-down (KD) two different CIV subunits (*COX5B* and *COX4*). Our results show that reduction of CIV activity during fly development promotes ageing, as indicated by a strong reduction in lifespan and stress resistance as well as reduction in mitochondrial oxygen consumption. These flies also show an adult phenotype reminiscent of mitochondrial-associated diseases, primarily characterised by their inability to store fat efficiently. However, KD of CIV subunits during adulthood has only a moderate effect on ageing rate regardless of the same severe reduction in mitochondrial respiration. In order to rescue the negative consequences associated with CIV dysfunction, we expressed the *Ciona intestinalis* alternative oxidase (AOX). AOX works as a bypass of complexes III and IV maintaining electron flow and preventing the excessive production of ROS by decreasing the leak of free electrons through reducing molecular oxygen to water. Our results show an improvement of the deleterious consequences of CIV dysfunction, when AOX is expressed during development. These results indicate that complex IV activity during development determines lifespan during adulthood and indicate that strategies to extend lifespan manipulating mitochondrial function must be produced during this critical period.

Keywords: ageing, complex IV, longevity, mitochondria, alternative oxidase

*Speaker

Modeling respiratory distress syndrome induced by SARS-CoV-2 genes in *Drosophila*

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The airway and alveolar epithelium play a significant role in protecting the respiratory tract from pathogens and environmental pollutants while maintaining airflow in the lungs. Injury/infection in the respiratory tracks results in inflammatory lung disorders like COPD (chronic obstructive pulmonary disorder), asthma, and ARDS (Acute Respiratory Distress Syndrome). A primary SARS-CoV-2 disease symptom is severe upper respiratory infections and pneumonia, often culminating in ARDS leading to impaired gas exchange and loss of lung compliance. Here, we aimed to decipher the candidate SARS-CoV-2 genes that underpin ARDS-like syndrome in a reductionist approach by using *Drosophila* as a model system. We screened for phenotypes induced by the expression of individual SARS-CoV-2 nonstructural and accessory proteins in the *Drosophila* larval trachea. In particular, we focused on those causing epithelial barrier perturbations in airways, underpinning larval hypoxia, and finally, identifying the cellular signaling pathway(s) targeted by the candidate SARS-CoV-2 nonstructural and accessory proteins. We will discuss our findings on the cellular signaling perturbations and our unraveling of the causal underpinnings of SARS-CoV-2-induced COVID-19-like respiratory distress.

Keywords: SARS, CoV, 2, epithelial barrier defect, ARDS, larval trachea

*Speaker

Modelling complex wound environments in *Drosophila*

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Wounds that fail to heal remain a major clinical and financial burden worldwide. Tissue damage is usually accompanied by recruitment of inflammatory cells that release an array of products, including growth factors and cytokines to drive repair, as well as reactive oxygen species (ROS) to fight infection. Although these inflammatory processes are critical to normal wound healing, they are also associated with chronic, non-healing wounds when not properly regulated. In recent years, the use of tractable model organisms such as zebrafish and *Drosophila* have enabled us to dissect many fundamental mechanisms underlying wound healing. However, the majority of these studies to date have focused on acute wounds in otherwise healthy individuals. Here, we develop *Drosophila* to model more complex wound environments, such as those that exist in diabetic individuals and infected conditions. Using high resolution live-imaging and cutting-edge omics, we explore how tissue repair and inflammation is affected in these more complex scenarios. We integrate these *in vivo* studies with our newly developed Genetic Epidemiology pipeline, to correlate gene expression (of human homologues of fly genes) with human disease. Together, this integrative approach enables the study of complex wound environments *in vivo* using *Drosophila* and exploration of its clinical relevance by exploiting publicly available GWAS data.

Keywords: *Drosophila*, Wound Healing, Inflammation, Genetic Epidemiology, Diabetes

^{*}Speaker

Natural variation of mitochondrial genome alters cell-mediated and humoral innate immune responses

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The role of mitochondria in both adaptive and innate immune responses is increasingly recognized. However, the role of mitochondrial genome (mtDNA) variation as an immunomodulatory factor has not attained attention. One reason for this is the difficulty of separating the effect of mtDNA from that of the nuclear genome. By utilizing the *Drosophila* model, we have created cytoplasmic hybrid, aka. cybrid lines, with unique mtDNA genomes on a controlled isogenic nuclear background. Cybrid lines enable us to disentangle the effects arising from mtDNA variation on various phenotypic traits. We have shown earlier that cybrid lines that carry "mtKSA2" mitotype manifest melanised blood cell aggregates which are considered as one of the hallmarks of the activation of cell-mediated innate immune system in *D. melanogaster*. This finding motivated us to harness the cybrid model to study the role of mtDNA variation on both cell-mediated and humoral innate immune responses. We show that mtDNA variation causes heterogeneity in infection outcomes upon parasitoid, bacterial and viral infection, both at larval and adult stage, respectively. Moreover, mtKSA2 cybrids had an elevated immune cell count prior to infection and upon infection these flies manifested enhanced immune responses and survival in studied infection models when compared to other cybrid lines. To elucidate the mechanism behind the enhanced immunocompetence of the mtKSA2 cybrids, we performed RNA sequencing of uninfected flies and flies challenged with bacterial and viral infections in comparison to controls that possess the same nuclear background and its original mtDNA. mtKSA2 flies produced unique transcriptomes infection type and duration specifically when compared to the control line. Overall, our results show that mtDNA variation can act as an immunomodulatory factor in both cell-mediated and humoral innate immunity and create heterogeneity in infection outcomes between individuals.

Keywords: Cybrid, Cytoplasmic hybrid, hemocyte, infection, mitochondria, oxidative phosphorylation, reactive oxygen species, resistance, respiration, survival, tolerance, transcriptome

*Speaker

Pathogenic tau and synuclein induce unique metabolic reprogramming in neurons distinct from normal aging that can be rescued by targeting GLS and PRAT

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Neuronal cells are highly specialized cells and have a specific metabolic profile to support their function. It has been demonstrated that the metabolic profiles of different cells/tissues undergo significant reprogramming with advancing age, which has often been considered a contributing factor towards age-related diseases including Alzheimer's (AD) and Parkinson's (PD) disease. However, it is unclear if the metabolic changes associated with normal aging predispose neurons to disease conditions or a distinct set of metabolic alterations happen in neurons in AD or PD which might contribute to disease pathologies. To decipher the changes in neuronal metabolism with age, in AD, or in PD, we performed high-throughput steady-state metabolite profiling on heads in wildtype *Drosophila* and in models of tau-driven AD and synuclein-driven PD. Intriguingly, we found that the spectrum of affected metabolic pathways is dramatically different between normal aging, Tau, or Synuclein overexpressing neurons. Genetic targeting of the metabolic pathway dysregulated in both old age and disease condition partially rescued the neurodegenerative phenotype associated with the overexpression of wildtype and mutant tau. Our findings support a "two-hit model" to explain the pathological manifestations associated with AD or PD where both age- and Tau/Synuclein- driven metabolic reprogramming events cooperate each other and targeting both could be a potent therapeutic strategy.

Keywords: Metabolism, *Drosophila*, aging, AD, PD, metabolomics, glutaminase, GLS, PRAT/PPAT, two, hit model.

*Speaker

Pathologically hyperactive BK channels act during a critical developmental window to disrupt motor control

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Hyperkinetic movement disorders (HMDs) such as dystonia and chorea are defined by sustained or transient involuntary muscle contractions that cause painful alterations in posture and a loss of motor control (1,2).

A wide range of mutations have been linked to inherited HMDs, providing insight to their molecular basis. However, despite these advances, neurobiological processes underlying these disorders remain unclear. Furthermore, many HMDs are refractory to pharmacological therapies, necessitating novel approaches to identify drug treatments. We have addressed these issues using a construct- and face-valid *Drosophila* model of a HMD caused by a gain-of-function mutation in the hSlo1 BK potassium channel (BK GOF) (3,4).

Neuronal BK channels regulate action potential shape, firing frequency, and neurotransmitter release (5), leading to recent suggestions that BK GOF disrupts motor control by altering the excitability of pre-motor regions such as the basal ganglia or cerebellum (6,7). To explicitly test for an adult-stage impact of BK GOF in *Drosophila* we developed a genetic strategy that allows spatial and temporal control of BK channel expression. To our surprise, adult-stage induction of BK GOF in neurons had no observable impact on movement, whereas inducing neuronal BK GOF solely for 24 h during the latter half of the pupal stage strongly disrupted movement and limb posture in resulting adult flies.

These data suggest that BK channel-linked HMD is fundamentally a *neurodevelopmental* disorder. Indeed, we found that both spontaneous neurotransmission and presynaptic maturation – two interlinked processes (7) – were perturbed during the pupal stage by BK GOF.

Finally, we tested whether we could pharmacologically rescue motor defects in mature BK GOF flies that have undergone neurodevelopmental alterations. Through unbiased screening we identified FDA-approved drugs capable of restoring normal motor function when fed to adult BK GOF flies. Importantly, to our knowledge these drugs have not previously been used to treat HMDs.

Collectively, our data suggest that alterations in spontaneous activity during critical neurodevelopment periods may contribute to HMD pathology, and provide proof-of-principle that *Drosophila* can be used to identify novel therapies for these debilitating diseases.

*Speaker

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Keywords: Dystonia, chorea, BK potassium channel, slowpoke

Positive effects of phosphodiesterase inhibitors in *Drosophila melanogaster* models for Friedreich's Ataxia

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Friedreich's ataxia (FA) is a neurodegenerative disorder caused by a lack of the protein frataxin. At the current time, the disease lacks an effective treatment. The absence of frataxin causes mitochondrial malfunction which in turn is associated with multiple cellular defects. One of such defects is alterations in the stability of the actin cytoskeleton. The stability of the actin filaments is regulated by the cofilin pathway. Importantly, defects on the regulation of the cofilin pathway have been already linked to the lack of frataxin. Class IV and V phosphodiesterase inhibitors have been shown to act upstream of the cofilin pathway and impact the cellular actin scaffold. In our work we aim to test the beneficial effect in a multicellular organism of two phosphodiesterase inhibitors such as Rolipram and Sildenafil that have shown to display positive effects in cellular models of the disease. However further studies in preclinical multicellular FA models are necessary. In order to prove the abilities of these drugs *in vivo*, we have downregulated fly frataxin in *Drosophila melanogaster* using the UAS/GAL4 system. Our experiments showed that phosphodiesterase inhibitors significantly improve the longevity and mobility of model flies. Furthermore, phosphodiesterase inhibitors displayed promising capabilities to reduce the neurodegeneration observed in the brains of frataxin-deficient flies. Interestingly, this recovery is not accompanied by improvements of mitochondrial function. We also found that both drugs are able to activate the Nrf2 transcription factor pathway and concomitantly recovering molecular alterations of the various components of the cofilin pathway. Since both, the Nrf2 and the cofilin pathways are key regulators of the actin cytoskeleton, our working hypothesis is that improvements of the actin cytoskeleton allow a better transport of the mitochondria in the cell facilitating the energy distribution. Our current experiments are focused on evaluating this hypothesis to further identify the mechanism through which Rolipram and Sildenafil benefit FA flies and establish a solid foundation for these inhibitors as a potential therapy in this disease.

Keywords: Friedreich's Ataxia, *Drosophila melanogaster*, Phosphodiesterase, Cofilin, Nrf2

*Speaker

Renal NF-kB activation impairs uric acid homeostasis to promote tumor-associated mortality independent of wasting

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Tumor-induced host wasting and mortality are general phenomena across species. Many groups have previously demonstrated endocrinal impacts of malignant tumors on host wasting in rodents and *Drosophila*. Whether and how environmental factors and host immune response contribute to tumor-associated host wasting and survival, however, are largely unknown. Here, we report that flies bearing malignant yki3SA-gut tumors exhibited the exponential increase of commensal bacteria, which were mostly acquired from the environment, and systemic IMD-NF-kB activation due to suppression of a gut antibacterial amidase PGRP-SC2. Either gut microbial elimination or specific IMD-NF-kB blockade in the renal-like Malpighian tubules potentially improved mortality of yki3SA-tumor-bearing flies in a manner independent of host wasting. We further indicate that renal IMD-NF-kB activation caused uric acid (UA) overload to reduce survival of tumor-bearing flies. Therefore, our results uncover a fundamental mechanism whereby gut commensal dysbiosis, renal immune activation, and UA imbalance potentiate tumor-associated host death.

Keywords: Cancer cachexia, Mortality, Gut bacteria, PGRP, SC2, Renal immune response, IMD signaling, Uric acid, insulin signaling, ImpL2, Pvf1, Upd3

*Speaker

Role of FOXO factors in maintaining airway homeostasis in *Drosophila melanogaster*

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The prevalence of chronic inflammatory lung diseases (asthma, COPD, lung fibrosis) is world-wide increasing, which makes the development of novel therapeutic concepts mandatory. Recent studies showed that members of the transcription factor family FOXO are involved in disease development. FoxO transcription factors are evolutionarily conserved and orchestrate various molecular processes, such as apoptosis, cell cycle arrest, immune function, growth regulation, lifespan, DNA repair, metabolism, and energy balance. In the airways, FoxOs are important for maintaining airway tissue homeostasis. Using tailored *Drosophila* models, we could show that the sole FoxO member present in *Drosophila* is activated by immune as well as by stress stimuli. In different cell types of the airway, the immune-induced FoxO activation led to different outcomes. Whereas a massive remodeling is seen in epithelial cells, progenitor cells undergo apoptosis, and terminal cells show structural defects. Whereas the underlying signal transduction pathway up and downstream of FoxO comprises various similarities, differences between progenitor cells and epithelial cells are evident in the signaling downstream of FoxO, leading to the different phenotypes. This study focuses on these differences and on the mechanisms of how FoxO orchestrates the reaction of different cell types in stressful situations.

Keywords: *Drosophila*, FoxO, airway

SARS-CoV-2 enteric infection causes intestinal damages and physiological disruptions in *Drosophila*

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The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in December 2019 has caused a global health crisis, resulting in more than 7 million deaths worldwide as of May 2023. While the associated disease, termed COVID-19, is primarily known to affect the respiratory system, multiple reports revealed that enteric symptoms are manifested in patients with a prevalence reaching as high as 50%. Such symptoms include diarrhea, nausea, vomiting, anorexia, and abdominal pain, suggesting that the gastrointestinal tract is also a target of extrapulmonary SARS-CoV-2 infection. Previous studies based on intestinal biopsies confirmed that SARS-CoV-2 can infect and replicate in human enterocytes, which co-express the angiotensin-converting enzyme 2 (ACE2) and trans-membrane protease serine 2 (TMPRSS2), two host factors that contribute to the entry of SARS-CoV-2 into target cells. Further clinical investigations showed that infected patients exhibited intestinal structural alterations associated with aberrant activation of local immune-inflammatory response, indicating that the gastrointestinal tract contributes to COVID-19 severity. In this perspective, and to better understand COVID-19 intestinal pathophysiology, we used *Drosophila melanogaster* midgut as a model system that highly expresses Ance, the orthologue of human ACE2. We showed that flies are permissive to enteric SARS-CoV-2 infection; the virus being detected by RT-qPCR and immunofluorescence assays. SARS-CoV-2-inoculated flies displayed noteworthy structural and cellular intestinal modifications, along with local metabolic and physiological disruptions. Furthermore, flies were highly susceptible and succumbed few days following enteric infection, suggesting that SARS-CoV-2 could induce additional harmful systemic effects contributing to death. Our study revealed direct deleterious impacts of SARS-COV-2 on the digestive system and paves the way for elucidating the molecular and cellular mechanisms underpinning SARS-COV-2 gut infection in a genetically tractable model organism.

Keywords: midgut, SARSCoV 2, metabolism, immunity, virus

*Speaker

Single-Stranded DNA Binding Protein Influence Neurodevelopment & Autism-Like Behaviors in *Drosophila*

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1p32.3 microdeletion/duplication has been implicated in several neurodevelopmental disorders, including intellectual disability, and autism. This region contains critical genes for development; however, none has been validated. From this region, using *Drosophila*, we investigated the role of the single-stranded DNA binding protein 3 (SSBP3). We show that SSBP3 over-expression causes morphological alterations, as well as affecting brain volume, glial cell number, and reactive oxygen species and display several ASD-like phenotypes, including anxiety, indecisiveness, sensory perception, social interaction, and feeding defects, which were partially rescued upon normalization of SSBP3 levels. Importantly, optogenetic manipulation of SSBP3-expressing neurons altered autism-associated behaviors. Our findings suggest that SSBP3 is a critical dosage-sensitive gene in the 1p32.3 region, highlighting the potential involvement of the canonical Wnt signaling pathway in SSBP3-mediated neurodevelopmental disorders. These results provide insight into the pathogenesis of neurodevelopmental disorders and highlight the importance of studying the genes in the 1p32.3 region.

Keywords: Neurodevelopment, Autism, Mitochondria, Oxidative Stress

*Speaker

The interplay between CFTR and mucus production in the *Drosophila* Cystic Fibrosis model

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Cystic fibrosis (CF) is an inherited disease of the *Cftr* gene and leads to an ion imbalance. Due to this imbalance, the clinical disease patterns are mainly obstructions of the respiratory lung epithelium and the absorptive intestinal epithelium particularly due to increased mucus formation. Still nowadays, the life expectancy of CF patients is significantly shorter than of healthy persons, as there is no cure but only symptomatic treatments.

However, the research on this is still in its infancy, which is why it is more important to advance research in this field. In the recent years *Drosophila* became a suitable and well-known model for many epithelial diseases. Thus, in this study we focus on i) the mucus production due to a *Cftr* knockdown in the enterocytes of the fly's intestine and ii) the investigation of two specific mucus-producing genes, *Mur29B* and *Muc68D*.

At first, we tested different staining methods to detect the mucus layer in the *Cftr* knockdown mutants. The fluorescence images with the lectin staining, using WGA, showed massive increase in mucus formation in the intestinal lumen compared to the control flies. In addition, we used TCEP (Tris(2-carboxyethyl) phosphine hydrochloride) a disulfide bond reducing drug and were able to detect a significant regression of mucus after drug administration.

Furthermore, we used the CRISPR-Cas9-system to create new fly lines to point the specific role of the single genes *Mur29B* and *Muc68D* in the mucus formation. Therefore, we used the pCFD6 plasmid for gene knockdown and the flySAM plasmid for overexpression of the genes.

First experiments with these lines are currently underway.

All in all, this work could bring CF research a step forward and enables further new ways to study and to find better treatments for CF either.

Keywords: Cystic fibrosis, mucins, CRISPR/Cas, TCEP

*Speaker

The role of autophagy in the rare genetic neurodegenerative disease BPAN

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The Beta-Propeller protein Associated with Neurodegeneration (BPAN) is a rare genetic neurological disease characterized by iron accumulation in the brain of patients. The clinical features are divided in two phases: a neurodevelopmental phase with epilepsy and intellectual deficiency, and a neurodegenerative phase associated with parkinsonism. BPAN is caused by mutations of the *WDR45* gene, known as a regulator of autophagy processes. The loss of WDR45 protein leads to autophagy defects in various BPAN cellular and animal models. Moreover, iron metabolism and ER-stress response are also dysregulated, and mice models show neurodegeneration and locomotor disorders. However, the molecular events leading to these phenotypes in *WDR45* mutants and particularly the possible causal role of autophagy are still largely unknown. To address this question, we have constructed a *Drosophila melanogaster* mutant for the putative *Drosophila WDR45* homolog (hereafter *dWDR45*). We have demonstrated that flies harboring *dWDR45* mutation mimic some hallmarks of BPAN, such as locomotors disorder, seizure-like phenotype, autophagy and ER stress response dysregulations. Importantly, the *Drosophila* BPAN model harbors iron accumulation in the whole body, a phenotype seen for the first time in an animal model. The establishment of this new and original BPAN model allows us to investigate the importance of autophagy in the establishment of the phenotypes associated with loss of dWDR45 functions. Our data will shed light on the biological mechanisms that link genetic mutations in *dWDR45* gene to behavioral defects in *Drosophila*. In the long term, our study will contribute to a better understanding of BPAN and bring valuable knowledge to the development of therapeutic molecules.

Keywords: Human disease, Neurological disease, Autophagy, Iron

*Speaker

Therapeutic exploitation of tissue-dependent phenotypes of the human leukemic oncogene *MLL-AF4* expressed in *Drosophila*

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Chromosomal rearrangements of the *MLL* gene are associated with development of high-risk acute leukemia that occurs in both children and adults. *MLL*-rearranged (*MLL-r*) leukemia is treated with aggressive chemotherapy, but patients often relapse and survival rates are dismal. Clearly, there exists a need for novel forms of therapy. However, development of new treatment is hampered by our limited understanding of the genetic framework that underpins *MLL-r* leukemia. Here we present that expression of the human oncogene *MLL-AF4* in the hematopoietic system of *Drosophila* leads to development of a leukemia-like phenotype, whereas expression of the very same oncogene in a non-hematopoietic tissue results in cell death through autophagy and caspase activity. We find that fat body cells expressing the leukemic oncogene initially develop as the surrounding wild-type cells, but thereafter their growth is restricted, and these cells are eventually eliminated. Autophagy plays an essential role in this context, whereas it is not required for the similar phenotype of cells with general aberrant transcriptional activity or another *Hox*-dependent leukemic oncogene. By understanding these contrasting effects induced by the leukemic oncogene in different tissues, our aim is to exploit this knowledge to rewire pro-tumorigenic cellular signals into anti-tumor defense signaling, with the ultimate goal of developing new therapies for patients suffering from *MLL-r* leukemia.

Keywords: *MLL*, *AF4*, leukemia, lymph gland, hemocytes, fat body, autophagy

*Speaker

Tumour heterogeneity and cell competition

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Mutations in multifunctional genes complicate treating their separate impact on cancer cell motility and their influence on tumour growth. Snail-related transcription factors, which are significant determinants of cancer, steer the course of cancer invasion and migration, also supporting cell survival while suppressing cancer cell proliferation. They accomplish these actions, in part, through crosstalk with epigenetic regulators. Still, not all cancer cells are created equal. In fact, typically only a fraction of the tumour mass, adopts an invasive phenotype, while their non-migratory counterparts divide rapidly. In a counterintuitive twist, research across various species has unveiled that apoptosis, a form of programmed cell death, also can contribute to tumour invasion. As such, therapies that promote cell death such as radiotherapy can inadvertently encourage resistant tumour cells to become invasive. Likewise, the inactivation of Snail-related genes suppresses primary cell invasion but can encourage the growth of micro-metastases. To investigate these problems, we used the co-activation of the epigenetic regulator Pipsqueak with the Snail-related gene *Escargot* to drive tumorigenesis. These tumours are highly invasive and metastatic, exhibiting the paradoxical trait of limited proliferation, accompanied by heightened cell death rates. Using mosaic analysis, RNAi screening, and functional assessment, we uncovered that cancer cell invasion and proliferation are antagonistic traits that likely reflect intratumoral clonal heterogeneity. Furthermore, we found that inhibiting Myc-driven cell competition may be a strategy to curtail tumour heterogeneity in Snail-mediated cancer traits.

Keywords: Cell invasion, Epigenetic, Cancer driver genes, Cell competition, Tumour heterogeneity

*Speaker

Tumour invasion initiates at Invasion Hotspots, an epithelial tissue-intrinsic microenvironment

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Carcinogenesis is understood as a stochastic process in which mutations in multiple genes accumulate over time, causing what was originally a normal cell to acquire new characteristics and evolve into a cancer cell. Our tumour model in *Drosophila* wing imaginal epithelia, however, shows that not only contingent accumulation of genetic mutations but also the tissue-intrinsic local structures play a key role in the carcinogenic process. We show that genetically mosaic clones of cells mutant for a neoplastic-tumour-suppressor gene in combination with the oncogenic Ras expression initiate invasion into the basal side of the epithelial layer at specific spots in the epithelial tissue. Through the ultrastructural analyses using particle image velocimetry (PIV) and serial block-face scanning electron microscopy (SBF-SEM), we found the patterns of planar-polarized cellular arrangement and the epithelial tissue organization are intrinsically disturbed at the "invasion hotspots." Our genetic experiments show that this local tissue disorganization is further enhanced by the oncogenic mutations, which results in basal mislocalization of the TNF receptor Grindelwald and following JNK-MMP1 signalling activation specifically at the invasion hotspots. Conversely, in other regions of the epithelial tissue, the oncogenic mutant clones do not strongly activate JNK-MMP1 signalling, deviate from the apical side of the epithelial layer, and show benign tumour growth in the lumen. These data indicate that the onset of tumour invasion is highly dependent on the tissue-intrinsic local architecture that is structurally vulnerable to oncogenic stimuli.

Keywords: tumour, cancer, invasion, epithelia, wing disc

*Speaker

Uncovering novel conserved functions for Rb proteins in neurons

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Rb (retinoblastoma) proteins are crucial cell-cycle regulators that control the G1-S phase transition via antagonism of E2F transcription factors. Mutations in the human Rb protein RBL2 have recently been shown to cause a complex neurodevelopmental disorder characterised by intellectual disability and motor dysfunction. To better understand the role of Rb proteins in the nervous system, we set out to characterise the neurological functions of the *Drosophila* RBL2 orthologue, *Rbf*. We have found that *Rbf* hypomorphs phenocopy various aspects of the human RBL2 phenotype, including morphological and behavioural defects. Interestingly, human RBL2 patients exhibit disrupted sleep, which we also observe in *Drosophila Rbf* mutants. Surprisingly, *Rbf* continues to be expressed post-mitotically in adult neurons, and neuron-specific knockdown of *Rbf* leads to severe movement and behavioural defects that are not correlated to increased cell death. Therefore, *Rbf* has novel neuronal functions outside of cell-cycle regulation and is a conserved regulator of complex behaviours such as sleep.

Keywords: Sleep, Disease, Neuron, Brain, Behaviour

*Speaker

Wing disc tumours promote gut atrophy through Upd3 in a *Drosophila* larvae model of cachexia.

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Cachexia, is an acute involuntary weight loss (> 5%), associated with different chronic illnesses, and several cancers (pancreas, colon...). It is a global metabolic syndrome triggering adipose tissue and skeletal muscles atrophy, accounting for 30% of cancer patients' deaths. We have developed *Drosophila* larvae models of cancer-associated cachexia based on localised Notch-driven wing disc overgrowth. In these models, Notch activation leads to hyperplastic overgrowth without muscle or adipose tissue wasting, while combining Notch activation with epithelial polarity impairment results in neoplastic growth and peripheral tissue wasting. Since these two tumour types are similar in size, these results suggest that neoplastic tumours produce specific pro-cachectic factors. Beside the adipose tissue and muscles, we observed that cachectic tumours also promote the atrophy of the larval gut. This is reflected in smaller width of the gut, and a change in its cellular composition. In particular atrophied guts have fewer stem cells, the adult midgut precursors or AMPs, which are normally put aside for metamorphosis, suggesting an untimely usage and depletion. Screening for the factors secreted by the cachectic tumours identified a role for the Jak/Stat pathway ligand Upd3 in mediating the atrophy of the gut and gut stem cell depletion. Preliminary results in mouse models suggest that gut atrophy is also observed in mammals during cachexia and that gut dysfunction might represent an important feature of cachexia.

Keywords: Cancer model, Interorgan communication, Larval gut, JAK/STAT signaling, Cachexia

*Speaker

dHNF4 acts in specialized enterocytes to promote lipid export and to suppress inflammation

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Inflammatory bowel diseases (IBDs) are characterized by chronic inflammation of the digestive tract and are associated with life-threatening complications, such as metabolic diseases and cancer. The prevalence of IBDs has increased steadily over the past decades, but the mechanisms driving these disorders remain poorly understood. Genetic variants in *Hepatocyte Nuclear factor 4A (HNF4A)* have been associated with IBDs in several genome-wide association studies. However, how this nuclear receptor interacts with inflammatory signaling remains to be elucidated. The *Drosophila* genome encodes for an ortholog of *HNF4A*, called *Drosophila HNF4 (dHNF4)*. The suppression of *dHNF4* in adult enterocytes recapitulates hallmark features of IBDs, including activated inflammatory networks and epithelial regeneration. We performed whole-midgut transcriptomic analyses to identify the mechanisms involved in these relationships. The loss of *dHNF4* induces global alterations in lipid metabolism and is associated with a marked accumulation of fatty acids. We show, however, that lipid accumulation is restricted to select enterocyte subpopulations upon pan-enterocyte *dHNF4* RNAi. We further demonstrate that these enterocytes are specialized in the digestion of dietary lipids, and in their export to peripheral tissues. Finally, we show that steatosis colocalizes with the activation of inflammatory signaling in *dHNF4*-deficient intestines, suggesting a causal link between the rupture in lipid homeostasis and inflammatory signaling. Thus, our studies in *Drosophila* add significantly to the existing knowledge on inter-organ lipid trafficking in insects, and could contribute to a better understanding of the relationships between intestinal lipid handling and inflammatory disorders.

Keywords: HNF4, Inflammatory bowel disease, inflammation, Intestine, Lipid metabolism, lipid trafficking

*Speaker

miR-210 is essential to retinal homeostasis in fruit flies and mice

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miR-210 is one of the most evolutionarily conserved microRNAs. It is known to be involved in several physiological and pathological processes, including response to hypoxia, angiogenesis, cardiovascular diseases and cancer. Recently, new roles of microRNAs are emerging in the context of visual system development and functioning as well as in eye diseases. Among them, miR-210 has been shown to be essential to *Drosophila melanogaster* visual system homeostasis and in the regulation of circadian rhythms. In particular, miR-210 loss in fruit flies results in a progressive retinal degeneration, which seems to be related with lipid droplets accumulation and alterations in lipid metabolism. However, the possible conservation of miR-210 knock-out (KO) effect in the mammalian retina remained to be investigated. Here we provide a deeper understanding of the lipid metabolism alterations in miR-210 KO flies and the first morphological (confocal microscopy and TEM) and transcriptomic characterization of the retina from miR-210 KO mice. We demonstrated the photoreceptors degeneration in miR-210 KO mice, but there is no evidence of an involvement of lipid metabolism, as previously demonstrated in miR-210 KO flies, suggesting a different mechanism of degeneration in mice than in *Drosophila*. Further characterization of these models will pave the way for a complete understanding of the functional role of miR-210 in the maintenance of the proper homeostasis of the visual system.

Keywords: microRNAs, miR, 210, visual system, eye, retina, photoreceptors, degeneration, lipid metabolism

*Speaker

smORF encoded peptides: potential newmarkers of cachexia

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Imbalance in the proliferation/differentiation of adult stem cells can lead to uncontrolled proliferation of these cells resulting in tumor formation. In some cases, the resulting tumor can impair the function of other organs and lead to significant weight loss. This organ wasting phenomenon, called cancer cachexia, is a multifactorial metabolic disorder that is found in more than half of all cancer patients. It is manifested by a decrease in the size of muscle and/or fat tissue. Although progressing, the mechanisms involved in the establishment of cachexia are still poorly understood. We hypothesize that diffusible molecules could be identified among the family of smORF-encoded peptides translated from ORFs of less than 300 nucleotides present in coding and non-coding RNAs. The identification of this type of peptides is particularly difficult because of their small size. Indeed, to avoid the annotation of millions of false ORFs, they have been considered as non-coding and remained ignored for a long time. They represent an unexplored reservoir of macromolecule regulators and could act locally or systemically. Using bioinformatics methods, RNA sequencing and ribosome profiling data from *Drosophila* samples, we identified novel genes encoding for smORF peptides and we are currently investigating their potential roles in tumor formation and inter-organ communication leading to cachexia.

Keywords: cachexia, smORF encoded peptides, gut

*Speaker

Cell stress, growth, proliferation &
death

A host-derived metabolite suppresses the nascent anticancer innate immunity

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Immune tolerance is a state of unresponsiveness of the immune system to pathogens, substances, tissues, or abnormal cells that naturally would elicit an immune response. Immune tolerance increases a host's resilience but decreases its ability to kill infected or abnormal cells. Inflammation is an important pathway for inducing immune tolerance although how inflamed cells induce and maintain host immune tolerance are poorly understood. Here, we show that Pten-mediated inflammatory Nitric Oxide released from tumour cells remotely upregulates the host tryptophan catabolism to overproduce 3-hydroxykynurenine metabolites that prompt immune tolerance to tumour cells. Silencing the aryl hydrocarbon receptor, the kynurenine receptor, in the host deters immune tolerance and thwarts tumour formation and progression. These data reveal how inflamed preneoplastic cells can survive and progress towards overt tumours by hijacking tryptophan catabolism to stop the nascent innate immune response

Keywords: cancer host interaction, Pten, innate immunity, metabolism, kynurenines

*Speaker

A positive feedback circuit that simultaneously drives cell death and proliferation

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Cell death and proliferation are at glance dichotomic events, but occasionally coupled. Caspases, traditionally known to execute apoptosis, play non-apoptotic roles but their exact mechanism remains elusive. Here, using *Drosophila* intestinal stem cells (ISCs), we discovered that activation of caspases induces massive cell proliferation rather than cell death. We elucidate that a positive feedback circuit exists between caspases and JNK, which can simultaneously drive cell proliferation and cell death. In ISCs, signaling from JNK to caspases is defective, which skews the balance towards proliferation. Mechanistically, two-tiered regulation of DIAP1 inhibitor *rpr*, through its transcription and its protein localization, exists. This work provides a conceptual framework that explains how caspases perform apoptotic and non-apoptotic functions *in vivo* and how ISCs accomplish their resistance to cell death.

Keywords: drosophila, cell death, apoptosis, intestinal stem cells, reaper, cell proliferation, jnk, yki, synr, gut

*Speaker

Cell death decision-making downstream of caspases *in vivo*

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Entry into apoptosis is mediated by caspase enzymes, particularly the terminal effector caspases. However, activation of these enzymes does not always trigger death in every cell, and can fulfil many non-apoptotic functions. The mechanisms by which cells decide whether to die or survive upon effector caspase activation are not clear. Preliminary results indicate that epithelial cells in *Drosophila* pupae have variable caspase sensitivities, and can commit to apoptosis across a broad range of effector caspase activation levels, suggesting that unknown factors modulate the likelihood of death upon caspase activation. Through live imaging of the *Drosophila* pupal notum, a single-layer epithelium, we use two approaches to quantitatively analyse how the probability of death is fine-tuned downstream of caspases *in vivo*. Using optogenetics to trigger mild caspase activation across the tissue, we tracked apoptotic death events to generate a coarse-grained spatial map of sensitivity to caspase in the notum, and characterised regions of high and low sensitivity to caspase. By transcriptionally profiling these distinct regions using Targeted DamID, we seek to identify candidate genes regulating caspase sensitivity. In parallel, through live imaging of a notum expressing an active caspase sensor, followed by analysis of cell morphometrics and caspase dynamics, we identified properties of cells which may account for variation in their individual caspase sensitivities. These results suggest that past activation of caspase primes cells to enter apoptosis more readily at a later time, suggesting the existence of a caspase memory effect which predisposes cells to death. Understanding the factors which influence a cell's probability of death downstream of caspase activation may allow us to predict which cells will die and survive in a developing or homeostatic tissue.

Keywords: apoptosis, caspase, epithelia, morphogenesis, live imaging, optogenetics

^{*}Speaker

Cellular and systemic growth control by HR3 acting as a cholesterol sensor and regulating the conserved nutrient sensor Target of Rapamycin (TOR)

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Emerging evidence indicates that cholesterol, an essential lipid, has a critical function in regulating cell growth and thereby plays a critical role in health and disease. We have recently shown that cholesterol is a regulator of the conserved intracellular nutrient sensor Target of Rapamycin (TOR) pathway as well as the superimposed insulin-like signaling system, the main hormonal system controlling cellular and systemic growth. Increasing intracellular cholesterol levels either by dietary supplementation or through knockdown of the lysosomal cholesterol exporter Niemann-Pick Type C-1a (NPC1a) increases TOR activity, leading to increased cell growth. Mechanisms for lysosomal cholesterol-mediated TOR activation have been reported in other systems, but those mechanisms are not entirely conserved. Any conserved upstream cholesterol sensor that regulates TOR activity remains unclear. The *Drosophila* nuclear receptor HR3 (also known as HR46) acts as a regulator of cell-autonomous growth through modulation of the TOR pathway, and in humans the close ortholog, retinoid-related orphan receptor (RORα) binds cholesterol, and has been suggested to be a regulator of TOR activity. We have found that the HR3 is activated by cholesterol and alters TOR activity. Our findings indicate that feeding *Drosophila* larvae cholesterol-containing food induces TOR activation in a HR3-dependent manner. The results indicate that cholesterol:HR3 induced TOR activation, alters RNA processing pathways within 1 hour, and within 6 hours transcription, translation, and ribosomal alterations are seen in phosphoproteomics data. In RNAseq data, we observe that the expression of 1773 genes is altered within ten hours of cholesterol feeding, of which 980 are dependent on HR3 and TOR, indicating that these pathways are mediating a main part of the response to cholesterol. Dysregulation of the TOR, DHR3 (ROR), and insulin/IGF signaling systems are frequently linked with cancers, and thus our findings may further our understanding of the links between cholesterol, hormonal signaling, and growth control in cancer development.

Keywords: Target of Rapamycin, TOR, HR3, Cholesterol

*Speaker

Chitin binding proteins regulate Minute cell competition.

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Damaged cells can be actively removed from tissues by their healthy neighbours through a process known as cell competition, which is thought to be important for maintaining tissue health and preventing diseases such as cancer. *Minutes* are animals that have lost one copy of a ribosome subunit encoding gene, and *Minute* cells are outcompeted by wildtype cells in mosaic tissues via cell competition. In an RNAi screen for modulators of *Minute* cell competition in *Drosophila* we identified *vajk2* and *vajk3*, two members of the *vajk* family of genes, as enhancers of *Minute* cell elimination. Knocking down Vajk2 or Vajk3 in otherwise wildtype cells leads to transcriptional activation of *xrp1*, a transcription factor required for *Minute* cell competition, and Xrp1 downstream responses such as increased eIF2a phosphorylation. Furthermore, *vajk2*- or *vajk3*-RNAi expressing clones are eliminated by wildtype cells suggesting that these cells behave as losers. Vajk family genes have previously been implicated in binding to chitin, a secreted polysaccharide, which plays an important structural role in the fly cuticle. This suggests that chitin regulation and/or macromolecule secretion regulates Minute competition.

Keywords: cell competition, chitin, xrp1, cancer, Minute, ribosome

*Speaker

Conserved elements of post transcriptional regulation in stress protection of *Drosophila*'s adult progenitor cells

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In *Drosophila*, most larval cells are polyploid and they die at the metamorphic stage. In contrast, Adult Progenitor Cells (APCs) survive throughout the developmental process and give rise to adult structures. These cells are specified during embryonic development, they undergo several mitotic divisions during larval stages, remain diploid, and finally proceed into their terminal differentiation during the pupal stages. Both APCs and larval tissue cells are exposed to the same nutritional and hormonal cues, thereby suggesting that unique molecular components act within the APCs to differentially regulate the effect of external and intrinsic stimuli in their unique setting and in particular to escape cell death at metamorphosis.

We have shown that the *headcase* gene (known as *hdc* and recently renamed as *heca* in Flybase), that was originally identified by its specific expression in *Drosophila* APCs, is one of the components that these cells use to survive, grow and terminally differentiate. Heca acts at a systemic level, controlling the hormonal response during development, while in APCs it modulates the effects of the dTOR pathway, acts as a tumor suppressor and is implicated in the control of the Unfolded Protein Response.

Heca is the founding member of a group of homolog proteins identified from *C. elegans* to humans. In humans, its homolog, has been found associated with different kinds of cancers but its function has not yet been identified and its role remains controversial. We provide evidence that both proteins interact with a conserved group of molecular elements that are known to control the RNA metabolic cycle, protect cells against stressful stimuli and form part of the post transcriptional mechanisms of gene regulation.

Keywords: stress, adult progenitor cells, RNA regulation

*Speaker

DEVELOPING NOVEL TOOLS FOR IN SITU VISUALISATION OF HETEROPLASMIC MTDNA VARIANTS.

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Mitochondria, the powerhouses in our cells, hold their own genome (mtDNA). Eukaryotic cells often contain many hundreds to thousands copies of mtDNA. When a mutation occurs in one or more of these copies, this results in a mixture of mutant and wild-type mtDNA, known as heteroplasmy. Developmental and ageing-related expansion of mutant mtDNA heteroplasmy is the main cause of adult-onset mitochondrial disease, affecting 1 in 5,000 people, and plays a role in neurodegenerative disorders like Alzheimer's and Parkinson's disease. The processes that cause this expansion are poorly understood, primarily due to lack of good *in vivo* models to measure and modulate mitochondrial mutations in somatic tissues. The main aims of this PhD project are to develop and optimize tools to visualise and measure mtDNA mutations in situ. Based on single-molecule fluorescent *in situ* hybridisation with hybridisation chain reaction (smFISH-HCR) for detection of RNA, we have established a novel approach to visualise and distinguish different mtDNA molecules in cells and tissues. Taking advantage of *Drosophila* heteroplasmic strains, we have applied this in vivo, in the developing larval brain, and found extensive cell-to-cell variability of heteroplasmy levels between individual neural stem cells (NSCs), and between NSCs and their postmitotic progeny. We are further optimising sensitivity and specificity using heteroplasmic *Drosophila* cell lines, to confidently quantify heteroplasmy levels through smFISH-HCR. We will use these tools to investigate how transmission of mtDNA mutations is regulated during NSC differentiation in the *Drosophila* brain. We next plan to extend this approach to cultured mammalian cells with mtDNA mutations. Together, this will allow us to reveal novel molecular mechanisms underlying the developmental and ageing-related expansion of mutant mtDNA heteroplasmy, and study new treatments aimed at reducing the mutational burden to prevent late-onset neurological disorders.

Keywords: Mitochondria, mtDNA, heteroplasmy, in situ hybridisation

*Speaker

Difference in protein synthesis levels between cells plays a crucial role in cell competition

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Cell competition is a context-dependent cell elimination via cell-cell interaction, whereby cells with lower fitness (losers) are eliminated by neighbouring cells with higher fitness (winners). One of the common features of loser cells is a reduction in protein synthesis levels, which is observed in *Minute*, *Mahjong*, or *Hel25E* mutant losers in *Drosophila* imaginal discs. However, whether the difference in protein synthesis levels between winners and losers is essential for driving cell competition or not is still elusive. We have performed a genetic screen in *Drosophila* for suppressors of cell competition. Specifically, we introduced CRISPR-Cas9-mediated knockout mutations (1,500 genes in total) in wild-type winners and screened for those that suppressed elimination of nearby *Hel25E* mutant losers. As a result, we isolated 24 suppressors of cell competition. Intriguingly, 16 mutants out of 24 suppressors had mutations in proteins required for mitochondrial respiratory function. Mitochondrial defect in wild-type cells led to a reduction in protein synthesis levels in winners and suppressed cell death in neighbouring *Hel25E* mutant cells. Our data suggest that the difference in protein synthesis levels between cells is essential, but not sufficient, for causing cell competition.

Keywords: cell competition, cell death, protein synthesis

*Speaker

Dynamics of chromatin accessibility and gene transcription in intestinal stem cells during aging

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During aging, both chromatin structures and gene expression change. How they interact is not completely understood. Here we analyzed chromatin accessibility and gene expression in intestinal stem cells (ISCs) during aging by performing ATAC-seq and RNAseq. We substantiate that there are drastic changes in both chromatin accessibility and transcription during aging. At the global scale there was very little correlation between these two distinct changes, while transcription of a limited group of genes was correlated with chromatin accessibility. Through transcription factor motif enrichment analysis, we found that Trithorax-like (Trl) target genes undergo closing of chromatin accessibility and suppression of transcription during aging. Inhibition of Trl or ced-6, a target of Trl, suppresses aging-induced ISC proliferation. This study provides new insight into interaction of gene expression and chromatin accessibility in tissue stem cells during aging.

Keywords: Intestinal stem cells, Epigenetics, ATACseq, Aging

*Speaker

Epithelial sensing of extracellular glutamate underpins cell competition

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Cell competition is an evolutionary conserved quality control process that eliminates sub-optimal or potentially dangerous cells. For ‘unfit’ cells to be detected, their competitive status needs to be compared to the collective fitness of cells within the tissue. Although differential metabolic states at the interface of competing clones act as direct drivers of cell competition, how these are measured across tissues is not understood. Here, we have identified that the glutamate signalling circuit functions as an *in vivo* sensor of cell competition. Autocrine secretion of glutamate drives activation of its receptor NMDAR and the downstream CaMKII, PKA and CrebB effectors, promoting survival of fitter cells. Mechanistically, we find that loser cells trigger the production of TNF, leading to autocrine-mediated TNF-receptor signalling and JNK activation, which in turn metabolically reprograms them to produce and transfer lactate to winners. Preventing loser cells from transferring lactate to their neighbours removes the fitness disparity and nullifies cell competition. Further, clones that express the dMyc proto-oncogene co-opt the glutamate > NMDAR signalling circuit to acquire super-competitor status and subdue their wild-type neighbours. Targeting glutamate signalling converts dMyc ‘super-competitor’ clones into ‘losers’, highlighting new therapeutic opportunities to restrict the evolution of fitter clones.

Keywords: cell competition, glutamate, NMDAR, fitness, lactate, Myc, VGlut, TNF, JNK

^{*}Speaker

Exploring the effects of mechanical constraints acting on *Drosophila* midgut using the StretchCo, a 3D-printed device

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Cells and tissues undergo changing mechanical constraints during development, due to physiological activities of organs, or during tumorigenesis. The effects of these constraints have raised more and more interests in the last decade and have been mostly studied in developmental or pathological context. However, these constraints and their consequences on cells have received far less attention on complex, adult tissues. Due to its physiology, the *Drosophila* midgut tissue undergoes variations of mechanical constraints *in vivo*, due to food transit and muscle activity. We propose to use this well-characterized tissue to investigate how its cells, enterocytes, enteroendocrine cells, and in particular stem cells, cope with varying mechanical constraints. To mimic physiological mechanical constraints, we developed the StretchCo, a 3D-printed device that allow applying precise stretch or compaction of the tissue *ex vivo*, while imaging it with an inverted microscope. Our set-up enabled us to investigate and characterize biomechanical properties of the midgut, both at organ, tissue, cellular and sub-cellular levels. Using this tool, we want to monitor various cell features behavior, starting with cell and nucleus shapes, upon variation of mechanical constraints. Furthermore, we aim to compare these behaviors among the different cell types of the gut to unravel potential cell-type biomechanic specificities. Our project aims to define adult intestinal responses to mechanical constraints and will have broader implications for our general understanding of epithelial biology.

Keywords: *Drosophila* midgut, mechanobiology, homeostasis

*Speaker

Exploring the role of long non-coding RNAs in apoptosis-induced proliferation

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Apoptotic cells can induce proliferation of the cells surrounding them to compensate for their removal, thus maintaining tissue homeostasis and aiding in wound healing and regeneration. This evolutionarily conserved process is known as apoptosis-induced proliferation (AiP). Studies in *Drosophila melanogaster* report that AiP in proliferating cells is mediated through the initiator caspase Dronc, the *Drosophila* caspase-9 ortholog, and activation of the c-Jun N-terminal Kinase (JNK) pathway. Further, Dronc-dependent F-actin polymerisation and cellular reactive oxygen species (ROS) production activate JNK. However, it is not yet clear how this process is regulated. It is rapidly becoming evident that the non-coding genome plays a key role in a wide range of cellular processes. Specifically, long non-coding RNAs (lncRNA), greater than 200nt and lacking a protein coding ability, have been implicated in many biological processes and pathologies. Despite the lack of protein coding, lncRNAs are widely expressed, interacting with RNA, DNA, and protein, to function as regulators and mediators of gene expression, chromatin organization, protein interaction, and cellular responses. The area of lncRNA research is rapidly expanding as the fundamental importance of the non-coding genome is becoming more apparent. Therefore, the exploration of key regulators, including a role for lncRNAs, remains critical for further understanding of the conserved process of apoptosis-induced proliferation.

Keywords: Apoptosis induced Proliferation, Long non coding RNA, JNK, Caspases

*Speaker

HIF-1 α restrains TOR signalling to prevent cellular stress arising from developmental growth-induced hypoxia

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In most instances, growth does not terminate abruptly but instead progressively decelerates as tissues and organs approach their final size. Despite the pervasiveness of this phenomenon, its molecular basis remains poorly understood. We are using the developing L3 *Drosophila* wing discs to investigate the molecular mechanism that could underpin growth deceleration. We used transcriptomic profiling of precisely timed L3 wing discs to identify genes that correlate with the decreasing growth rate. This revealed that growth deceleration is accompanied by a gradual decrease in mRNAs encoding components of aerobic respiration, suggesting that perhaps, wing discs become mildly hypoxic during growth. To assess this possibility, we developed a highly sensitive reporter of Sima/HIF-1 α , the master regulator of the response to environmental hypoxia. Despite larvae being raised in normoxia, the reporter activity was found to rise steadily from early L3 before reaching a maximum at the time of pupariation. Using RNAi, we provide evidence that Sima/HIF-1 α down-regulates TOR signalling activity during normal development. Conversely, we found that excess TOR activity leads to increased hypoxia. Thus, growth increases the demand on oxygen, which becomes limiting with increasing tissue size. We suggest that developmental growth-associated hypoxia contributes to growth deceleration and that Sima/HIF-1 α , which mediates the response to environmental hypoxia, ensures that TOR activity does not impose impossible demands on oxygen availability. In the absence of Sima/HIF-1 α , growth is still constrained by oxygen availability but the lack of a brake on TOR activity leads to cellular stress. In summary, we propose that limited oxygen availability could contribute to growth deceleration and that Sima/HIF-1 α has been co-opted to ensure that tissues grow within their means. Modulation of TOR activity by the combutant (oxygen) mirrors the well documented effects of the comburant (nutrient).

Keywords: Sima, HIF1 α , oxygen, hypoxia, TOR signalling, cellular stress, stress, development, growth, growth rate, growth deceleration, RNA, Seq, reporter, wing disc

^{*}Speaker

Identification of novel venom proteins required for *Drosophila* epithelial cell death by the parasitoid wasp *Asobara japonica*

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Parasitoid wasps are one of the most diverse groups of animals. Their ecological and taxonomic characteristics have been actively studied for a long time. However, the molecular mechanism of parasitism is poorly understood due to many technical limitations.

To address this issue, we use *Asobara japonica*, a Braconidae parasitoid wasp, and *Drosophila* species as a model of parasitoid wasp-host interaction. *A. japonica* parasitizes a wide range of *Drosophila* species. The adult wasp lays an egg inside the host *Drosophila* larval body. The wasp infection does not interfere with the process of pupariation, but eventually the adult wasp emerges from the host pupal case.

We found that host imaginal discs shrank after *A. japonica* infection, which was mediated by apoptosis, autophagy, and inhibition of cell proliferation, while other tissues looked intact. Furthermore, we found that this specific cell death of host imaginal discs was caused by the venom injected into the host by the wasp. To elucidate the molecular mechanism of the venom-mediated imaginal disc shrinkage, we performed whole genome sequencing and developed a double-stranded RNA injection-based gene knockdown method in *A. japonica* (Kamiyama, Shimada et al., 2022, *DNA Res.*). Transcriptomics, proteomics, and comparative genomics approaches allowed us to narrow down 63 genes as candidates that are highly and exclusively expressed in the *A. japonica* venom gland. After screening these candidates using double-stranded RNA injection-based gene knockdown, we identified two candidate genes, both of which encode novel secretory proteins. When each of the two identified genes was knocked down in the wasp, the venom-induced apoptosis, autophagy, and inhibition of cell proliferation in the host imaginal discs were suppressed. These results suggest that these two genes are required for the host imaginal disc shrinkage after *A. japonica* infection. Our results provide new insights into the venom function of the parasitoid wasp to manipulate its host development.

*Speaker

Keywords: parasitoid wasp, cell death, autophagy, venom, secretory protein

Insights into mechanical cell competition in epithelia: uncovering the role of growth and interfacial tension to generate compaction-driven cell death

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Epithelial tissues are present in multiple parts of the animal body and consist of one or multiple layers of joined cells that separate organs from the external environment. The maintenance of epithelial integrity is crucial as its regular disruptions can lead to chronic diseases. While most studies on homeostasis and development in epithelia have focused on changes in size and shape mediated by cell growth and division, the contribution of cell survival and cell death (apoptosis) has received little attention.

Cell competition is the process by which cells with low proliferation rate and survival properties are forced to die through apoptosis by the surrounding cells. While this limits the appearance of (genetic) errors in our organs, the activation of some oncogenes related to cell growth and cell survival in clones can drive the elimination of healthy wild-type (WT) neighboring cells and promote the expansion of the pretumoral population.

Previous studies in the *Drosophila* pupal notum and mammalian cells have shown that fast-growing and apoptosis-resistant clones can induce cell death in neighboring WT cells through the local compaction of the WT tissue, a process called mechanical cell competition. However, the biological conditions that can generate such compaction and the specific cellular processes through which compaction modulates survival probability are not yet fully understood.

In this project, I aim to characterize the mechanical perturbations that promote local compaction and apoptosis of cells. Through genetic perturbations combined with live imaging, I identified both growth and interfacial tension as drivers of cell compaction. These results are based on precise spatiotemporal localisation of cell death, clone shapes and growth quantification, live-sensor-based caspase activity measurements and force inference techniques. We then combine these results to create an in-silico vertex-based model in which we validate the mechanical parameter that can generate cell compaction. Finally, I validated these findings by the identification and use of two genetic conditions that modify growth and tension independently. I also determined that mechanical cell competition might lead to different final states for the tissue, either completely overrun by the oncogenic clone or not, depending on the genetic condition through which compaction is generated. These findings shed new light on the roles of mechan-

^{*}Speaker

ical forces in epithelial tissue homeostasis and may have implications for the understanding of tissue growth and of the first phases of cancer development.

Keywords: apoptosis, mechanical cell competition, epithelia, compaction

Investigating growth control in the *Drosophila melanogaster* abdomen using transcriptomics

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Growth and pattern formation during development are regulated by a complex network of signals that act together to ensure robust and reproducible growth. The coordinated effect of these inputs is captured in gene expression changes that instruct the morphogenetic behaviours of individual cells. Here, we utilise bulk and single-cell transcriptomics to explore the signals that regulate developmental growth in the *Drosophila melanogaster* abdominal histoblast model system. We examined five stages of histoblast development, to reveal the transient cell states and the mechanisms that drive progression through development, and, eventually, to growth arrest. Analysis of gene expression changes between proliferative and arrested states revealed a decrease in the expression of glycolytic enzymes concomitant to growth arrest (at 26 h after puparium formation), indicating a switch from glycolysis to oxidative phosphorylation. We also observe an increase in the expression of gap junction components along histoblast development (16-36 h after puparium formation). We utilise live imaging of histoblast development in mutants for relevant differentially expressed target genes, coupled with our bespoke image analysis pipeline, to identify the effect of metabolism and cell-cell communication in histoblast proliferation dynamics and transition to growth arrest.

Keywords: development, growth control, growth arrest, proliferation

*Speaker

Investigating the metabolism of genetically-induced tumors in the *Drosophila* midgut

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Cancers are usually considered of monoclonal origin, i.e arising from a unique cell, and their energy metabolism considered relying on glucose fermentation to lactate rather than on mitochondrial respiration. Either characteristic has been challenged by a number of studies. The goal of my PhD project was to decipher metabolic needs of a *Drosophila* midgut tumor model and to demonstrate their polyclonality. These tumors are induced at a given time and express GFP, facilitating their monitoring. They result from the loss of the tumor suppressor Apc (Adenomatous polyposis coli) and the ectopic expression of an oncogenic form of Ras (RasV12). These two genetic events are typically found in human colorectal cancers. By RNA-interference knockdown targeted to the tumor cells, we have evaluated the requirement of 60 metabolic enzymes and follow the tumor polyclonality. Our results suggest that despite their genetic identity, these tumors are always polyclonal and exhibit a metabolic heterogeneity. We propose that these intestinal tumors arise from several clones that fulfill different metabolic functions, thereby allowing tumor progression.

Keywords: *Drosophila*, tumor, metabolism, polyclonal, heterogeneity

*Speaker

Lack of apoptosis causes cellular senescence and tumorigenesis in *Drosophila* epithelial cells.

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Programmed cell death (apoptosis) is a homeostasis program of animal tissues designed to remove cells that are damaged by physiological insults or unwanted during development. To assess the functional role of apoptosis, we have studied the consequences of subjecting *Drosophila* epithelial cells defective in apoptosis to stress or genetic perturbations that normally cause massive cell death. We find that many of those cells acquire persistent activity of the JNK signaling pathway, which drives them into senescent-like phenotype, characterized by arrest of cell cycle progression, cell hypertrophy, Senescent-Associated β -gal activity (SA- β -gal), ROS production and Senescent-Associated Secretory Phenotype (SASP). These cells also show migratory properties. These JNK-dependent, senescent cells are responsible for the tumor overgrowth generated in apoptosis-deficient tissue. Furthermore, we have identified two classes of senescent cells in the wing imaginal disc: 1) those that localize to the appendage part of the disc, express the *upd*, *wg* and *dpp* signaling genes and generate tumor overgrowths, and 2) those located in the thoracic region, which do not express *wg* and *dpp* neither induce tumor overgrowths. These tumor overgrowths depend on the activation of the Wg, Dpp and JAK/STAT pathways. Whether to become tumorigenic or not seems to depend on the original identity of the cell prior to the transformation. We also find that the *p53* gene contributes to senescence by enhancing the activity of JNK, being a major activator of the pathway after stress or DNA damage.

Keywords: Apoptosis, Senescence, JNK pathway, tumorigenesis

*Speaker

Non-cell autonomous autophagy that drives cell competition

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Cell competition is a quality control process that selectively eliminates unfit cells from the growing tissue. For instance, cells with reduced protein synthesis ("losers") are eliminated from *Drosophila* imaginal epithelium when surrounded by wild-type cells ("winners"). Cell competition can be triggered by various gene mutations, but the upstream events of cell death during cell competition has still remained elusive. We have previously found that loser cells such as *ribosomal protein*, *RNA helicase (Hel25E)*, or *Mahjong* mutant cells are eliminated via autophagy-mediated induction of the cell death gene *hid* when surrounded by wild-type cells. Moreover, we have recently found that 'super-competition' in which oncogenic mutant cells such as Hippo pathway mutant cells eliminate neighboring wild-type cells is also regulated by non-autonomous induction of autophagy and subsequent induction of *hid* in wild-type losers. These findings suggest that non-autonomous induction of autophagy in loser cells is a common mechanism that drives cell competition. Therefore, we tried to elucidate the mechanism by which autophagy is induced specifically in loser cells at the boundary between winner and loser clones. A series of genetic analyses identified the upstream mechanism of non-cell-autonomous autophagy in loser cells, which will be discussed.

Keywords: Cell competition, Cell death, Autophagy

*Speaker

PWP1 mediates intestinal stem cell homeostasis in a nutrient dependent manner and affects aging

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One of the hallmarks of aging is the loss of regenerative capacity and homeostasis in tissues with high cellular turnover, where integrity is maintained by self-renewal and differentiation of resident stem cells. Importantly, the ability to replace damaged cells is gradually reduced as stem cells age and nutrition appears to have a prominent effect on the maintenance of their functions. The intestinal epithelium, characterized by high cellular turnover and remarkable plasticity in response to nutrient intake, is an optimal model for studying stem cell nutrient regulation.

It is known that the nutrient-dependent mTOR signalling pathway is activated in intestinal stem cells (ISCs) during regenerative growth, and unpublished work from our lab has shown that nutrient-induced mTORC1 activation contributes to ISC differentiation. These data suggest that this pathway might exert a pivotal role in the regulation of intestinal homeostasis in response to nutrients. Previous research from our lab discovered a nutrient-dependent role for Periodic tryptophan protein 1 (PWP1) downstream of the nutrient-responsive Insulin/mTOR signalling pathway in *Drosophila* fat body. PWP1 is known to be a chromatin-binding protein implicated in germline stem cell regulation, but its role in somatic stem cells still remains concealed.

My research focuses on exploring the regulation and function of PWP1 in intestinal epithelium homeostasis during aging. I have found that PWP1 is expressed in fly intestine in a region-specific manner and that its function is strongly regulated by nutrient intake. Its genetic modulation in ISC-derived cells affects both cellular differentiation and growth, thus altering stem cell processes that are crucial for promoting and maintaining a healthy intestinal homeostasis. Interestingly, the effects of PWP1 in the intestinal epithelium appear to be especially dramatic in aging flies, and any unbalance in its cellular expression is detrimental for organismal lifespan. Altogether, my work highlights a new role for PWP1 in the in the context of nutrient regulation of ISCs and will contribute to a better understanding of the relationship between aging, cellular homeostasis and nutrient availability.

Keywords: Aging, intestinal stem cell, nutrient sensing, lifespan, homeostasis, cell proliferation

*Speaker

Pvf1-Pvr signaling and its role in homeostasis and regeneration.

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Intestinal homeostasis is important for organism health, with steady state turnover required, to balance cell loss with newly divided and differentiating cells. We identified Pvf1, a growth factor related to platelet-derived growth factor (PDGF) and vascular-endothelial growth factor (VEGF), as a hit in an unbiased genetic screen for niche-derived signals required for optimal resistance to oral infection. Previous work has shown that autocrine Pvf2/Pvf3-PvR signalling is required for intestinal stem cell (ISC) turnover in homeostatic conditions. Building on this, we have worked on elucidating the role of Pvf1-PvR signalling during homeostasis and determined a key function in controlling enteroblast-to-enterocyte maturation in homeostatic conditions. We have further determined that this homeostatic role of Pvf1 is specific to the R2 region of the midgut, therefore demonstrating a potential function for Pvf1 in gut regionalisation. Recently, our interest has turned to tissue regeneration. Consistent with a function of Pvf1 in regeneration, we have shown that knockdown of its receptor, PvR, in ISCs, is not only required during homeostasis, as previously shown, but is also critical in mediating the proliferative response associated with tissue repair.

Keywords: gut, homeostasis

*Speaker

ROLE AND REGULATION OF NF- κ B - DRIVEN TUMORIGENESIS

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Seminal studies in flies and later mammals established that NF- κ B transcription factors direct development and are master regulators of inflammation and innate immunity. Additionally, there is extensive evidence that NF- κ B is active in mammalian cancer cell lines where it promotes survival, proliferation, epithelial-to-mesenchymal transition (EMT), invasion, angiogenesis and metabolic reprogramming. In flies, NF- κ Bs have been implicated in the phenomenon of cell competition where NF- κ B drive Hid or Rpr-driven cell death in losing neighbouring cells adjacent to either Myc high, or Yki high (Fat-/-) hyperplastic cells. However, evidence for cell-autonomous functions of NF- κ Bs in tumorigenesis is lacking. Using a *Drosophila melanogaster* carcinoma model, we identified an inflammation signature in the tumor cell population. Functional screening identified NF- κ B/Dorsal as being required for growth of RasV12, scribRNAi tumors. Here we describe the activation, regulation and downstream functions of Dorsal in growth and invasion in this model, extending the similarities of NF- κ B functions between mammals and flies to also apply to tumorigenesis.

Keywords: NF κ B, Cancer, survival, invasion

Role of the trachea in the formation of abdominal epidermal layer during metamorphosis of *Drosophila*

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Development requires a tight coordination between tissues and organs in order to generate a complete adult organism. Such coordination is particularly relevant during insect metamorphosis when larval cells are removed whereas the adult progenitor cells proliferate and proceed to the formation of the adult structures. One of the tissues undergoing deep remodeling during the metamorphosis is the tracheal system. The *Drosophila* larval tracheal system consists of more than 10000 interconnected tubular segments. The major signaling pathway regulating the branching pattern of the larval tracheal system is the FGF pathway, where branchless (*bnl*) acts as the ligand and breathless (*btl*) as the receptor. *bnl* acts as a chemoattractant driving the outward migration of *btl*-expressing tracheal cells ultimately shaping the larval trachea and generating a network of interconnected tubes that will serve and form the larval tracheal system. During metamorphosis, the tracheal system of *Drosophila* suffers a deep remodeling. Whereas, the abdominal larval tracheal branches degenerate, FGF/*bnl* signaling dependent proliferation and posterior migration of the quiescent tracheal progenitor cells form the adult/pupal abdominal tracheae (PAT). Surprisingly, depletion of the adult trachea by inactivation of FGF/*bnl* signaling, abolishes the abdominal epidermal formation, suggesting a connection between remodeling of the trachea and development of the adult abdomen. Adult abdominal epidermis form from the adult progenitor cells called histoblasts, specified during embryogenesis, and remain quiescent in small nests during the juvenile stages. At the onset of metamorphosis, the histoblasts undertake a rapid proliferation that allows them to expand and make a continuous layer at the expense of the surrounding larval epidermal cells (LECs), which ultimately commit programmed cell death. Our current work focuses on examining how pupal abdominal trachea and adult ectoderm develop during metamorphosis and identifies the signals that coordinate these processes.

Keywords: Trachea, Spiracular branch, Tracheoblasts, FGF, Histoblasts, Larval epidermal cells, Cell migration, Metamorphosis

*Speaker

Roles of Tetraspanins in apoptosis-induced proliferation

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Apoptosis is a critical process that eliminates damaged cells in response to environmental stress. It has been revealed that apoptotic cells can stimulate additional proliferation of their neighbouring healthy cells to maintain tissue homeostasis, a phenomenon termed apoptosis-induced proliferation (AiP). Uncontrolled AiP can lead to tissue overgrowth. Therefore, AiP is relevant to both regeneration and tumourigenesis. Caspases, key executioners of apoptosis, can trigger AiP via activation of the stress response c-Jun N-terminal kinase (JNK) signalling pathway and its downstream secretion of the mitogens such as Wingless (Wg/Wnt), Spitz (Spi/EGF) and Decapentaplegic (Dpp). However, our understanding of AiP is still far from complete. To address this, we have conducted a genetic screen with identification of Tetraspanins, a family of transmembrane proteins involved in diverse biological processes including intracellular signalling, as critical regulators of AiP. I will present our systematic analysis of 36 tetraspanins in *Drosophila* and their roles in AiP.

Keywords: Apoptosis, induced proliferation, Tetraspanin, Caspase, JNK

^{*}Speaker

TNFR Wengen activation is ROS-mediated in *Drosophila* wing imaginal disc regeneration.

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The family of tumor necrosis factor receptors (TNFR) consists of cytokine receptors capable to bind to the tumor necrosis factors (TNF) via a cysteine-rich extracellular domain to control apoptosis, survival, and differentiation. In *Drosophila* two TNFR's, Wengen (Wgn) and Grindelwald (Grnd), have been identified. We first studied the involvement of Wgn and Grnd in survival and apoptosis. Our findings demonstrate that Wgn is required for survival by protecting the cells from apoptosis. This protective role is mediated by Traf1 and MAP3K Ask1 by the activation of the MAPK p38. In contrast, Grnd plays an apoptotic role via the activation of JNK-dependent apoptosis as previously described by others (1,2). We wondered whether these two TNFR are involved in regeneration. We induced cell death in imaginal disc epithelia, by temporarily activation apoptosis in specific zones (genetic ablation), to monitor regeneration. We found that Wgn but not Grnd is necessary to regenerate the apoptotic zone. Grnd requires the Eiger TNF ligand to activate JNK-dependent apoptosis (1,2). We found that apoptotic cells accumulate Eiger, likely to reinforce the apoptosis induced. At the molecular level, epithelial cells involved in regeneration activate Wgn in a ligand-independent manner. In these regenerating cells, Wgn is activated by the oxidative stress generated by the reactive oxygen species (ROS) produced after genetic ablation and not by Eiger. We found that Wgn is required for p38 activation in regeneration. Furthermore, based on protein 3D structural conservation, the extracellular Cys-rich domain of Wgn is highly homologous to the vertebrate TNFR1A, a TNFR also involved in cell survival in development and cancer. Taken together, our results show a novel role for Wgn triggered by ROS that ensures the p38-survival response in regeneration.

1. Andersen, D., Colombani, J., Palmerini, V. et al. The *Drosophila* TNF receptor Grindelwald couples loss of cell polarity and neoplastic growth. *Nature* 522, 482–486 (2015).

2. Palmerini, V., Monzani, S., Laurichesse, Q. *et al.* *Drosophila* TNFRs Grindelwald and Wengen bind Eiger with different affinities and promote distinct cellular functions. *Nat Commun* 12, 2070 (2021).

Keywords: Wengen, survival, regeneration, ROS, p38

*Speaker

The PIWI protein Aubergine regulates intestinal stem cell proliferation via piRNA-independent mechanisms

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Adult stem cells are essential actors in the maintenance of basal homeostasis and damage-induced regeneration in self-renewing tissues. This relies on an accurate regulation of the proliferation and the differentiation of stem cells by highly conserved and coordinated signalling pathways. Consistently, disorders in signalling activity may lead to homeostasis disruption inducing age-associated tissues dysfunctions and a wide range of cancers.

To identify and understand the molecular mechanisms controlling and adjusting stem cell behaviour to fit the proliferative demands of the intestinal epithelium, we use the adult *Drosophila* midgut. Previous pioneer works shed light on multiple conserved signalling pathways involved in the regulation of intestinal stem cells (ISCs) proliferation such as Wnt, JAK/STAT or EGFR signalling. Unexpectedly, the PIWI pathway, known for its role in the repression of transposable elements in the germline, is also essential for ISCs maintenance and function. In addition, we discovered that Aubergine (Aub), a component of the PIWI pathway, is specifically expressed in ISCs and required for their proliferation upon intestinal damage and upon tumorigenesis. Our work revealed that Aub acts independently of its canonical role described in the PIWI pathway. Instead, Aub positively regulates the expression of multiple subunits of the eukaryotic translation initiation factor 3 (eIF3). eIF3 is a key complex in the initiation of protein translation, a main feature of ISCs proliferation. In absence of Aub, the expression eIF3 complex is downregulated leading to a decrease of protein translation and ISCs proliferation.

Interestingly, analysis in humans revealed that PIWIL1, the homolog of Aub in mammals, is also increased in colorectal cancer patients. These discoveries raise the interesting question about the conserved role of Aub in the regulation of protein translation in humans. Altogether, our works reveal a new mechanism involved in the regulation of stem cell behaviour and may lead to the identification of new therapeutic targets to prevent stem cell dysfunction in colorectal cancer patients.

Keywords: Intestinal Stem Cell, Regeneration, PIWI pathway, Tumorigenesis

*Speaker

The Role of Lipid Droplets in *Drosophila* Necrotic Germ Cell Death

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The dogma that apoptosis is the only form of cell death involved in the elimination of super-numerary cells during development and pathologies has been challenged with the understanding that necrotic cell death is not only accidental but can be genetically controlled. It is now accepted that programmed necrosis regulates cell homeostasis during differentiation. This is for example the case of the elimination of germ cells, which involves programmed necrotic cell death during fly spermatogenesis. To study the kinetics of events in programmed necrosis, we identified and characterize novel testes-specific markers of necrosis including the release of nuclear localized proteins and lipid droplet (LD) accumulation in dying germ cells. We have observed that lipid droplets (LDs) particularly accumulate in necrotic germ cells at the apical tip of the *Drosophila* testis, which could be a consequence of a modification of LD turnover during the necrotic cell death process. In addition, to determine if LDs contribute to necrosis regulation in germ cells, we examined the role of regulators of LD lipolysis and biosynthesis. We showed that lipase brummer (*bmm*) mutant reduces, while germ cell overexpression of *bmm* enhances necrotic germ cell death. Furthermore, the germ cell inhibition of SREBP, a major transcription factor that controls lipid metabolism and LD formation, enhances germ cell death. Collectively, these results suggest a protective role of LDs in germ cells to limit necrotic germ cell death. We are further investigating the relationship between lipid droplet homeostasis (biosynthesis and degradation) in the control of germ cell elimination.

Keywords: *Drosophila* testis, germ cell, lipid droplet, programmed necrosis

*Speaker

The deubiquitinating enzyme dUSP36 functions non-catalytically to promote cell growth

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Deubiquitinating enzymes (DUBs) are specific proteases removing ubiquitin moieties from ubiquitinated proteins. They regulate many cellular processes including protein stability, cell signalling or endocytosis. Their dysregulation has been linked to many human pathologies, enlightening the importance of understanding their mechanisms of action.

In *Drosophila*, the DUB dUSP36 is involved in several physiological processes including cell and organismal growth, immunity, stem cell maintenance and autophagy. We have shown that a nucleolar isoform of dUSP36 promotes cell growth and proliferation by regulating the stability of dMYC, the *Drosophila* homolog of the MYC oncogenic protein. The control of MYC stability by USP36 is conserved in human cells indicating that the MYC-USP36 complex represents an evolutionary conserved regulatory node.

To further understand dUSP36 functions *in vivo*, we have generated a catalytically inactive version of the endogenous dUSP36 protein by mutating by CRISPR-Cas9 its catalytic cysteine to a serine residue. Compared to *Drosophila dUsp36* null mutants, which produce no dUSP36 protein and display strong cell growth defects leading to larval lethality, *dUsp36* catalytic dead mutants display very mild cell growth defects and survive to adulthood. However, they are more sensitive to dMYC dosage than wild-type control flies. These results show that the catalytic dead version of dUSP36 can promote cell growth almost as well as the wild-type protein, and alter our understanding of the mechanisms by which dUSP36 regulates dMYC.

Keywords: ubiquitination, deubiquitination, MYC

*Speaker

Transferrin 2 affects homeostasis and ageing of the *Drosophila melanogaster* midgut

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Loss of the balance between cell death and production, which often occurs with old age, can lead to a variety of diseases. As the intestine is a tissue with a high turnover rate, homeostatic control is especially important. Stem cells are responsible for maintaining tissue homeostasis, and previous work from our lab and others has shown that proteins secreted by the stem cells play important roles in the control of *Drosophila* intestinal homeostasis. A screen of conserved intestinal stem cell-secreted proteins identified Transferrin 2 (Tsf2) as a regulator of homeostasis. We have found that changing the levels of Tsf2 expression in stem and progenitor cells in the *Drosophila* midgut disrupts homeostasis and alters lifespan, and that Tsf2 expression changes with age. Furthermore, although Tsf2 acts as a septate junction protein in other tissues, changing expression levels in the *Drosophila* midgut does not impact its barrier function, suggesting an alternative mechanism of action. Our findings may have relevance to human health and disease as the mammalian homologue of Tsf2 has been associated with tumorigenesis.

Keywords: intestinal stem cells, transferrin 2, homeostasis, ageing

*Speaker

Understanding Contact Inhibition Locomotion Switching in *Drosophila* *Melanogaster*

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Immune cells typically use a surveillance mode of migration so that pathogens, injuries or apoptotic cell body can be detected early for clearance. This surveillance mode means that migrating immune cells are spread apart from each other, and even upon contact will not clump together. Studies in *Drosophila* have shown that their macrophages are spread throughout the organism due to the phenomenon of Contact Inhibition Locomotion (CIL). CIL is defined as the occurrence where cells, upon contact, reorganise their cytoskeleton network resulting in deflection or repulsion away from each other. Our *in vivo* studies have shown that these same *Drosophila* macrophages switch CIL off in the presence of apoptotic cell clearance. However, it is still unclear how cells switch between the CIL-on and CIL-off state, hereon termed *CIL switching*. Here, we show that CIL switching can be mimicked in *ex vivo* and uncover its mechanism. We found that macrophages seeded on glass naturally exhibit CIL. Whereas when we then seeded the macrophages on a substrate mimicking an apoptotic environment, CIL off behaviour was exhibited. From RNAseq results, we find that microtubule (MT)-related pathways are heavily involved in the role of CIL switching. We found that by inhibiting MT polymerization of cells were seeded on glass, CIL is lost. Our RNAseq data also highlighted a difference in metabolic pathways regulation between cells seeded on glass and on the apoptotic substrate. Our studies elucidated that glutamate metabolism influenced CIL behaviour as knocking down a key metabolic gene rescued CIL, despite the macrophages being seeded on the apoptotic-mimic environment. We anticipate that this our *ex vivo* set up offers new means of studying migratory behaviours in cells during exposure apoptotic eat-me signal. While the role of MT, metabolism and migration has been exceptionally studied individually, what connects MT activity, metabolism and how they affect cell-cell interaction has yet to be bridged. Given the importance in proper apoptotic cell clearance, especially during development, our study offers new insight into how immune cells interact with each other during apoptotic clearance.

Keywords: Apoptosis, Contact Inhibition Locomotion, metabolism, macrophage

*Speaker

Understanding the cellular and molecular mechanisms of growth control by the steroid hormone ecdysone.

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The precise and coordinated regulation of organ growth ensures proper adult body size and proportions. During development, organs rely on a specific combination of autonomous and non-autonomous inputs to achieve their appropriate size. At the systemic level, hormones play a key role in orchestrating growth among body parts by modulating organ-intrinsic growth programs according to developmental and environmental signals. In insects, the steroid hormone ecdysone serves as a major timer controlling the emergence of developmental events. Beside this well-established function, ecdysone is also emerging as a growth-promoting hormone controlling and coordinating the size of organs. Yet, the tissular, cellular and signaling mechanisms mediating steroid-dependent growth control are largely unknown. This constitutes an important limitation to our understanding of recently identified ecdysone-dependent processes such as interorgan growth coordination and organ size adjustment. Using genetic tools to modulate intracellular ecdysone levels specifically in the wing pouch during larval development, we analyzed tissue and cellular growth parameters. Our results indicate that ecdysone impacts growth of imaginal tissues by acting primarily on cell growth. Because cell growth is coupled to cell divisions during the feeding larval stages, increasing intracellular ecdysone leads to an increase in cell number, while lowering it eventually reduces cell number by decreasing cell proliferation rate. Accordingly, our findings indicate that ecdysone is required to sustain protein synthesis, opening new perspectives to identify its downstream molecular effectors for growth control.

Keywords: Ecdysone, Growth control, Wing imaginal disc, Cell growth

*Speaker

When to stop: new perspectives on organ growth in the *Drosophila* wing

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Organ size determination relies on complex mechanisms of growth control during development. Two main parameters govern the final size of a tissue: its growth rate and the duration of the growth period. In addition, growth must be coordinated with morphogenetic processes to ensure proper organogenesis. While tissue growth relies on established, conserved signaling pathways, the cellular and molecular mechanisms underlying growth arrest remain largely unknown. The imaginal discs of *Drosophila* have been extensively used as a model to unravel the autonomous and systemic signals involved in organ size determination. Discs are highly proliferative during the larval stages and undergo morphogenesis during the pupal stage. So far, growth of wing primordia has been mainly assessed through cell proliferation. Using volume measurements as a proxy for tissue mass, we observed that growth of wing primordia continues after larval development and is uncoupled from cell proliferation. These findings question the classical dogma that growth is restricted to the larval stage and followed by pupal morphogenesis. In addition, we used perturbations of wing development as an alternative approach to the underlying mechanisms of size determination. We found that growth perturbations early in development are corrected by a catch-up growth mechanism operating in early prepupa. This suggests that wing primordia detect and correct growth defects at the prepupal stage according to a yet unknown mechanism of "sizostat". I will present our recent findings supporting a new model for growth, growth arrest and size determination of the wing primordia.

Keywords: size control, organogenesis, growth arrest, *Drosophila* wing, catch up growth

*Speaker

smORF peptides molecular functions in *Drosophila* growth and fertility

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Recent technological breakthroughs have revealed that small ORFs, with size inferior to 100 codons and considered as non-coding, can be translated into thousands of small ORF (smORF) peptides in all organisms. Some studies have shown that smORF peptides can interact and regulate canonical protein activities. Therefore, they constitute a large regulator pool with unknown functions. Our previous work identified developmental regulator smORF peptides, of which several were encoded by polycistronic genes. We thus focused our functional analyses on both the highly conserved smORF peptide Sem1/DSS1 and unknown smORF peptides encoded by a bicistronic RNA that we named Tic and Tac. Sem1 is an intrinsically disordered protein that is known to interact *in vitro* with several macro-molecular complexes and it regulates their activity. First, we showed that Sem1 is essential for development and its deletion leads to apoptosis. Its structure-function analysis *in vivo* highlights amino-acid regions necessary for its function and we deciphered the involvement of each part of the peptide in growth and tumoral development. On the other hand, we demonstrated that Tic and Tac are two peptides translated from the *tictac* bicistronic gene that is expressed in male reproductive organs. Transcriptomic data on these organs reveals that the absence of *tictac* leads to transcriptional deregulation, with 10% of differentially expressed genes encoding seminal fluid components. Moreover, the absence of *tictac* in males affects female Post-Mating Response (PMR), showing that *tictacKO* males produce a less efficient seminal fluid. Finally, deletions of each of these smORF peptides show that Tic and Tac are both necessary in regulating PMR but are not functionally redundant. These studies highlight that smORF peptides constitute a pool of misunderstood regulators that can be involved in human pathologies like cancer or fertility.

Keywords: smORF, peptide, *Drosophila*, growth, bicistronic RNA

*Speaker

Population genetics & evolution

Adaptation of insect Endogenous Retrotransposons to the Transcriptional Network of their host

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Endogenous retroviruses (ERVs) play an important role in the evolution of their host's genomes by providing cis-regulatory elements (CREs) to enrich host gene regulatory networks. But how and under which pressure ERVs first adapted their CREs for expression and replication in their host remain poorly understood.

An ideal system to study the diversification of viral CREs is the *gypsy/gypsy* clade of insect ERVs (iERVs), which are expressed in multiple cell type niches of the *Drosophila* ovary. We recently reported that most of these iERVs are expressed in the somatic cells that surround the developing germline. However, during evolution, some iERVs have lost their infectivity gene, and this was accompanied by a transition from somatic expression to exclusive germline expression. Our previous work indicated that the different iERV expression patterns are encoded within the LTR and 5'UTR sequences of retrotransposons, suggesting that they have adapted their CREs to the diverse ovarian "ecosystem" (1).

We will present our ongoing efforts towards understanding how the CREs of iERVs changed in response to the loss of infectivity in order to drive expression specifically in the germline. Towards this end, we generated an array of transgenic reporter lines expressing lacZ under control of the LTR and 5'UTR sequences of somatic and germline expressed iERVs. We confirmed that the relevant CREs are contained within the respective iERV control regions as they recapitulate the respective endogenous expression patterns. Notably, we found the transition from somatic expression to germline expression requires sequences present specifically in the 5'UTRs, which are mostly dispensable for somatic expression. This suggests that the switch to germline expression required loss of soma-specific CREs in the LTR and gain of germline-specific CREs in the 5'UTR.

1. Senti et al., 2013, BioRxiv

Keywords: Transposable elements, retrotransposons, transcriptional regulation, oogenesis

*Speaker

Contribution of transposable elements in the sex gap longevity of different *Drosophila* species

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In *Drosophila*, like in many other animal species, females tend to live longer than males, a phenomenon known as sex gap in longevity (SGL). One of the possible causes underlying this phenomenon could be related to the high number of transposable elements (TE) in the Y chromosome (toxic Y effect). TE activity is normally repressed by epigenetic mechanisms. However, it is known that this regulation is disrupted with age. Since the Y chromosome is rich in TEs, more TEs may become active in old males than in old females, generating more somatic mutations, and reducing longevity in males. In this work, we studied the natural variation in SGL in several natural populations of three different *Drosophila* species that vary in their TE content: *Drosophila melanogaster*, *Drosophila simulans*, and *Drosophila sukukii*. Furthermore, we found that the replacement of the Y chromosome between strains with different SGL reduces male lifespan over generations and thus increases SGL, suggesting an important role of the Y chromosome in male longevity. Finally, RNA-seq analysis from old and young flies suggested that there is an increased number of upregulated TE families in old samples, and more specifically in old males compared to old females, and that the total fraction of transcripts derived from repeats increase during aging depending on the species and the population tested. Overall, this work tries to better understand the genomic differences that lead to variation in longevity patterns between sexes, and emphasizes the importance of TEs in male longevity.

Keywords: Transposable Elements, longevity, natural populations, Y chromosome

*Speaker

Exploring the role and evolution of male Accessory gland's Secondary Cells in Drosophilids

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In *Drosophila*, the male accessory glands produce most of the proteins found in the seminal liquid that are transferred to females during mating. Among these proteins, the Sex-Peptide is known to be the main inducer of Post-Mating Responses (PMR) in females including the stereotypical increases in ovulation/oviposition, modification of circadian rhythms and food preference, and a drastic decrease in receptivity to additional matings. The gland is composed of two cell types: main cells and secondary cells. Sex-Peptide is only produced by main cells. Previous studies in *Drosophila melanogaster* have shown that secondary cells are necessary for the PMR to persist for periods longer than ~24 hours, as the proteins produced by these cells bind the Sex-Peptide to the sperm, prolonging its activity for as long as the sperm remains stored in the female sperm storage organs. However, investigating genomic and physiological data from other *Drosophila* species has shown that both Sex-Peptide and secondary cells are not universally present in the Drosophilidae family. By assessing PMRs in various species, this study aims to establish the role of secondary cells in organisms that lack Sex-Peptide and potentially identify other mechanisms that may have evolved to compensate for the loss of one or the other component of the Post-Mating response. The results of this study should not only contribute to our understanding of *Drosophila* mating behaviour, but also shed new light on the evolution of reproductive strategies in insects.

Keywords: Accessory Gland, Sex Peptide, Post, Mating Response

*Speaker

Genomic dark matter of cactophilic *Drosophila* species: The contribution of transposable elements into host shift

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It has been proposed in insects that reproductive isolation between allopatric populations might be an outcome of successive adaptations driven by a host shift, as a result of divergent selection between different ecological environments. The adaptations that have allowed such shift are associated to genetic variability and selective pressure under genes from populations that use different hosts, which may have its origin either on point mutation, transcription levels, or epigenetics. Transposable elements (TEs), which are ubiquitous structural variants from eukaryotic genomes, have been considered as an important source of local adaptation in species and populations, giving rise to genomics and transcriptomics novelties. However, the extent of TEs contribution to such host shift remains unexplored. In this work, we selected seven cactophilic *Drosophila* species with different host preferences and incipient reproductive isolation to investigate the role of TEs on their host shift. Through long reads genomics data, we identified bursts of transposition, as well as a high prevalence of TEs in the promoter region of genes associated with host location. Furthermore, through RNA-seq, we also investigated gene expression divergence in head and larvae tissues between the seven species/subspecies. Our results indicate that divergence in expression are associated with host location in the head, and detoxification for the larvae tissue. In addition, we also found evidence of mRNA derived from genes and TEs, hereafter called as chimeric transcripts. Taken together, our results demonstrate for the first time that TEs have substantial contribution to transcriptomic variability through chimeric transcripts among cactophilic *Drosophila* species, reinforcing their role in rapid evolution. Our combined genomics and transcriptomics approaches provide new insights regarding the role of TEs into the host shift between species/subspecies that use different cacti-hosts.

Keywords: transposable elements, chimeric transcripts, exaptation

*Speaker

Inversion karyotype controls gene expression profile in a context-specific manner

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A central question in evolutionary genetics is the role of chromosomal inversions, which suppress recombination in heterozygous state, in adaptation. In *D. melanogaster*, the inversion polymorphism, *In(3R)Payne*, is found worldwide in natural populations and shows strong latitudinal frequency clines on multiple continents. We have previously found that this inversion affects viability, body size, stress resistance, and life span. However, it is not well understood how these fitness-related traits might be regulated at a gene expression level. Here, we compared the transcriptomes of inversion homokaryotypes (INV), standard arrangement homokaryotypes (STD), and heterokaryotypes (HET) isolated from a single natural population in Florida (USA). We found that many of the genes that were differentially expressed between INV and STD during embryogenesis were also differentially regulated between HET and STD, while there were only few expression differences between HET and INV. This indicates that the INV karyotype has a dominant effect on gene expression in HET embryos. Surprisingly, the opposite was true when we compared the transcriptomes of wing discs, where the STD karyotype largely determined gene expression in HET. Overall, many of the differentially expressed genes were found in the genomic region spanned by *In(3R)Payne*. When comparing chromatin accessibility in wing discs, we found that accessible regions shared between all karyotypes were evenly distributed across the genome, while *3R* had the highest density of uniquely accessible regions in STD and INV. Moreover, the accessibility profile was more similar between HET and STD than between HET and INV. Our findings show that chromosomal inversion polymorphisms can result in major changes in chromatin accessibility which in turn may influence gene expression. Understanding this regulation will ultimately be very important for understanding how inversions contribute to adaptation.

Keywords: inversion, gene expression, transcriptional regulation, chromatin accessibility

*Speaker

MITO-NUCLEAR EPISTASIS MODULATES RAPAMYCIN'S LONGEVITY EFFECT

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"Mito-nuclear" interactions between the nuclear and mitochondrial genomes are emerging as a major source of genetic variation. In *Drosophila*, these interactions affect fitness, disease and ageing. The macrolide drug rapamycin is one of the most translationally promising anti-ageing compounds. However, whether the benefits (and costs) of rapamycin vary genetically is understudied. We have characterised how mito-nuclear epistasis modifies benefits of rapamycin for *Drosophila* lifespan, and corollary costs to egg laying, using a panel of lines bearing replicated and fully-factorial mito-nuclear variation. Rapamycin's impact was determined by mito-nuclear epistasis: benefits for lifespan were not universal among mito-nuclear backgrounds, and some mito-nuclear backgrounds resisted costs to egg-laying. We assess evaluate the relative contribution of variation in mitochondria, nuclei, and mito-nuclear epistasis. These results indicate that rapamycin's impacts can be contingent on the combination of mitochondrial haplotypes and their nuclear genomic context.

Keywords: mitonuclear, rapamycin

*Speaker

Making more than one type of sperm – how do flies and moths do it?

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Sperm heteromorphy (the production of more than one type of sperm) has evolved independently in a wide range of animals from insects to molluscs to fish. In these species the distinct sperm morphs differentially function in fertilisation and post-mating processes in the female reproductive tract indicative of specialisation within a fundamentally critical biological process. However, very little is known about how the morphology of different sperm types is reproducibly generated.

We used *Drosophila pseudoobscura* (*D. pse*) - a species that makes three sperm morphs (short (50um), medium (100um) and long (300um)) in parallel – as a model system to interrogate the molecular mechanisms acting within the male germline that ensure that similar, but distinct, differentiated sperm cell types are made. RNAseq of single, cytologically identical, pre-meiotic spermatocyte cysts identified genes that are differentially expressed or alternatively spliced between cells with different developmental fates in *D. pse* testes. Many genes predicted to function in transcription, cell cycle regulation, spermatid elongation and morphology were differentially expressed between primary spermatocytes destined to become short, medium, or long sperm. We discovered recently duplicated genes that showed differential expression of paralogues between morphs, suggesting that gene duplication and sub- or neo-functionalisation could be a driver of sperm specialisation. Among the differentially expressed putative transcriptional regulators were homologues of genes known to regulate transcription in *Drosophila melanogaster* spermatocytes, including *kmg* - an accessibility regulator, and *tgif*, *mip40*, and *caf1* - subunits of testis meiotic arrest complex (tMAC). A *kmg*-GFP reporter construct confirmed differential accumulation of this protein in late spermatocyte cysts. We have conducted ChIP-seq to identify *Kmg* binding sites to reveal whether it is acting as a transcriptional activator or repressor.

Lepidoptera have independently involved sperm heteromorphy; each testis first makes eupyrene sperm (fertilisation competent) before switching during pupal stages to making apyrene sperm (fertilisation incompetent). To investigate this convergent evolution of sperm heteromorphy we determine the transcriptomes of individual spermatocyte cysts from *Galleria mellonella* (wax moth) larval and pupal testes. As with *D. pse*, we identified differentially expressed genes implicated in transcriptional regulation, cell cycle and spermatid differentiation. Intriguingly, some genes were differentially expressed in both the *D. pse* and *G. mellonella* system.

Keywords: Spermatogenesis, gene expression, sperm, moths

^{*}Speaker

Odyssey: ancient origin and neofunctionalization of the *Drosophila* speciation gene *Odysseus* (*OdsH*)

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Odysseus (*OdsH*) was the first gene described in *Drosophila* related to speciation and hybrid sterility between *D. mauritiana* and *D. simulans*. Its origin is attributed to the duplication of the gene *unc-4*, in the ancestor of the subgenus *Sophophora*.

By using a much larger sample of *Drosophila* species, we show that contrary to what has been previously suggested, *OdsH* occurrence is widespread in species of the Drosophilinae subfamily (Drosophilidae). Bayesian inference indicated its monophyly and estimated its origin around 62 million years ago. Sequence analyses showed that *OdsH* evolved at higher rates, in comparison to its paralogue *unc-4*. We identified innovative expression of *OdsH* in *Drosophila* male reproductive tracts and evidences of its regulation by transcription factors related to male reproduction. Furthermore, the analysis of *OdsH* protein allowed the identification of mutations in DNA- and protein-binding domains specifically of *D. mauritiana*.

We looked then for the expression of *OdsH* in the spermatocytes of the recently diverged sister species *D. arizonae* and *D. mojavensis*, and in their hybrids. Our data indicate that *OdsH* expression is not atypical in their sterile hybrids.

In conclusion, we demonstrated that the origin of *OdsH* is older than previously proposed and its neofunctionalization in male sexual functions occurred rapidly after its origin. Our results also suggest that its role as a speciation gene, as in the *melanogaster* subgroup, may be restricted to specific taxa, resultant from mutations in the binding motifs of its encoded protein, leading to incompatibilities in the hybrids.

*Speaker

Keywords: Gene duplication, Homeodomain, Transcription factor, OdsH expression, Drosophilidae

P elements in *Drosophila willistoni* group species

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The *P* transposable element invaded the *Drosophila melanogaster* natural populations between 1950 and 1970, following a horizontal transfer, very likely from *Drosophila willistoni*. We analyzed by genomic DNA PCR and sequencing the *P* element status in *willistoni* group species (*D. willistoni*, *D. equinoxialis*, *D. paulistorum*, *D. insularis*, *D. nebulosa*, *D. tropicalis*) in order to investigate what can be equilibrium states of the *P* family TEs on their long term in species (still active family or not). We found that potentially autonomous *P* elements appear to be still present in most of the *D. willistoni* and *D. equinoxialis* natural populations, whereas a large proportion of *D. paulistorum* and *D. tropicalis* collected lines apparently lack complete *P* copies. In addition, we have found in all except one more distant species (*D. nebulosa*) the presence of numerous *P*-MITes (#200bp) with conserved *cis*-regulatory sequences to be mobilized in *trans* and which appear to be identical by descent in all *willistoni* group species tested so far. They appear therefore to be old genome components present before species radiation. Strikingly, *D. insularis* appear to have retained only *P*-MITes but no long *P* copies. We have found that most of the *willistoni* group species carry a common *P* element variant, named "protocanonical *P* element" (pc*P*), which differs from the canonical *P* element (c*P*) of *D. melanogaster* by three diagnostic marks including a SNP variation in the 3' Transposase Binding Site (TBS) which becomes therefore symmetrical with the 5' TBS, a situation distinctive from that of c*P* elements. Finally, small RNA sequencing in *D. willistoni*, *D. equinoxialis* and *D. tropicalis* ovaries showed the presence of sense and antisense *P*-homologous piRNAs which exhibit a ping-pong signature, indicating a *P* repression based on a canonical germline double-strand piRNA mechanism. The evolutionary perspectives of the *P* family in the *willistoni* group species will be discussed.

Keywords: P elements, *Drosophila willistoni*, long term homeostasis

*Speaker

Pervasive insect behavioral changes in response to sub-lethal concentrations of agricultural toxins

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Insect biomass is declining across the globe at an alarming rate. Global warming and the widespread use of pesticides are considered two underlying causes. However, the lack of systematic experimental studies-which so far have mostly been restricted to bees-limits our causal understanding of this problem. Here, we employed a chemical library encompassing 1044 different molecules to explore how insect populations are affected by sublethal concentrations of pesticides. This library includes insecticides, herbicides, fungicides, inhibitors of plant growth, and other chemicals that are or have been used in agriculture. Strikingly, we found that a substantial number of pesticides affect the behavior of the larvae, even at sub-lethal concentrations. For some of these molecules, the effect became even stronger when we increased the temperature at which the larvae were exposed to the pesticides. Furthermore, we tested the effects of these pesticides on different *Drosophila* genetic backgrounds, using not only lab strains but also natural isolates, and found that whilst lab strains react to the pesticides similarly, the natural isolates appear to be more resistant to specific families of insecticides employed in the region where the wild flies were captured. Finally, we adapted our experimental design for working with mosquito larvae and detected similar behavioral alterations triggered by pesticides at sub-lethal concentrations. Taken together, these results suggest that pesticide exposure might have been overlooked as a main explanatory factor behind the global decline in insect populations.

Keywords: pesticides, behavior, insecticide resistance, toxins, natural isolates

*Speaker

Segregation Distorter requires Overdrive for gamete elimination

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Intragenomic conflict involving selfish chromosomes is a powerful force in evolution that shapes the evolution of genomes, cells, and species. Despite decades of studies, few of the underlying genes and mechanisms are known. *Overdrive (Ovd)* is necessary for both segregation distortion and male sterility in *Drosophila pseudoobscura Bogota-USA* hybrids. Here, we show that *Ovd* is a non-essential gene that is necessary for the selfish action of *Segregation Distorter* in *D. melanogaster*. In particular, *Ovd* is required for the targeted elimination of competing gametes by SD. Our results suggest that tricking germline checkpoints may be a common mechanism of selfish chromosomes.

Keywords: segregation distortion, speciation, chromatin, spermatogenesis

*Speaker

Single-cell dissection of olfactory system remodelling in the ecological specialist *Drosophila sechellia*

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Sensory systems display remarkable adaptability, rapidly evolving in response to environmental changes. *Drosophila sechellia*, with its close relationship to *D. melanogaster* but unique specialisation on *Morinda citrifolia* (noni) fruit, offers an ideal model for examining how ecological adaptations shape sensory system evolution. Prior studies have observed alterations in the *D. sechellia* olfactory system, notably the expansion of olfactory sensory neuron (OSN) populations responsive to noni odours. We have used single-nucleus RNA-sequencing (snRNA-seq) to systematically characterise changes in the *D. sechellia* adult olfactory system. Our comparative single-cell transcriptomic analysis not only confirmed known changes but also predicted novel features, such as loss of OSN classes and neuronal fate conversion, which were validated through *in situ* expression analysis and electrophysiological recordings of OSN responses. To explore the developmental origins of these changes, we conducted snRNA-seq on developing antennal imaginal discs, mapping developmental divergences that contribute to the unique composition of *D. sechellia* olfactory system. Together, our work illuminates the molecular, genetic, and developmental mechanisms driving olfactory system remodelling - as well as the potential functional consequences - in response to ecological specialisation.

Keywords: Ecological specialisation, Olfactory system remodelling, EvoDevo, *D. sechellia*, Comparative single cell transcriptomics

*Speaker

Spatial coupling of food and mates in *Drosophila*

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In many taxa, individuals aggregate at food resources. This generates a co-localization of foraging and mating opportunities, which potentially promotes assortative mating. Here, we show that food and mates are spatially coupled in the fruit fly, *Drosophila melanogaster*, and we also explore the sensory and behavioural mechanisms underlying this co-localization. We track the mating location of flies in environments containing heterogeneous food patches and observe that *D. melanogaster* and several of its sibling species generally choose to mate on patches containing yeast - the primary diet of fruit flies. *D. melanogaster* is an exception with virgins primarily mating away from yeast, but previously mated females re-mating on yeast. Flies either alone or in pairs with another sex locate on yeast at night, but individuals' tendencies to be on the yeast during the day depend on several variables including sex, light conditions and presence of another sex. Mating location preference involves attraction to yeast-derived chemical cues (the combination of acetic acid and protein) and is modulated by the male-derived sex peptide received by females during mating. Preference for mating on yeast-containing patches is stronger at night than during the day, and increases with the passing of time since the first mating. We also find that *D. melanogaster* pairs exert preferences for mating on certain yeast species over others when exposed to patches containing different yeasts. Our study demonstrates a mechanism by which divergence in food preference can directly lead to assortative mating.

Keywords: Fly, yeast interaction, spatial segregation, dietary adaptation

*Speaker

Viral infection impacts transposable element transcript amounts in *Drosophila*

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Transposable elements (TEs) are genomic parasites that are found in all genomes, some of which display sequence similarity to certain viruses. In insects, TEs are controlled by the Piwi-interacting small interfering RNA (piRNA) pathway in gonads, while the small interfering RNA (siRNA) pathway is dedicated to TE somatic control and defense against viruses. So far, these two small interfering RNA pathways are considered to involve distinct molecular effectors and are described as independent. Using Sindbis virus (SINV) in *Drosophila* and high throughput RNA sequencing, we show that viral infections affect TE transcript amounts via modulations of the piRNA and siRNA repertoires, with the clearest effects in somatic tissues. These results suggest that viral acute or chronic infections may impact TE activity and, thus, the tempo of genetic diversification.

Keywords: transposon, piRNA, siRNA, virus, small RNAs

*Speaker

Neural development, circuits & behaviour

A Notch-dependent transcriptional mechanism controls the expressions of temporal patterning factors in the optic lobe medulla of *Drosophila melanogaster*.

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A Notch-dependent transcriptional mechanism controls the expressions of temporal patterning factors in the optic lobe medulla of *Drosophila melanogaster*.

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Temporal patterning is an important mechanism for generating neural diversity from a seemingly homogenous progenitor pool in vertebrates and invertebrates, whereby the same neural stem cell forms different subtypes of neurons depending on its age at the time of birth of the neuron progeny. *Drosophila* neuroblasts in the optic lobe medulla are temporally patterned by a series of sequentially expressed Temporal Transcription Factors (TTFs), which are inherited in their progeny neurons and presumably direct the subsequent development of these neurons into distinct subtypes. Based on the phenotypes observed when TTFs are mutated it is inferred that earlier TTFs activate the expressions of TTFs expressed immediately after and are in turn repressed by them, thus forming a transcriptional cascade. However, direct transcriptional regulation between TTFs has not been verified in most cases. Furthermore, it is unknown how transitions between successive TTFs are coupled with generating an appropriate number of neurons at each stage. We use neuroblasts of the *Drosophila* optic lobe medulla to address these questions and show that the expressions of TTFs Sloppy-paired 1 and Sloppy-paired 2 (Slp1/2) are directly regulated at the transcriptional level by two TTFs that are expressed earlier and by cell-cycle dependent Notch signalling acting through two *cis*-regulatory elements around the *sloppy-paired* coding locus. We also show that supplying constitutively active Notch can rescue the delayed transition into the Slp1/2 stage in cell cycle-arrested neuroblasts. Our findings reveal a novel Notch-pathway-dependent mechanism through which the cell cycle progression regulates the timing of a temporal transition within a TTF transcriptional cascade.

*Speaker

Keywords: temporal patterning, neuroblasts

A SLC7A amino-acid transporter, MINIDISCS, involves in amino acid-dependent neuronal activity in the *Drosophila melanogaster* Mushroom Bodies.

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In all living organisms, nutrition is the key element for organism’s energy needs while providing essential molecules necessary for the proper functioning of cells and tissues. Specifically, essential amino acids (EAAs) play multiple roles within the cell. To enter cells, including neurons, amino acids such as EAAs use specific amino acid transporters, among which are members of the Solute Carrier 7 (SLC7A) family (Ren *et al.*, 2007). These transporters are heterodimeric protein complexes consisting of two subunits: a heavy chain, responsible for the localization of the complex to the membrane (SLC3A), and a light chain which determines the specificity of the transport (SLC7A) (Wagner *et al.*, 2001). In *Drosophila melanogaster*, the heavy chain is encoded by a single SLC3A gene, CD98hc, and the light chain by five SLC7A potential genes: *Minidiscs* (*mnd*), *Genderblind*, *JhI-21*, *Sobremesa*, or *CG1607* (Martin *et al.*, 2000; Featherstone 2011; Augustin *et al.*, 2007).

To decipher the role of SLC7A transporters in the adult central nervous system (CNS), we focused on the putative orthologue of SLC7A5: *Minidiscs*.

We have previously demonstrated that MND is expressed in larval brain neurons that secrete insulin-like peptides (DILPs) to regulate sugar metabolism in response to Leucine presence (Manière *et al.*, 2016). Here, we discovered that *Minidiscs* is expressed in adult brain cells, including neurons forming the α/β and γ lobes of the Mushroom bodies (MBs). By performing live calcium imaging experiments, we showed that MND is required for MB activity in response to various amino acids such as L-Leucine, L-Isoleucine, or L-Glutamate. Additionally, our findings suggest that the TOR pathway and not the GDH pathway is involved in MB response to L-Leucine. As MND regulates the MB response to L-Glutamate, we hypothesized that MND acts directly or indirectly on glutamate receptors. Through genetic intersectional approaches, we identified that *Minidiscs* is co-expressed with various glutamatergic receptors: NMDARs, KAINATE receptor, GLUC α receptor, and DmGLURA in neurons of the MBs.

Overall, these findings support the hypothesis that MND plays a crucial role in amino acid-dependent MBs activity, and that the response of the MBs to L-Leucine involve MND and the TOR pathway. These results suggest that MND could act as a sensor of AAs in the MBs and could potentially modulate MB-related behaviors such as feeding behaviour, memory or learning.

*Speaker

Keywords: Amino acid transporter, SLC, Minidiscs, Central Nervous System, Mushroom bodies

A new experimental approach to studying path integration in *Drosophila melanogaster*

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Many species rely on path integration to compute and maintain a stable representation of the shortest route home. So far, the search for neural substrates of this type of vector-based navigation has been restricted to nesting species, such as honeybees or desert ants, which use their nest as a stable point of reference. In the present study, we create the illusion of a nest in the genetically yielding, but a priori nest-less, fruit fly *Drosophila melanogaster*. By optogenetically activating sugar-sensing gustatory neurons whenever the fly occupies a defined place within a chamber, we train it to revisit this position. Flies keep returning to the reinforced location over a few minutes, even after the optogenetic reward is discontinued. Previous studies report that this behaviour does not rely on visual or olfactory cues. Future experiments will determine the influence of proprioceptive feedback on the homing behaviour and the neural substrate of the path integrator.

Keywords: path integration, drosophila, vector, based navigation, optogenetic stimulation, proprioception

*Speaker

Acetylcholine receptor diversity as a postsynaptic regulatory mechanism in *Drosophila* synapses

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Chemical synapses are extremely dynamic communication sites, a characteristic primarily owed to the variety of regulatory mechanisms separately imposed at their pre- and post-synaptic sites. In *Drosophila melanogaster*, the majority of neuronal connections are excitatory cholinergic ones, which results in a wide spectrum of acetylcholine receptors. Specifically, two receptor categories, fast ionotropic and slow metabotropic are expressed at T5 direction selective neurons of the visual system. Nonetheless, acetylcholine receptor diversity in synapses among the four excitatory columnar input neurons Tm1, Tm2, Tm4, Tm9 and T5 neurons has not been addressed. In this study, we found a differential expression of nicotinic acetylcholine receptor alpha subunits and the expression of the muscarinic acetylcholine receptor type B on T5 dendrites. To tackle receptor localization in synapses of interest, we employed the vast *Drosophila* genetic toolbox, acquiring cell-type specific genetic access. To better visualize synapses with the resolution restrictions that light microscopy imposes, we screened for the most suitable genetic approach, finally choosing methods of transsynaptic labelling. So far, our experimental strategy suggests the differential expression of acetylcholine receptor subunits across the four cholinergic input neurons. Ultimately, we aspire to link receptor topology to receptor function, so as to further understand the molecular mechanisms of direction selectivity

Keywords: Receptors, Synapses, Visual system

Are the functions of Netrin and Frazzled to guide axons conserved among insects?

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The Netrin-Frazzled/DCC signaling pathway promotes midline crossing of axons in the nervous system of insects and other bilaterian animals. Netrin-Frazzled is the main midline attractive pathway that guides axons to cross the midline in *Drosophila*. Orthologs of the pathway ligands (Netrins) and receptors (known as Frazzled (Fra) in insects and Deleted in Colorectal cancer (DCC) in vertebrates) are widely conserved in bilaterians. However, the regulatory roles of the above-mentioned in insects other than *Drosophila* are not yet well understood. This project aims to compare the midline attractive roles of the Frazzled receptor in the flour beetle (*Tribolium castaneum*) and fruit fly (*Drosophila melanogaster*) using CRISPR/Cas9-mediated gene replacement. The research approach includes CRISPR modifications to replace the *Drosophila* frazzled gene with HA-tagged cDNAs encoding *Drosophila* Frazzled (DmFra) or *Tribolium* Frazzled (TcFra). We compare the expression of DmFra and TcFra in the *Drosophila* embryonic CNS, and examine midline crossing of axons to see if TcFra is able to rescue DmFra's role in promoting midline crossing of axons in *Drosophila*. Our project aims to gain insight into the evolutionary conservation of axon guidance in insects.

Keywords: axon guidance, axon, neural development, Frazzled, Netrin, DCC, Bilaterians, Midline, crossing axons, CRISPR

*Speaker

Beyond wings: Roles for apterous gene in gut development, feeding initiation and adult survival

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Apterous (*ap*) encodes a transcription factor of the LIM homeodomain family and is best known for its important role in D/V patterning of imaginal discs. *apnull* flies display the following phenotypes: no wings and halteres and precocious adult death. The precocious death syndrome is characterized by adult death within 72 to 96 hrs after eclosion, and correlates with female sterility and abnormal adipose tissue. Using *Drosophila*'s powerful toolbox of classical genetics and modern methods, we have studied *cis*-regulatory elements of *ap*. We were able to map and characterize an *ap* regulatory region, the *Life Span Enhancer* (*LSE*), which is required and sufficient to rescue all of the phenotypes associated with the precocious death syndrome. However, a model for the comprehensive explanation of this syndrome and the connection between the individual disorders is still missing. We are currently investigating the genetic and cellular basis of the precocious adult death phenotype. *ap-null* flies (and flies lacking *LSE*) do not excrete their meconium and are not able to defecate. Furthermore, the mutant flies barely eat, implicating that feeding initiation is also disrupted. We found that during metamorphosis, both mutant fly lines have dramatic defects in mid- and hindgut remodeling processes resulting in a bloated and shortened adult midgut and lack of all rectal papillae in the hindgut ampulla. At the conference, we hope to present a model about how the various phenotypes of the syndrome are connected to each other.

Keywords: gut, feeding initiation, feeding, gut development, gene regulation, rectal papillae, midgut development, hindgut development

*Speaker

Body Symmetry Relies on Interhemispheric Communication and Piezo-mediated Proprioception input

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Body symmetry exemplifies the resilience of juveniles to perturbations, yet the circuitry and logic that ensure the matching between the left and right sides remain ill-defined. Juveniles of *Drosophila melanogaster* use the relaxin ILP8 hormone to continuously send information about growth status (or deficits) to keep body symmetry (1) via its receptor Lgr3 (2-6). Still, does the brain's perception of a circulating hormone suffice to accurately locate the mismatch's position and direction (left or right)? Using functional assessment, calcium imaging, MCFO mosaic analysis, automatization, and AI, we delineate the circuitry responsible for assuring bilateral symmetry. Notably, we found the body symmetry relies on an Lgr3 ensemble that is different from the neurons regulating ecdysone for delaying developmental timing in response to injury. We also discovered that proprioception, a sense that detects the position and orientation of body parts, via the Piezo channel provides crucial feedback for stabilizing left-right symmetry. These discoveries offer a new perspective on the mechanisms through which developing juveniles can detect and rectify even minute variations to keep impeccable body symmetry.

Keywords: Developmental homeostasis, circuit, relaxin, mechanosensory input

^{*}Speaker

COP9 Signalosome Subunits CSN1b and CSN7 are essential for neural development

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The nervous system consists of the brain and associated structures that are replete with highly specialized neurons and glia. Central to the development of such a complex and highly organized system are Neural Stem cells (NSC), which self-renew to maintain their number and also give rise to differentiated progeny. To identify genes involved in the maintenance of NSCs, we used *Drosophila* Neuroblasts (NB) as a model system. We performed a protein expression screen followed by a secondary protein knock down screen in NBs. Through this, we identified CSN7, a COP9 signalosome (CSN) subunit, which we found to be enriched in NBs and essential for neural-development. CSN is a highly conserved multi-protein complex that is known to regulate proteasome mediated degradation via modulation of Cullin-RING E3 ligases. Using a combination of MiMIC technology and NB specific knockdown, we demonstrate that loss of *CSN7* and *CSN1b* lead to a small brain phenotype, decrease in NB size and a reduced mitotic index. Signalling pathways such as InR/Akt, dTor and the Hippo pathway are known to regulate NB growth in *Drosophila*. Our results show a decrease in Akt and pAkt upon loss of *CSN7* and *CSN1b*. Our ongoing investigation suggests the CSN regulates Akt via Cullin 1 in the developing larval brain.

Keywords: neural development, neuroblast, neural stem cell, CSN, Akt, Cullin

*Speaker

Calcium dependent mechanisms to establish neuronal homeostatic setpoints

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The properties of neuronal networks required for normal function are specified during a phase of development that is referred to as a critical period. Transient activity perturbations during a critical period cause mis-specification of neuronal properties with lasting consequences to network function. In humans, suboptimal critical period experiences are thought to be associated with neuro-developmental or neuro-psychiatric conditions. How critical period activity patterns are translated into structural and physiological changes, is currently not well understood. We are studying this fundamental question using the *Drosophila* larval locomotor network, which also has a clearly defined critical period in late embryogenesis. *Drosophila* is susceptible to temperature changes, which, when experienced during the critical period, cause lasting morphological, physiological and behavioural changes. For example, transient embryonic experience of heat (29°C or 32°C) cause changes in neuromuscular junction growth, transmission and larval crawling behaviour. Experiencing 32°C during the critical period reliably leads to increased presynaptic growth and bouton number at the larval neuromuscular junction. I am particularly interested in exploring roles for calcium in translating critical period experiences and regulating adaptive mechanisms that contribute to establishing homeostatic setpoints. Preliminary data suggests, that this critical period-specified presynaptic terminal growth relies on calcium signalling. To determine how changes in synaptic size and morphology correlate with altered physiology, I am using two electrode voltage clamp to analyse critical period-induced changes to synapse physiology, either caused by transient changes in calcium signalling during the critical period and/or 32°C temperature exposure.

Keywords: Setpoint, Critical Period, Calcium, Voltage gated calcium channels

*Speaker

Ceramide synthase Schlank is required for development and function of astrocytes

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Glial cells in *Drosophila* and mammals have a crucial role in maintaining neurons and the brain healthy. Basic glial cell biology and function are conserved between mammals and *Drosophila*. Mammalian astrocytes and *Drosophila* astrocyte-like glia (ALG) both provide metabolic and structural support to neurons and are vital to neural remodeling. However, we know little about astrocyte-derived lipids in glia-neuron interactions. The nervous system is enriched with certain classes of Sphingolipids (SLs), e.g. ceramide and its derivatives that play important roles in cell-cell-signalling and signal transduction. Alterations in the regulation of ceramide levels were shown to influence the risk of developing neurometabolic diseases. Ceramide synthases (CerSs) are central enzymes of SL metabolism catalyzing the formation of ceramide, the precursor for all complex SLs. *Drosophila* encodes for only one CerS called Schlank. To identify whether CerS is also required in neuron-associated glia in the central nervous system, we induced a glia subtype specific *schlank* knock-out (KO) in cortex glia, ensheathing glia and ALG. The strongest impact was found in adult ALG specific KO mutants (*ALG> KO*), which display a drastically reduced locomotion ability and lifespan. Primary ALG (pALG) have crucial functions in neuronal circuit refinement as they transform into phagocytes during early metamorphosis and clear pruned synapses. We could show that pALG in *ALG> KO* have noticeably altered morphology but still promote engulfment and clearing of synaptic material in early pupae. However the infiltration of the neuropil by secondary ALG (sALG) at later pupal stage is less pronounced in *ALG> KO* compared to control. Furthermore, in adult flies the soma of sALG is enlarged and the neuropil area covered by ALG processes is reduced. We hypothesize that communication between ALG and neurons is disturbed in *ALG> KO* by impaired function of sALG like regulating synapse formation. In a murine primary astrocyte culture we found already evidence for altered lipid trafficking. Our future goal is to study the effects of CerS on lipids and signals secreted by astrocytes for glia-neuron communication and finally for the assembly of adult neural circuits.

Keywords: glia, astrocytes, Ceramide synthase, Schlank, Ceramides, glia neuron interaction

*Speaker

Characterization of gustatory second order neurons in *Drosophila melanogaster*

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Animals, including the fly *Drosophila melanogaster*, continuously receive and process sensory information from the surrounding environment via different sensory systems, which ultimately direct appropriate behavioral responses. Among those behaviors, feeding is essential as it is how animals get all the needed nutrients to support their lives. In order to discriminate between nutritious and potentially toxic food, a set of specialized neurons, Gustatory Receptor Neurons (GRNs), housed in gustatory sensilla along the body, express a combination of chemosensory receptors responsible for the detection of food chemicals and project their axons to the subesophageal zone (SEZ), the primary taste center in the brain. While much is known regarding the gustatory receptors and the role of GRNs, it is not yet clear how the gustatory information conveyed by GRNs to the SEZ is processed. We have characterized molecularly by RNAseq the gustatory second-order neurons (GSONs) receiving direct input from sweet, bitter and mechanosensory (GRNs) in fed and starved conditions. The gene expression analysis shows that GSONs receiving input from sweet, bitter, and mechanosensory neurons segregate molecularly and that their molecular profile varies with the metabolic state of the fly (fed vs. starved). Furthermore, GSONs express a complex combination of neurotransmitters and neuropeptides, indicating that those neurons are not homogenous even when receiving information from the same taste quality. We are currently characterizing the role of specific neuropeptides whose expression depends on the fly's metabolic state, which could be involved in the integration of sweet and bitter information.

Keywords: Neural circuit, behaviour, feeding, gustatory neuron

*Speaker

Deciphering the machinery of muscle innervation maintenance

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Locomotion is a stereotyped behavior that allows animals to carry out actions necessary for survival, such as finding food, mating or escaping from predators. Movement is the result of a coordinated muscle contraction pattern, which is finely regulated by the complex architecture of the locomotor system: specific connections between motor neurons (MNs) and muscles are built during development, and are maintained throughout the entire lifespan. Our goal is to understand how locomotor systems maintain their specific architecture, focusing on the *Drosophila* leg neuromuscular system.

MNs differentially express a code of transcription factors (morphological transcription factors, mTFs), which regulates their unique connection with muscle fibers during development (Enriquez et al. 2015). We hypothesize that the molecules that are shaping the specific wiring of each MN during development are also preserving it in adult life and that, therefore, each muscle must express a unique set of molecules in order to interact specifically with MNs axon terminals.

To decipher the molecular bases of muscle innervation maintenance we use a biased approach by exploring the function in adult flies of genes known to be involved in late axonal targeting during development. In particular, we are investigating the role of interacting transmembrane Ig superfamily proteins DIP- α and Dpr10 in axonal wiring maintenance, as they have been demonstrated to establish terminal axon branching patterns in a subset of MNs during pupal development (Venkatasubramanian et al. 2019): our preliminary results indicate that DIP- α can rescue terminal axon branching defects when selectively expressed in adult flies.

Moreover, we use an unbiased strategy, which consists in profiling the transcriptome of each of the 14 T1 leg muscles by single nuclei RNA-sequencing (snRNA-seq). We performed snRNA-seq on T1 femur in adult flies and identified a set of genes differentially expressed in myonuclei. So far, we validated the expression of three genes to be specific of two different muscles in adult femur: Oct α 2R and Oct β 2R in Tibia Reductor Muscle and Zfh-1 in Tibia Depressor Muscle. This data altogether supports our hypothesis, which we will test by selectively ablating candidate genes in adult *Drosophila* to determine their function on terminal axonal wiring and locomotion.

Keywords: Locomotion, axonal wiring, motoneurons, muscles, maintenance

*Speaker

Decoding modality-specific function and neuromodulation in the *Drosophila* nociceptive network

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Fast and efficient escape behaviour in response to noxious stimuli is essential for the protection and survival of all animals. In *Drosophila* larvae, specific sensory neurons, so-called nociceptors, detect noxious stimuli including heat and touch triggering a rolling escape response. Despite the extensive characterization of nociceptors across organisms, how noxious stimuli including harsh touch and heat are processed at the neuronal network level remains poorly understood. The recently reconstructed central nervous system of the *Drosophila* larva now provides insight into the organization of the circuits underlying nociceptive behaviour. In addition, neuromodulatory peptidergic signals play an important role in shaping stimulus-specific network responses by influencing neuronal activity. In the larval nociceptive circuit, short Neuropeptide F (sNPF) and its receptor (sNPF-R) are required for both, mechano- and thermo-nociception, yet sNPF signaling seems to occur in different neurons for each modality. We are investigating the diverging circuits and sNPF function underlying thermo- and mechano nociception at the behavioral and functional level in this network. We found that distinct 2nd order neurons downstream of the nociceptors are required for escape responses to noxious mechanical or thermal stimulation. However, at higher-order levels, both modalities rely on the same neuron(s) suggesting divergent and convergent sensory processing by the underlying network. By mapping the expression of sNPF and its receptor in combination with their genetic manipulation and functional imaging, we aim to assess the modality-specific requirement of peptidergic signaling in these circuits. Our study thus provides the basis for a detailed understanding of the divergent mechano- and thermonociceptive circuitry and neuromodulatory signaling underlying larval escape behavior.

Keywords: Nociception, C4da, sNPF, behavior, neural network

*Speaker

Dendritic growth of motion sensitive T4/T5 neurons of *Drosophila* is affected by knocking down cell surface receptor Alk

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Motion-sensitive T4/T5 neurons of the *Drosophila* visual system exist in four subtypes (a,b,c,d) with each subtype being tuned to visual motion only in one of the four cardinal directions. The dendrites of the four subtypes are of identical size and receive information from the same input neurons, but are oriented in a subtype specific way such that their tips point opposite to the neurons preferred direction. Although the transcription factor combination that gives rise to each subtype is known thanks to scRNA sequencing studies, specific mechanisms responsible for the oriented growth is yet to be explained. We hypothesize that cell surface proteins respond to extrinsic cues relaying spatial information differently in each subtype which can result in subtype specific oriented dendrite growth. In this study we are screening differentially expressed cell surface proteins and the ones with a known function in neuron differentiation by knocking down their expression in T4/T5 neurons using RNAi tools. Then, we analyze the size, the growth length along different axes, and the final orientation of adult T4/T5 dendrites. Our results show that knocking down Anaplastic lymphoma kinase (Alk), which is a protein belonging to one of the major families of cell surface proteins Receptor Tyrosine Kinases, causes T4a and T4b subtypes to overgrow only along the dorso-ventral axis and show no effect in T4c and T4d subtypes. This result suggests the involvement of Alk in T4/T5 growth as well as the existence of separate mechanisms for the growth along different axes and in different T4/T5 subtypes.

Keywords: Dendritic morphology, T4/T5 neurons, cell surface proteins

Differing modes of epigenetic repression in the *Drosophila* brain revealed by large-scale Targeted DamID profiling and chromatin state analysis

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Brain development is a complex process requiring precise control of a multitude of cells and their genomes. Epigenetic changes have become established as a key driver of cell fate, with spreading of HP1 heterochromatin seen as a mark of terminal differentiation. However, little is known in *Drosophila* about the epigenetic landscapes of the primary cell types in the brain, neurons and glia.

Here we show that the epigenomes of brain cells are highly varied, with glia in particular exhibiting unexpected approaches to gene repression. We used Targeted DamID (TaDa) to generate cell-type specific binding profiles of 7 key chromatin proteins, as well as two histone marks, in neuron and glial cells at both larval and adult stages. Using these data, we determined chromatin states via a Hidden Markov Modelling approach and identified the epigenetic differences between cell populations. In neurons we saw repression driven primarily by spreading of HP1 associated heterochromatin, a trait expected in a terminally differentiated cell. In glia, however, we saw a relative absence of HP1 heterochromatin. Instead, genes in these cells were repressed primarily in chromatin states with potential for later activation. These findings were consistent across gene bodies, promoter regions and enhancer elements identified from previous single-cell RNA-seq data.

From our analysis we identified a selection of glial specific developmental genes, which were then targeted in a reverse genetic screen. Using RNA interference to knock-down the expression of these targets we saw impaired cell proliferation and brain development in several candidates. Our data show that repression of genes in terminally differentiated cell types is more complex than previously thought. As the first comprehensive profiling of chromatin states within the larval and adult brain, our datasets also represent a major resource for the *Drosophila* community.

Keywords: glia, glial cells, brain development, epigenetics, chromatin states, chromatin state, repression, DamID, differentiation

*Speaker

Elucidation of the role of dopamine in the *Drosophila* antennal lobe

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Dopamine, a neurotransmitter of the monoamine family, is one of the key modulators of the mammalian nervous system. It is involved in many functions, such as learning, motor coordination, sleep, and reward perception. Death of dopaminergic neurons results in Parkinson's disease (PD), a neurodegenerative disease that causes severe motor impairments, dementia, and finally, death. The fruit fly, *Drosophila melanogaster*, is widely used as a model animal to study dopamine signaling *in vivo*. Multiple studies have revealed many processes in the *Drosophila* brain in which dopamine signaling plays an important role, among them learning and memory, courtship, stress responses, sleep, and aggression. However, although it was shown that all four *Drosophila* dopamine receptors are expressed in the antennal lobe (AL), the first relay in the olfactory pathway, and that few of its intrinsic neurons express the enzyme required for dopamine synthesis (Tyrosine hydroxylase, TH), the role of dopamine in early olfactory processing in *Drosophila* is not known. Here, using *in vivo* two-photon functional imaging and genetically encoded indicators for Ca²⁺ and dopamine, we show that dopamine has an excitatory effect on the output of different AL glomeruli, and verify that dopamine is secreted in the AL. In addition, using genetic approaches and confocal microscopy, we examine the expression of dopamine receptors in different classes of AL neurons, and identify TH-positive cells that innervate the AL.

Keywords: Olfaction, Dopamine, Antennal Lobe

Evolution of taste preference in drosophilids

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Feeding is an essential requirement for survival, and the sense of taste plays a crucial role in determining whether to accept or reject potential food sources. While we have gained considerable knowledge about the molecular and neural circuit properties of this sense in both invertebrate and vertebrate models, our understanding of how taste preferences evolve to explore new food sources during speciation remains limited. To address this question, we investigate three closely-related species of *Drosophila*: *D. sechellia*, *D. simulans*, and *D. melanogaster*. While the latter two are generalists, capable of feeding on various fruits, *D. sechellia* is a specialist that exclusively relies on the fruit of the tropical shrub *Morinda citrifolia* (Noni) throughout its life cycle. We used quantitative feeding assays to characterize the gustatory preference of the three species for Noni and we identified single bitter compounds which induce feeding aversion in *D. melanogaster* and *D. simulans* but not in *D. sechellia*. By comparative genomics, we discovered a mutation unique to *D. sechellia* in a bitter Gustatory receptor (Gr39a) that we demonstrate to be responsible for the reduced bitter aversion in inter-species allele-swap experiments. Interestingly, this mutation maps to the receptor's intracellular anchor domain suggesting that formation of bitter receptor complexes, rather than alterations in ligand affinity, may underlie ecological adaptation. Together with a comprehensive characterization of the neurophysiology of taste circuits in all three species, we establish a link between genetic changes, taste circuit neurophysiology and feeding preferences to understand how animals adapt to defined ecological niches.

Keywords: *D. sechellia*, *D. simulans*, gustation, flyPAD, Gustatory receptors, Gr39a

*Speaker

Flexible neural control of transition points within the egg-laying behavioral sequence in *Drosophila*

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The drive to reproduce is a dominant motivator of behavior in all species. For oviparous animals that do not brood, such as the fruit fly, *Drosophila melanogaster*, considerable pressure is imposed on the selection of the appropriate time and place to deposit eggs. During egg laying in the fly, females evaluate the local environment prior to expressing an ordered motor sequence – abdominal bending, ovipositor digging, and egg expulsion – that culminates in egg deposition subterraneously within a nutritive substrate. We have characterized the structure of this sequence in detail and found significant variability in the transitions between component actions that affords the organism an adaptive flexibility. We have demonstrated that this flexibility arises from the fact that the individual components of egg deposition are not simply motor acts but also acts of sensory evaluation of the internal and external world that sculpt the behavioral progression. Specifically, using a combination of high-resolution behavioral observations, machine-vision based behavioral classification, in vivo calcium imaging, and action-triggered optogenetics, we uncovered the logic of an interoceptive feedback mechanism that governs both the timing and direction of sequence transitions during ovipositor digging (i.e., whether to persist in digging, to advance to ovulation upon successful egg deposition, or to revert to an earlier step in the egg-laying sequence if unsuccessful). The mechanism is based on a newly discovered cluster of internal sensory neurons that encircle the uterus and monitor the internal position of the egg during deposition. Moreover, we also identified and characterized the critical roles of two additional neuronal elements: abdominal bristle sensory neurons provide tactile information about the substrate during abdominal bending and a pair of uterine motor neurons enact the final transition to egg expulsion. This work reveals an elegant strategy by which the nervous system flexibly assembles elemental actions into complex sequences, a process at the foundation of nearly all goal-directed behavior.

Keywords: behavior, neuroscience, sensory feedback, sensorimotor integration, interoception, optogenetics, behavioral sequences, egg laying, neuroethology, in vivo calcium imaging, machine learning, NOMPC, motor neurons

^{*}Speaker

Functional and connectomic analysis of a sensory circuit bridging larval metabolics and brain

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Feeding is a universal behavior highly regulated and modulated by neuropeptides. Past research had the *Drosophila melanogaster* feeding and neuropeptidergic system reconstructed and analyzed in great detail at synaptic resolution with the help of a high-resolution EM volume of the larval brain (Schlegel et al., 2016; Miroschnikow et al., 2018; Hückesfeld et al., 2021). However, it remains unknown which and how sensory inputs are integrating into the circuits involved. Since we now have a new EM volume of the whole entire larva at our disposal, reconstruction of complete neural circuits is now possible, starting from sensory inputs in the periphery – such as chemo- and mechanosensory sensors of the enteric nervous system and external sensing organs – to the release sites of neuropeptides, including hugin neuropeptides secreting and insulin-producing cells (IPCs) in the larval brain. A first such a complete analysis was performed on the serotonergic system of larval feeding control (Schoofs et al., 2023, bioRxiv). Here, we present a monosynaptic circuit of sensory cells with sensory fields at the anterior larval aorta. These span from the anterior aorta opening close to the ring gland into the subesophageal zone and pars intercerebralis of the larval brain and make strong connections onto both IPCs and interneurons. The implied function of such a circuit is a conduit of information about the metabolic state of the animal to both direct output neurons releasing insulin and into central data processing. Thus, these cells are to be analyzed both by assessing connectivity and function, the latter initially by screening for the expression of different neurotransmitters and receptors. In addition, the downstream synaptic connectivity of the cells is examined and the effects of circuit activity studied. After obtaining these insights, it will be possible to generate a complete understanding of this circuit from input to output. Since we are part of the FlyWire consortium (Dorkenwald, ..., Flywire Consortium, 2023, bioRxiv) and have access to a whole brain EM volume of the adult fly, it is possible to determine how a circuit evolves in adulthood after metamorphosis and also ultimately search for similar circuits in vertebrate systems.

Keywords: neurobiology, connectomics, insulin, metabolism, neuroethology

^{*}Speaker

Identifying activity-regulated genes required for long-term memory

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For long-term memory (LTM) formation, neuronal activity needs to be modulated through experience-dependent changes at the synapse. This synaptic plasticity includes modulation of neurotransmitter release and receptor rearrangement on the post-synaptic membrane. These changes require rapid regulation of gene expression during memory formation and consolidation. Genes rapidly expressed upon neuronal activity are classed as activity-regulated genes (ARGs), which include transcription factors required for synaptic plasticity (e.g., *c-fos*). However, how the targets of these transcription factors contribute to synaptic plasticity remains unclear. Recently, numerous ARGs were discovered in the fly *Drosophila melanogaster*, but many have not yet been characterised for a role in LTM. To understand the role of ARGs in LTM, we performed an RNAi knockdown screen with a variety of ARGs using appetitive olfactory conditioning to measure LTM performance and potentially found two ARGs required for LTM. To understand ARG dynamics upon learning, endogenous fluorescent tags were engineered for one of these genes. Imaging allowed us to confirm its expression in the olfactory learning and memory centre (the mushroom body) upon exposure to sensory stimuli. This research will help us further understand the molecular regulation of LTM. I will present our latest findings.

Keywords: memory, LTM, arg, conditioning, transcription factor

*Speaker

Identifying metabolic interactions between NSCs and glial cells

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Metabolism and mitochondrial homeostasis are key elements to normal functioning of cells and organisms. Mitochondrial dysfunction plays a key role in the development of many disorders, but how it affects neural stem cells (NSCs) of the nervous system remains largely unknown. Brain development requires a delicate balance between NSC proliferation and subsequent differentiation, which are strongly influenced by their environment in the stem cell niche. Recent studies have provided controversial results on the dependence of NSCs on mitochondrial Oxidative Phosphorylation (OxPhos). In the course of this research project, we are assessing which conditions may render NSCs in the developing *Drosophila* brain more, or less, sensitive to mitochondrial dysfunction *in vivo*. We found that induction of mitochondrial dysfunction in all NSCs compromises NSC proliferation significantly. On the other hand, when this induction is limited to a few NSCs within the *Drosophila* brain, via mosaic analysis, this proliferation defect is partially rescued. Our main hypothesis is that glial cells provide metabolites to NSCs, to support and compensate for the NSC-specific mitochondrial dysfunction. In order to identify which metabolic interactions provide a buffer against mitochondrial dysfunction between different cell types, we have conducted an RNAi-based small-screen to knock down metabolic genes in the glial niche, while simultaneously inducing OxPhos-inhibition in NSCs. In addition, we performed single cell RNA sequencing to investigate transcriptional differences in cells within the niche in response to mitochondrial dysfunction in NSCs. Better understanding of these events will advance our understanding of mitochondrial disorders and neurodegeneration and potentially lead to novel therapies aimed at reducing metabolic stress caused by mitochondrial dysfunction.

Keywords: Neural Stem cells, Mitochondrial dysfunction, Metabolism

*Speaker

In vivo dissection of behaviorally-relevant neuronal relaxin signaling pathway

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Relaxin-like peptides (relaxins) belong to the insulin-like family of peptides. Instead of acting via receptor tyrosine kinases as insulin and insulin-like growth factors do, relaxins typically exert their biological effects by binding to G protein-coupled receptors. The relaxin signaling pathway is found both in invertebrates and vertebrates, including humans, and plays important roles in the reproductive, circulatory, skeletal, renal, and nervous systems. The therapeutic potential of human relaxins, for instance, are being explored due to their vasodilator, antifibrotic, and antidepressant properties. However, the biology of relaxin receptors is not fully understood. Here, our primary aim is to identify new conserved regulatory mechanisms of relaxin receptor activity. For this, we are using a relaxin-receptor-dependent *Drosophila* phenotype to screen for new relaxin pathway components and regulators. In *Drosophila*, lack of the relaxin-like peptide, Dilp8, or its neuronal G protein-coupled receptor, Lgr3, compromises the formation of the puparium, a sort of cocoon generated by the larva from its own external cuticle to protect itself from desiccation and predators during metamorphosis. During puparium formation, or pupariation, the cuticle is actively remodeled by stereotyped muscle contractions and then hardened enzymatically. Reduced Lgr3 receptor signaling in six ventral nerve cord interneurons leads to abnormally shaped puparia, a phenotype that can be easily, cheaply, and quickly scored by eye at the same time that it is highly informative about the integrity of the Dilp8-Lgr3 relaxin signaling pathway, the presence and integrity of the critical Lgr3-positive interneurons, and the complex behavior they mediate. Taking advantage of this, we are performing a large cell-type-specific RNAi screen in vivo using a UAS-inducible RNAi stock collection for genes expressed in the central nervous system. In this presentation we will provide an update on the ~1500 genes screened up to now and an initial dissection of the candidate hits identified.

Keywords: Relaxin, *Drosophila*, genetic screen, pupariation, Lgr3 receptor

*Speaker

Inseparable: a new player in neuroblast maintenance

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De-regulated neural stem cell maintenance has profound consequences on brain development that can lead to neurodevelopmental disorders. To unveil the molecular players maintaining neural stem cell homeostasis, we performed genetic screens using *Drosophila* neuroblasts as a model system. We isolated an evolutionarily conserved gene that we named *Inseparable*. *Inseparable* is an uncharacterized protein-coding gene and its role in brain development is not known. We show that loss of *Inseparable* leads to developmental delay and larval lethality with a small brain phenotype. The mutants neuroblasts display cytokinesis defects and grow smaller in size. They lose apical basal polarity and prematurely differentiate into neurons leading to a reduction in the pool of neural progenitors. The mutant neuroblasts also show accumulation of rab11 vesicles. Our data suggests that *Inseparable* is involved in vesicle trafficking.

Keywords: Brain, neuroblast, vesicle trafficking

*Speaker

Investigating the contribution of Notch signalling in diversifying lamina neuron identities

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The development of a complex nervous system relies upon precise spatio-temporal patterning to generate diverse cell types in the right place and at the right time. Much work has focused on cell-intrinsic programs that drive cell fate decisions, but extrinsic signals can also impact cell fate decisions. My project is focused on understanding how cellular diversity is generated in the lamina, the ‘simplest’ processing layer of the optic lobe, made up of only five local neuron types (L1-L5). During lamina development, post-mitotic lamina precursor cells (LPCs) are arranged into pre-cartridges or lamina units called columns and differentiate in an invariant spatio-temporal pattern, such that the LPC located most distally in a column differentiates as an L2, followed by an L3, L1, L4 and the L5 is located most proximally. Recently our lab showed that post-mitotic LPCs in the youngest columns respond to a Hedgehog (Hh) morphogen gradient polarised from high to low along the distal-proximal axis, such that L2s and L3s are specified by high levels of Hh, L1s and L4s by intermediate levels of Hh and L5s by low levels of Hh. Here we explore how the neuron types are further diversified within the high and intermediate Hh signalling domains. Our recent data indicates that Notch signalling is also required for proper lamina neuron patterning, and may act as a binary switch to further diversify lamina cell identities. I will present data on the impact of disrupting Notch signalling activity in the lamina and my experimental plans to elucidate the molecular mechanisms responsible for Notch-dependent lamina neuron specification.

Keywords: neuron, Notch, Hedgehog, Hh, lamina, development, signalling, signaling, patterning, specification, differentiation, optic lobe

*Speaker

Investigating the links between neuronal reproductive plasticity and sexual identity in the intestine.

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The gastrointestinal tract and its neurons (so-called "second brain") have emerged as key regulators of physiology and nutritional decisions. The intestinal epithelium has a sexual fate that plays important roles in food intake, reproductive output and tumour susceptibility. Our most recent work identified gut-innervating neurons that are specifically active during reproduction in females. This provides an opportunity to investigate, for the first time, links between the sexual fate and reproductive plasticity of enteric neurons. During my postdoc I plan to investigate how the sexual fate of gut enteric neurons impacts reproductive status and plasticity. I am currently characterizing the genetic programmes associated with sex and reproduction specifically in gut-innervating neurons. I plan to focus on a subset of genes with sex and reproductive-biased expression to investigate their physiological significances. I will combine genetics, behavioural assays and live imaging to determine how the sex of enteric neurons impact reproduction. My work will shed light on the individuality of the "second brain", identifying mechanisms that, if conserved, could become relevant to the known sex biases in gastrointestinal/metabolic disorders.

Keywords: Enteric Neurons, Intestine, Gut, Sex differences

*Speaker

Investigating the role of miRNA/mRNA networks in neuronal maturation in *Drosophila*

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The development and maturation of neurons take place in a step-wise and synchronised manner. Therefore, fine-tuning the gene expression is a fundamental requirement for regulated spatial and temporal development across thousands of diverse neuronal cells. In such developmental processes, microRNAs (miRNAs) represent an attractive mechanism for controlling gene expression programs due to their ability to fine-tune the expression of multiple mRNA targets simultaneously. However, their role during the neuronal maturation processes is unclear. Therefore, we aim to investigate how different active modules of miRNAs coordinate the activity of gene networks involved in the maturation of neurons in *Drosophila*. Using the MARCM technique, we have determined and characterized the time interval for different neuronal maturation steps in the **central brain** and the **ventral nerve cord** and observed that the global inhibition of miRNA biogenesis steps impacts some of these maturation processes. We are now isolating active miRNAs in these time intervals using Argonaute protein-Affinity Purification by Peptides (**Ago-APP**) to perform **miRNA sequencing**, which, on comparison with the **mRNA sequencing** can determine which miRNAs might play a role in the maturation of neurons. Since one miRNA could have several mRNA targets and one mRNA could be targeted by several miRNAs, the effect of perturbing the function of miRNA can be minimal. So, we will investigate how knocking down or overexpressing the miRNA clusters can affect the development of the nervous system in *Drosophila*. Understanding these mechanisms opens a way to control the manipulation of gene networks for therapeutic applications in regenerative medicine or cancer.

Keywords: miRNA, neuronal maturation, Ago, APP, miRNA, mRNA network

^{*}Speaker

Kenyon cell subtypes display different odour preference and odour discrimination capabilities

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The mushroom body (MB), the center for olfactory associative learning, can discriminate between even similar odours. We examined mechanisms underlying odour discrimination in the MB by combining connectome analysis, network modelling and functional imaging. We revealed that different Kenyon cells (KC) subtypes sample the olfactory space differently, deviating from the theoretical optimum for odour discrimination.

In the MB calyx, KCs receive combinatorial input from different types of olfactory projection neurons (PNs). In the presence of inhibition, KCs display sparse population coding. The PNs-to-KCs connectivity determines the KC's pattern separation ability: it is theoretically optimal if KCs receive random inputs from the PNs.

Contrary to the common belief that different KCs subtypes receive similar information about the odour for various memory processes, our analysis of multiple connectomics datasets revealed that α/β and α'/β' KCs receive highly biased inputs from food-odour-responding PNs, while the γ main KCs receive slightly biased inputs from mating-odour-responding PNs. With the use of a MB network model that incorporates realistic PN-to-KC-subtype connectivities, we showed that biased connectivity of α/β and α'/β' KCs to food-odour-responding PNs could increase their response overlap between various food odours. In contrast, the biased connectivity of the γ main KCs to mating-odour-responding PNs results in further decorrelation of the KCs response. These predictions are supported by our functional imaging experiments. Altogether, these results suggest that the connectivities of α/β and α'/β' KCs favour generalisation of novel food odours, while the γ main KCs have reduced response overlap among food odours, which could enhance food odour discrimination.

Keywords: Mushroom body, Circuit organization, Pattern separation, Odour discrimination

*Speaker

Larval microbiota primes the *Drosophila* adult gustatory response

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The survival of animals depends, among other things, on their ability to identify threats in their surrounding environment. *D. melanogaster* lives on rotten fruits populated by microbes that produce many metabolites. Detecting these molecules helps flies to find nutrient-rich food, select egg-laying sites and avoid pathogens. While the molecular mechanisms by which invading microorganisms are detected by the fly's immune cells following infection are well known, the mechanisms by which flies detect those present in the external environment remains largely unknown. The fly's senses of smell, sight, and taste play an essential role in assessing its environment. My work focuses on the mechanisms of detection of bacteria by *Drosophila* gustatory system. We demonstrate that the peptidoglycan (PGN) that forms the cell wall of bacteria triggers an immediate feeding aversive response when detected by the gustatory system of adult flies. Although we identify ppk23+ and Gr66a+ gustatory neurons as necessary to transduce fly response to PGN, we demonstrate that they play very different roles in the process. Time-controlled functional inactivation and *in vivo* calcium imaging demonstrate that while ppk23+ neurons are required in the adult flies to directly transduce PGN signal, Gr66a+ neurons must be functional in larvae to allow future adults to become PGN sensitive. Furthermore, the ability of adult flies to respond to bacterial PGN is lost when they hatch from larvae reared under axenic conditions. Recolonization of axenic larvae, but not adults, with a single bacterial species, *Lactobacillus brevis*, is sufficient to restore the ability of adults to respond to PGN. Our data demonstrate that the genetic and environmental characteristics of the larvae are essential to make future adults competent to respond to certain sensory stimuli such as PGN.

Keywords: behavior, taste, priming, bacteria

*Speaker

Markerless tracking of subtle locomotor defects in tethered adult *D. Melanogaster*

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Precise joint and appendage tracking has the potential to accelerate the disentanglement of neural correlates of motor control, notably in the genetically tractable and numerically simple nervous system of the adult fly, *Drosophila melanogaster*. Pose estimation accuracy is particularly important when studying specific motor neural circuits or single genetic manipulations contributions to locomotion. The advancement of deep learning-based methods in the past years has largely revolutionized computer vision techniques and now gives the potential to precisely track user-defined features in challenging experimental setups as small as they can be for adult fly behavioral studies. Therefore, we took advantage of *DeepLabCut* – an open-source toolbox recently developed by colleagues that allows training of a deep neural network by using limited training data – to track *D. Melanogaster* locomotion without markers. We have built a "fly treadmill" surrounded by a multicamera system to record a tethered yet freely walking adult fly and reconstructed the 3D pose estimation from the automated 2D annotations of each camera view thanks to triangulation methods. Here we describe the crafted treadmill setup and propose a simple working pipeline to precisely extract relevant adult fly kinematics. We aim at leveraging this system to identify potential disease modifying targets in *tbph*^{-/-} flies for the motor neuron disease Amyotrophic Lateral Sclerosis.

Keywords: motor control, locomotion, behavior, motor neuron disease, deep learning, computer vision

^{*}Speaker

Methylating sleep: a role for H3K9 methylation in sleep function and homeostasis

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In all species, sleep and wakefulness appear to correlate with specific changes in gene expression, thus providing a potential handle to the identification of "sleep genes", a first pivotal step towards understanding the yet mysterious cell biological functions of sleep. Following this path, we used transcriptomics to analyse how gene expression changes in the brain upon acute and chronic sleep deprivation and found a clear role for histone methylases in regulating sleep-specific changes in gene expression. Prolonged sleep-deprivation – whether mechanical or genetically driven – leads to an overall decrease in gene expression driven by enhanced histone methylation activity (H3K9) in the brain and adult-specific removal of H3K9 methylases (SuVar3-9, G9a) in neurons interferes with this process, blocking any sleep-specific changes in gene expression and cancelling the fly's internal homeostatic control of sleep. As we write this abstract, it is yet not clear whether this effect is systemic or whether it is coordinated by specific sleep centers in the brain, such as the *Exlf2* neurons. The answer is likely to provide information on whether sleep in *Drosophila* is a coordinated behaviour driven by a master regulator brain center, or rather an emerging behaviour in which each neuron sleeps independently.

Keywords: Sleep, H3K9 methylation, Su(Var)3, 9, sleep homeostasis

*Speaker

Modulatory enhancement of input maintains reinforcement of aversive learning in hungry *Drosophila*

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Maintaining flexibility of neural circuit function under different internal states is a critical brain process. In hungry *Drosophila*, aversively reinforcing dopaminergic neurons (DANs) are inhibited to provide part of the hunger-dependent control of the expression of food-seeking memory. Multitasking the reinforcement system for this purpose potentially undermines its ability to function in aversive learning. Here we show that chronic hunger enhances aversive learning and that normal and hunger-enhanced learning require adipokinetic hormone (AKH) signaling, the fly functional homolog of the vertebrate glucagon. AKH is produced and released in the hemolymph by the corpora cardiaca and it influences aversive learning via its receptor expression in two neurons per hemisphere located in the subesophageal zone of the brain. Connectomic approaches reveal that the AKH receptor-expressing neurons are presynaptic to ascending neurons that relay shock and bitter taste information to aversively reinforcing DANs. All of the cells comprising this AKH regulated pathway are required for efficient aversive learning and its enhancement by chronic starvation. We propose that coordinated AKH-mediated modulatory enhancement of input compensates for the hunger-state dependent suppression of reinforcing dopaminergic neuron activity to preserve aversive reinforcing function when required.

Keywords: aversive olfactory learning, immediate memory, aversive dopaminergic neurons, Adipokinetic hormone (AKH), starvation

*Speaker

Molecular logic of building a visual map

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Precise mapping of neural networks is critical for navigating and perceiving the external world around us. In the visual system for instance, axons of photoreceptors (PRs) in the retina form precisely matched connections with their synaptic targets in the brain: PRs collect and transmit visual information from the retina to different brain regions while preserving its spatial information, a phenomenon known as retinotopy. Although progress has been made in understanding some of the basic molecular mechanisms controlling retinotopy, it remains unclear how different regions of the visual system are developmentally coordinated to transfer spatially intact images across multiple processing layers in the brain. To address this fundamental question, I use the *Drosophila* visual system that sequentially processes visual information from the retina in subsequent brain structures, analogous to the vertebrate visual system. During retinal development, a wave of differentiation, the morphogenetic furrow, sweeps through the eye imaginal disc in a posterior-to-anterior (A-P) direction. As the morphogenetic furrow progresses anteriorly in the eye disc, differentiating PR axons sequentially arrive in the optic lobe neuropil in the order of their birth i.e., the most posterior (oldest) PRs target first, and the most anterior (youngest) PRs target the later born columns. Furthermore, each row of PRs that differentiates simultaneously along the dorsal-to-ventral (D-V) axis of the eye disc also retains its positional information when retinotopically projecting into the optic lobe. Consequently, both the A-P and D-V position of PRs is preserved while they innervate medulla columns and lamina cartridges of optic lobe. I am currently using genetic and single cell transcriptomic approaches to identify and characterize the spatial and temporal positional cues that build retinotopy. From preliminary analysis of a single cell RNA-seq dataset of the retina, I have identified putative D-V markers based on co-expression with dorsal genes (e.g., *mirr*) that are currently being screened for their role in retinotopy. These studies will reveal principles that may be conserved and applicable to build similar neural maps used in processing other sensory stimuli in flies and across species. Overall, my research will have broader implications in ocular pathology and ultimately treating eye disorders.

Keywords: *Drosophila*, optic lobe, retina, photoreceptors

*Speaker

Multiple modes of post-transcriptional regulation determine the timing of neuronal commitment

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Developmental timing is a key yet understudied aspect of nervous system development. Here, we focus on understanding the molecular mechanisms that regulate the timing of neuronal commitment using the well-characterised lamina of the *Drosophila* visual system. During lamina development, post-mitotic precursor cells differentiate into neurons (or commit to neuronal fate) in response to ERK/MAPK signalling activity, which is triggered by extrinsic signals from glia. Neuronal commitment is marked by expression of the pan-neuronal protein Elav (a Hu protein homolog), neuron sub-type-specific proteins and extensive morphological changes including neurite outgrowth. Previously, we showed that the transcriptional effector of ERK signalling, Pointed-P1 (Pnt-P1) is both necessary and sufficient to drive neuronal commitment, arguing that transcriptional targets of Pnt-P1 drive this process. Surprisingly, we find that though pan-neuronal protein expression correlates with ERK activity, Pnt-P1 expression and the onset of neuronal commitment, their transcripts do not. Instead, post-mitotic precursors express pan-neuronal transcripts (such as *elav*) independently of Pnt-P1. Thus, Pnt-P1 does not directly regulate the expression of pan-neuronal genes but instead must indirectly regulate expression of the proteins they encode. Consistent with this hypothesis, we find that global rates of mRNA translation increase just prior to neuronal commitment in a Pnt-P1-dependent manner. Moreover, increasing mRNA translation independently of Pnt-P1 drives premature neuronal commitment. We are now working to identify the direct transcriptional targets of Pnt-P1 that promote mRNA translation to drive neuronal commitment. Interestingly, Pnt-P1 has been reported to bind to the lncRNA:CR31044 locus, which encodes the microRNAs miR-279/996. These target the 3'UTRs of several pan-neuronal mRNAs including *elav* to repress protein expression. lnc:CR31044 is expressed in uncommitted post-mitotic precursors and its expression is inhibited upon ERK activity and Pnt-P1 expression, suggesting that it is a negatively regulated target of Pnt-P1. In sum, we have uncovered multiple modes of post-transcriptional regulation (*i.e.*, at the level of global mRNA translation and of miRNAs targeting specific transcripts), which drive neuronal commitment through pan-neuronal protein expression in response to ERK activity. This regulation is essential for precise spatio-temporal patterning as it enables precursors to rapidly commit to a neuronal fate in response to extrinsic signals.

Keywords: neuronal commitment, posttranscriptional regulation, translation, microRNA, PntP1

*Speaker

Nepriylsin 1 controls SIFamide Levels to Regulate Feeding-related Behavior in *Drosophila melanogaster*

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Feeding-related behavior in *Drosophila melanogaster* encompasses olfactory and gustatory detection of odors, foraging, and food uptake. Peptidergic neurons are known to modulate feeding behavior. One of the peptides, SIFamide (SIFa) has been shown to translate hunger signals into feeding behavior in *Drosophila*. Specifically, activation of SIFamide signaling increases appetitive olfactory and gustatory behavior as well as food uptake. However, it is not known how SIFamide is subsequently inactivated. We hypothesized that a member of the neprilysin M13 family of metalloendopeptidases is involved. We first used biochemical analyses to show that Nepriylsin 1 (Nep1) is the enzyme responsible for degradation of the SIFa neuropeptide. We next characterized the expression pattern of Nep1 and found prominent staining for Nep1 in the adult mushroom bodies. Furthermore, we showed that SIFamidergic neurons project to the mushroom bodies. Given the known role of mushroom bodies in controlling food-seeking behavior, we hypothesized that Nep1 in the MB may modulate SIFa levels, thereby impacting food-seeking behavior. Our behavioral results show that overexpression of Nep1 via P-element insertion in the *Nep1* gene impaired hunger driven food-seeking behavior, while revertant flies displayed a feeding behavior indistinct from wildtype control flies. On the other hand, ablation of SIFa neurons (SIFamide > rpr) in the brain showed significantly prolonged seeking times compared to wild control flies, similar to what we observed in the P-element insertion flies. Our study identifies Nep1 as a novel regulator of food-seeking processes through the modulation of SIFamide levels, shedding light on the interplay between neuropeptidergic signalling and neural circuits governing feeding behavior and opening avenues for further exploration into the molecular mechanisms underlying this essential behavior in fruit flies.

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Keywords: Drosophila, Feeding behavior, Neprilysin, Neural circuits, SIFamide

Neurogenetics of confinement-induced behavioral alterations

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The molecular and neural mechanisms underlying the translation of physical confinement to internal state changes and how these alter behavior are poorly understood and very difficult to study in natural settings due to an excessive number of possible confounding factors. Innate behaviors, which are complex, genetically-encoded behaviors that proceed with a predictable sequence, provide a unique window to study the effects of confinement on behavior. Adding this to the power of a genetically tractable model organism, we aim to help unravel both the molecular and cellular foundations of behaviorally-relevant, confinement-induced changes in internal states. Here, we start to genetically dissect the wing expansion behavior that *Drosophila* flies perform upon eclosing from their puparium. The execution of this behavior, which is triggered by the hormone Bursicon, is strongly negatively-regulated by spatial confinement. Namely, wing expansion behavior occurs within 30 min when animals eclose in normal, unconfined conditions, yet it is delayed to > 180 min upon eclosion into a confined environment. The molecular mechanisms controlling this wing expansion decision process upon confinement are poorly understood. By chance, we found a background mutation segregating in *Drosophila* stocks where wing expansion behavior is severely compromised specifically under confinement, not under normal unconfined conditions. We are mapping this mutation in the hope that it provides a unique entrance into the molecular and neural underpinnings of confinement-induced behavioral control. In parallel, we have performed two studies to identify factors that are affected in the central nervous system of animals undergoing spatial confinement: a genome wide time-series transcriptomic study of confined and unconfined animals and a small-scale genetic screen for neuropeptides and receptors affecting this confinement-induced behavioral response. We will present the preliminary analyses of these studies. We hope that these approaches will help clarify how spatial confinement alters wing expansion behavior, and provide further insight on how environmental changes affect behavior and health.

*Speaker

Keywords: Innate behavior, genetic screen, wing expansion

Plasticity of adult intact neural tissue under stress conditions

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Our knowledge about the capacity for plasticity of adult neural circuits is limited. Neural plasticity includes axonal regeneration when a neuron is damaged, but also the reshaping of surrounding intact nervous tissue. Understanding neural plasticity and how to modulate it will be essential to restore the functionality of circuits. We use *Drosophila melanogaster*'s digestive tract as a model system where we induce stress in the organ and assess the plasticity response of the intact nervous system that innervates it. To this end, we have developed an automated morphometric analysis pipeline that allows us to quantify the amount of neural tissue and the complexity of the neural network in whole mount preparations. We have also set up a feeding protocol using Dextran Sodium Sulfate (DSS), a chemical which is widely used in flies to alter intestinal stem cell proliferation. It also affects basement membrane structure, muscle contractibility and induces trachea sprouting, features that are reversible after a recovery period. Contrary to mammals, DSS causes minimal cell death in the fly. With this feeding protocol we show neural plasticity in the enteric neural network, with a sustained increase in neural tissue and its complexity. We have focused on the anterior midgut innervation and the increase in neural tissue observed takes place without changes in the number of neurons or glial cells present in the hypocerebral ganglion, the major source of neurons innervating this region of the gut. We are characterizing the cellular and molecular mechanisms that regulate the neural growth observed. Experiments in this direction include analysis of the dynamics of change in the dendritic and axonal compartments, assessment of neural plasticity stability following a recovery period after DSS feeding, as well as identification of the signaling pathways behind the observed neural growth. Our work unveiling the nature of organ-neural tissue interactions under stress conditions can shed light on how to modulate neural plasticity.

Keywords: neural plasticity

*Speaker

Post-transcriptional control of neurotransmitter receptors during synaptogenesis in vivo

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Neuronal circuit function is defined by synaptic wiring with subcellular precision, which is itself dependent on the distribution of Neurotransmitter Receptors (NTRs) onto postsynaptic dendritic arbors to align with the neurotransmitter identity of the presynaptic neuron. However, little is known on the developmental mechanisms that establish such synaptic subcellular distribution. We address this question by exploring *in vivo*, the cellular interactions between synaptic partners, and the post-transcriptional mechanisms that control in space and time NTR recruitment to post-synaptic terminals during synaptogenesis. As a paradigm, we use the T4 neurons in the *Drosophila* optic lobe, the fly's visual processing center. Each T4 receives input from different upstream neurons by clustering distinct NTRs to discrete dendritic areas. We found that developing T4 dendrites express mRNAs coding for GABA NTRs subunits 3 days before their protein is produced and synapses are formed. This suggests a previously underestimated tight and specific coordinated regulation of NTR distribution at the transcript level. Using circuit tracing tools, we found that different T4's presynaptic inputs establish synaptic contacts with T4 neurons at distinct time points during development. We hypothesize this temporal circuit assembly influences partner selection, and contributes to the establishment of subcellular synaptic domains in T4 dendrites by regulating post-transcriptional mechanisms that synchronize NTR mRNAs-translation and recruitment to the corresponding synapses during synaptogenesis. We are currently using single-cell approaches combined with endogenous tags, single molecule RNA detection techniques, and *in vivo* SunTag methods to monitor translation to (i) dissect the mechanisms and molecular players of the spatiotemporal post-transcriptional regulation of NTRs, and (ii) test the role of cell contacts and developmental patterned stimulus-independent neural activity (PSINA) between synaptic partners in regulating NTR local translation and recruitment in developing T4 dendrites. Our multi-level approach will identify cellular interactions and molecular players that orchestrate synapse formation, and provide seminal insights into the function of activity and mRNA regulation during neural circuit assembly.

Keywords: neuronal development, neural circuits, RNA, post, transcriptional control, optic lobe, dendrites, synaptogenesis

*Speaker

RNAi screen identified CG1701, CG11600 and Acp26Ab as re-mating delay factors in the *Drosophila* post-mating response

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Seminal fluid proteins (SFPs) are components of the male ejaculate that are transferred together with sperm to the female reproductive tract during copulation. *Drosophila melanogaster* is an excellent model organism for unraveling the function of SFPs secreted by the male accessory gland (MAG). *Dm*SFPs, such as sex peptide (SP) and ovulin, are central mediators of several noticeable changes in female behavior and reproductive physiology after mating, collectively termed "post-mating responses" (PMRs). PMRs include reduced female receptivity, stimulation of ovulation, and sperm storage. Furthermore, our previous data suggests that a seminal fluid component other than SP modulates the female wing song during copulation (1). The physiological function of the bulk of secreted SFPs and the identity of the seminal fluid component that possibly elicits the female copulation song remain poorly understood. Wild type females that are mated with the Hox gene *Abdominal-B* enhancer deletion mutant *iab-6cocu* exhibit a severe reduction in female copulation song (1) and are prone to re-mating (2). The *iab-6cocu* mutant has compromised secondary cells (specialized secretory cells of the MAG), which leads to alterations in their secreted seminal fluid during mating (2). Based on a differential seminal fluid proteome and transferome data between the *iab-6cocu* mutant and wild type flies, we performed an RNAi screen of candidate SFPs that were transferred in significantly lower or higher amounts from the *iab-6cocu* mutants to females. While no effects on female copulation song were observed, we revealed three novel candidates, CG1701, Acp26Ab and CG11600, affecting remating and confirm lectin-46Cb as a remating factor. Mates of the males with tissue-specific RNAi-mediated knockdown of CG1701, Acp26Ab, and CG11600 in the main and/or secondary cells of the MAG show defects in long-term egg laying and suppression of receptivity. In addition, we investigate whether these phenotypes are caused by a defect in the sperm storage in female mates of the RNAi-mediated knockdown males. Our results suggest that the female copulation song and the long-term PMRs observed in mated females are not solely induced by main cell or secondary cell SFPs secretions of the male reproductive system, but rely on the complex interplay of several factors.

(1) Kerwin P, Yuan J, von Phillipsborn AC. *Female copulation song is modulated by seminal fluid*. Nat Commun. 2020 Mar 18;11(1):1430. doi: 10.1038/s41467-020-15260-6. PMID: 32188855; PMCID: PMC7080721.

(2) Gligorov D, Sitnik JL, Maeda RK, Wolfner MF, Karch F. *A novel function for the Hox gene Abd-B in the male accessory gland regulates the long-term female post-mating response in*

*Speaker

Drosophila. PLoS Genet. 2013 Mar;9(3):e1003395. doi: 10.1371/journal.pgen.1003395. Epub 2013 Mar 28. PMID: 23555301; PMCID: PMC3610936.

Keywords: Seminal fluid proteins, post, mating responses

Regulation of temporal patterning during development and evolution.

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The central nervous system contains numerous neurons divided in several cell types that can be defined by their morphology, connectivity, molecular identity etc. This complexity is achieved by the interplay of two main mechanisms, termed temporal, and spatial patterning. Temporal patterning refers to the sequential expression of a series of temporal transcription factors (tTFs) in neuronal stem cells, which alter their capacity to generate neuronal types. Spatial patterning corresponds to the differential expression of transcription factors in neuronal stem cells that come from different neuroepithelial domains. Both spatial and temporal patterning of neuronal stem cells affect neuronal fate and can be found in both vertebrates and invertebrates. While temporal patterning appears to be conserved in animals, the transcription factors that implement it are different.

Here, we use the *Drosophila melanogaster* developing visual system to understand how different temporal series may evolve.

Most neuronal cell types of the *D. melanogaster* optic lobes originate from a neuroepithelial structure, which is called Outer Proliferation Center (OPC). The OPC can be divided into two parts, the main OPC (mOPC) and the tips of the OPC (tOPC), that implement two distinct temporal series. Our goal is to understand what regulates the differences in the temporal series and how these differences have evolved. Our preliminary results suggest that the expression of a signalling molecule, *wg*, and two conserved transcription factors in the tOPC, *Sp1* and *button-head* (*btd*), may be responsible for the differentiation of the tOPC temporal series from that of the mOPC. Moreover, using immunohistochemistry and fluorescence *in situ* hybridization, we show that both transcription factors are expressed in the tOPC of insects that have diverged from *Drosophila melanogaster* up to 390 million years ago.

In future experiments, we want to test the scenario where the expression of two spatially restricted transcription factors, Sp1 and Btd, caused the divergence of the tOPC temporal series from the mOPC one, which, in turn, led to the generation of different neuronal types.

Keywords: Neuronal development, Neuronal identity, Evolution, Temporal patterning, Spatial patterning, Optic lobe

*Speaker

Reorganization strategy of the dopamine neuron network architecture during pupal metamorphosis of *Drosophila*

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Dopaminergic neurons (DANs) play crucial roles in the control of behavior in vertebrates and invertebrates. In *Drosophila* Larvae and adults occupy different ecological niches and display significant differences in morphology with largely different behavioral characteristics, requiring an adaptation of the according neuronal networks. While the architecture of DAN circuits in larval and adult *Drosophila* is well understood, our knowledge about their transition during pupal metamorphosis remains scarce.

To understand the reorganization of the dopaminergic system during metamorphosis, we established reference brain templates for nine representative time points during pupal development at 6-h intervals between 6h-48h after puparium formation (APF) and at 72h APF. Together with the commonly used brain templates for the starting point (late third-instar larva; Muenzing et al., 2018) and end point of metamorphosis (adult, 2018 Janelia Standard brain; Bogovic et al., 2020), we can now register and compare DANs of different samples throughout developmental stages using immunohistochemical labeling against tyrosine hydroxylase (TH). Previous studies revealed two scenarios of DAN reorganization. When the larval mushroom body (MB) lobes are pruned and adult-specific lobes are formed, (1) PAL cluster DANs retract from the peduncle and project to the adult optic lobes (Truman et al., 2023). In contrast (2) adult-specific PAM neurons send projections to newly developing neuropil (MB γ -lobe) before the MB intrinsic neurons re-extend their axon branches, and then mature to adult PAM DANs (Bornstein et al., 2018). We identified all other clusters of DANs in the flyEM dataset in adult *Drosophila* (Zhang et al., unpublished) and examined their development. We found three additional general scenarios of DAN reorganization during metamorphosis: (3) Larval-specific PAM cluster (pPAM) DANs undergo apoptosis shortly after their target neuropil is pruned being a prerequisite for timely adult PAM DAN maturation as mentioned above. (4) In the brain regions that are unique to the adult brain, i.e., the central complex, DANs become TH positive already before reaching newly emerging neuropils (PPM3, PPL1-, PPL2- subclusters). (5) In the brain regions that do not show extensive pupal reorganization, DANs innervating these target neuropils remain with little reorganization (e.g., PPM1/2) but display an increase in cell numbers (PPL1 subclusters, SEM and SEL).

Our study revealed that DAN development from larva to adult can be categorized into 5 major reorganization strategies that are tightly correlated with the reorganization patterns of their target neuropils.

*Speaker

Keywords: dopamine, pupal metamorphosis, DANs, brain template, reorganization

Requirements for both Dcr-2 and cGlr1/Sting dsRNA-activated signaling for aberrant innate immune induction in *Adar5G1* null mutant flies lacking adenosine to inosine editing in dsRNA

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Drosophila Adar is mainly expressed in the nervous system (Jepson et al. 2011) and carries out A-to-I RNA editing in dsRNA hairpins in pre-mRNAs. Edited mRNAs are numerous in CNS and enriched in ion channels and neurotransmitter receptor subunits which produce new edited proteoforms (Duan et al. 2017). Loss of Adar RNA editing activity in *Adar5G1* null mutant flies leads to a severe locomotion defect, consistent with loss of edited CNS proteoforms, and also to aberrant innate immune AMP induction (Deng et al. 2020). The AMP induction is suppressed by silencing of *Dicer-2* in cholinergic neurons (Deng et al. 2020) and may be similar to aberrant activation of Dicer-related vertebrate cytoplasmic antiviral dsRNA sensors by unedited dsRNA. We sought to determine whether knocking down the antiviral cGas-Like Receptor1 (cGlr1), recently shown to be activated by dsRNA in *Drosophila*, or Sting receptor which acts downstream of cGlr1, can rescue aberrant AMP induction and other defects in *Adar5G1* flies. We found that ubiquitous RNAi knockdown of *cGlr1* in *Adar5G1*, *arm>cGlr1* RNAi flies rescues the aberrant immune induction in heads; however, it does not rescue the locomotion defect or reduced survival to eclosion. Similar RNAi knockdown of Sting improves survival and rescues aberrant immune induction but not locomotion defects. Furthermore, the double null mutant *Adar5G1; cGlr1 KO* prevents the immune induction and significantly improves the locomotion. It is important to note that *cGLR1 KO* alone has no effect on wildtype locomotion. These data suggest that the innate immunity and neuronal defects in *Adar5G1* null mutant involve both Dcr-2 and cGlr1/Sting pathways, perhaps working together.

Keywords: Adar, Sting, cGLR1, immunity, locomotion

*Speaker

SWI/SNF subunit Snr1 regulates neural stem cell determination and differentiation

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In the developing brain, neural stem cells spatially and temporally regulate gene expression. These patterns are integrated during differentiation to generate the diverse types of cells that build the mature brain, while failure to integrate multiple factors leads to defective brain structures or tumor formation. Previous studies suggest coordinated change of chromatin state are needed to direct neural stem cell patterning and differentiation, but the mechanisms remain unclear. Analysis of the SWI/SNF chromatin remodelling complex protein Snr1 identified a key role for Snr1 in regulating the transition of neuroepithelial cells into neural stem cells and subsequent differentiation of neural stem cells in the *Drosophila* optic lobe. Loss of Snr1 in neuroepithelial cells lead to premature neural stem cell formation. Additionally, loss of Snr1 in neural stem cells resulted in inappropriate perdurance of neural stem cells into adulthood. Using single-cell RNA-sequencing, we found that reduced expression of Snr1 in neuroepithelial or neural stem cells lead to the differential expression of target genes involved in neural specification. In particular, Notch signalling was identified as a target of Snr1 regulation in the neuroepithelial to neural stem cell transition. We found that Snr1 was associated with the actively transcribed chromatin region of Notch pathway genes. Thus, Snr1 regulates the chromatin state in neuroepithelial cells and maintains chromatin state in neural stem cells for proper brain development.

Keywords: Snr1, SWI/SNF, neural stem cell, neuroblast, optic lobe, chromatin, development

*Speaker

Sex- and strain-specific effects of age on sleep/wake behaviours in *Drosophila*

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Drosophila are a major discovery platform in the biology of ageing due to their balance of relatively short lifespan and relatively complex physiology and behaviour. Among behavioural approaches in *Drosophila*, sleep/wake behaviours are particularly well studied: as such, these assays offer an attractive approach for assessing nervous system function and overall health with age in *Drosophila*. However, both the ageing and sleep biology fields have occasionally suggested generalised conclusions from studies using only one sex or one strain of *Drosophila*. In an attempt to establish a baseline for studying sleep/wake changes with age between sexes and across strains, I have used the DAM5H system (a widely-used behavioural system from Trikinetics) to characterise age-related changes in activity and sleep behaviours in female and male flies from three different commonly-used lab strains: *Canton-S*, *Dahomey*, and *w1118*. My results highlight a surprising degree of divergence among behavioural changes with age, with some features of activity and sleep patterns showing strongly different patterns of ageing between sexes and among strains. At the same time, some features of age-related change in activity patterns and sleep quality are more consistent, suggesting that these may be more robust measures across sexes and strains. As a whole, my results suggest that both sex and background strain need to be carefully considered in project design for studies on age-related behaviour changes in *Drosophila*.

Keywords: ageing, lifespan, sleep, circadian biology, sexual dimorphism

*Speaker

Single-cell RNA sequencing of motoneurons identifies regulators of synaptic wiring in *Drosophila* embryos

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The correct formation of neuronal circuits during development is one of the most complex processes, as precise and specific connections were made out of a huge number of possibilities. While circuit wiring is in general genetically determined, the mechanisms guiding axons to innervate target cells are not well understood. We studied embryonic motoneurons and their targets in *Drosophila* to shed light on this process. Specific driver lines labeling embryonic motoneurons and muscles were profiled using single-cell RNA sequencing; the spatial position of the cells in the embryos were mapped using bioinformatic approaches as well as imaging; functional changes were investigated by genetic manipulations. We found that a combination of homeodomain transcription factors (TFs) and downstream immunoglobulin domain proteins (normally cell-surface-proteins, CSPs) is expressed in individual cells and plays a key role in determining cell-specific connections between motoneurons and their targeted muscles. We functionally showed five homeodomain TFs and four immunoglobulins (CSPs) that are crucial for the neuromuscular wiring. Knockdown and ectopic expression of these five homeodomain TFs resulted in cell-specific synaptic wiring defects that were partially phenocopied by genetics modulation of their immunoglobulin targets. These findings suggest that expressions of homeodomain TFs and immunoglobulins (CSPs) are closely linked and they function as a critical determinant of neuron circuit formation. Our study provides new insights into the process of neural circuit formation in single cell level and highlights the role of homeodomain TFs and downstream CSPs in this process.

Keywords: single, cell RNA sequencing, circuit wiring, homeodomain transcription factors, Ig domain encoding proteins, embryonic motoneuron

*Speaker

Single-cell transcriptome profiles of *Drosophila* fruitless-expressing neurons from both sexes

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Drosophila melanogaster reproductive behaviors are orchestrated by *fruitless* neurons. We performed single-cell RNA sequencing on pupal neurons that produce sex-specifically spliced *fru* transcripts (*fru P1* neurons). Uniform Manifold Approximation and Projection (UMAP) with clustering generates an atlas containing 113 clusters. While the male and female neurons overlap in UMAP space, more than half the clusters have sex-differences in neuron number, and nearly all clusters display sex-differential expression. Based on an examination of enriched marker genes, we annotate clusters as circadian clock neurons, mushroom body Kenyon cell neurons, neurotransmitter- and/or neuropeptide-producing, and those that express *doublesex*. Marker gene analyses also shows that genes that encode members of the immunoglobulin superfamily of cell adhesion molecules, transcription factors, neuropeptides, neuropeptide receptors, and Wnts have unique patterns of enriched expression across the clusters. In vivo spatial gene expression links to the clusters are examined. A functional analysis of *fru P1* circadian neurons shows they have dimorphic roles in activity and period length. Given that most clusters are comprised of male and female neurons indicates that the sexes have *fru P1* neurons with common gene expression programs. Sex-specific expression is overlaid on this program, to build the potential for vastly different sex-specific behaviors. Current functional studies that are based on this single cell RNA-seq data set will be presented. PMID: 36724009

Keywords: Courtship, fruitless, doublesex, reproduction, behavior

*Speaker

Social and dopaminergic influences on preferential self-administration of methamphetamine in *D. melanogaster*

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Social isolation is a stressful condition in social animals and humans that negatively impacts adaptive behaviors and can induce maladaptive behavior, such as increased consumption of addictive substances. This has been shown in rodents, and in *Drosophila* where social isolation affects behaviors such as sleep, locomotion, courtship, aggression, and increases resistance to sedation to ethanol. Social experience modulates activity of dopaminergic neurons, and dopaminergic signaling is important in behaviors that are modulated by social isolation, such as sleep and aggression. Our aim was to investigate if social isolation modulates preferential self-administration of METH, and if the change in preference can be explained by dopaminergic signaling.

We have previously shown that *Drosophila* voluntarily and preferentially self-administers METH-laced food in the FlyCafe where singly housed flies have a choice between liquid sugar-based food versus one laced with METH. METH preference is present from the first day of the experiment and can persist for at least seven days. Flies with mutation in the dopaminergic transporter, *fumin*, show no preference over multiple days in FlyCafe, like the wild type flies that are fed 3-iodo tyrosine, a dopamine synthesis inhibitor. Surprisingly, a single day of isolation before Fly Café results in negative preference during first two days. One day of social isolation resulted in decreased dopamine measured in the head homogenates measured by the HPLC-MS method. However, the preference for METH on the first day of testing in flies that do not experience social isolation before or during the test as in the CAFÉ assay have on average lower METH preference than flies in FlyCafe.

These results show that the modulation of dopamine signaling is permissive for the expression of METH preference. Decreased dopamine due to social isolation can temporarily decrease METH preference. Test design (FlyCafe versus CAFÉ) influences initial METH preference emphasizing the need for better understanding of social and other environmental effects on behavioral outcomes.

Keywords: social isolation, dopamine, methamphetamine, *fumin*, addiction

*Speaker

Testing Causality between Developmental Neuronal Remodeling and Behavior Using a New Model System in *Drosophila*

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Developmental neuronal remodeling is essential for the precise formation of the mature nervous system. *Drosophila* is an important model for studying neuronal remodeling. However, existing experimental systems are not suitable for examining neuronal remodeling at the circuit level and its effect on behavior. We present a new model system within the *Drosophila* nervous system, the neuronal circuitry controlling backward locomotion, to investigate neuronal remodeling. We show that the Moonwalker SEZ neuron (MooSEZ), which elicits backward walking in adult flies, also functions in larva and is part of a conserved motor circuit persisting from larval to adult stages. Furthermore, neuronal remodeling is crucial for maintaining the behavioral output of the circuit. The well-characterized connectivity of the circuit, combined with the low number of elements per neuronal subtype, and availability of subtype-specific driver lines, provide an appealing system to mechanistically study developmental remodeling of neuronal circuits. Moreover, as each of the neuronal elements of the backward locomotion circuitry directly controls the motor output, the impact of developmental neuronal remodeling on the behavioral outcome could be readily tested using this system. Hence, we establish a new model system in *Drosophila* to investigate basic yet largely unknown principles of neural circuit remodeling and their relation to behavior.

Keywords: Developmental neuroscience, *Drosophila*, metamorphosis, neural circuits, neuronal remodeling, motor control, backward locomotion, MooSEZs, MDNs, ecdysone

*Speaker

The TCF7L2 homolog, Pangolin, is essential for *Drosophila* Insulin producing cell development.

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Drosophila insulin-producing cells (IPCs) are a group of neuroendocrine cells situated in the pars intercerebralis of the brain that produce *Drosophila* insulin-like peptides 2, 3 and 5. The IPCs are the functional equivalent of the pancreatic beta cells. Previous work has identified a few homologous transcription factors to act in IPCs and pancreatic beta cells, suggesting that the regulation of development and function of these cells is conserved. Multiple genome-wide association studies have independently found that variants in TCF7L2 are strongly associated with increased type 2 diabetes risk and several studies show that it is required for beta cell function. We here show that Pangolin, the fly homolog of TCF7L2, is involved in the regulation and development of IPCs. IPC-specific expression of a dominant-negative form of Pangolin results in smaller flies that are developmentally delayed. This is the result of reduced insulin-like peptide expression in the IPCs. The IPCs additionally show prominent morphological defects in neurite complexity and cell size. We further show that Pangolin acts upstream of Dimmed, a transcription factor that promotes a prosecretory cell fate, of Eyeless which regulates IPC morphology and controls insulin-like peptide transcription, and of Hth which in the larval stage is also involved in insulin-like peptide transcriptional control. Our work identifies an additional regulator of the IPCs that is conserved in the mammalian pancreatic beta cell, lending credence to the hypothesis that both cell types are evolutionarily related.

Keywords: Insulin, producing cells, Pangolin, neuroendocrine

The role of Imp and Syp RBPs in precise neuronal elimination by apoptosis through the regulation of TFs

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Neuronal stem cells generate a limited and consistent number of neuronal progenies, each possessing distinct morphologies and functions. These two parameters, involving the precise production of neurons with distinct identities, must be meticulously regulated throughout development to ensure optimal brain function. In our study, we focused on a neuroblast lineage in *Drosophila* known as Lin A/15, which gives rise to motoneurons (MNs) and glia. Interestingly, Lin A/15 neuroblast dedicates 40% of its time to producing immature MNs that are subsequently eliminated through apoptosis. Two RNA-binding proteins, Imp and Syp, play crucial roles in this process of neuronal elimination. We found that Imp+ MNs survive, while Imp-, Syp+ MNs undergo apoptosis. Our results indicate that Imp promotes survival, whereas Syp promotes cell death in immature MNs. Furthermore, our investigations revealed that late-born motoneurons face elimination due to their failure to express a functional code of transcription factors (mTFs) that control their morphological fate.

Late-born MNs possess a unique and distinct set of TFs compared to early-born MNs. By manipulating the expression of Imp and Syp in late-born motoneurons, we observed a shift in the TF code of late MNs towards that of early-born MNs, resulting in their survival. Additionally, introducing the TF code of early MNs into late-born MNs also promoted their survival. These findings demonstrate that the differential expression of Imp and Syp in immature MNs establishes a connection between generating a precise number of MNs and producing MNs with distinct identities through the regulation of mTFs.

Importantly, both Imp and Syp are conserved in vertebrates, suggesting that they play a central role in determining the number of neurons produced during development. The *Drosophila* model, along with its genetic tools, provides a unique opportunity to further explore and decipher the functions of these RNA-binding proteins in neural stem cells versus immature neurons. The insights gained from these studies could shed light on the broader mechanisms of neurogenesis and neuronal identity determination in more complex organisms.

^{*}Speaker

Keywords: Motoneuron, RNA binding protein, Imp, Syp, Programmed Cell Death, Neurodevelopment, transcription factor

The role of PHGPx in the developing *Drosophila* CNS during oxidative stress

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Developing animals rely on the external supply of oxygen and nutrients from the environment. Early-life malnutrition or hypoxia inhibit the growth of the central nervous system much less severely than other organs. This effect is called brain sparing and has been documented in mammals for several decades, yet the underlying mechanisms remain largely unknown. *Drosophila* larvae display brain sparing in response to nutrient restriction, hypoxia or chemical oxidative stress (Cheng et al 2011) (Bailey *et al.* 2015). In the presence of oxidative stress, polyunsaturated fatty acids (PUFA) such as linoleic acid (C18:2) undergo lipid peroxidation chain reactions that cause membrane, DNA and protein damage through adduct formation and can lead to ferroptosis. Here we investigate the role of glutathione peroxidase (PHGPx), an enzyme responsible for the detoxifying lipid peroxides, during oxidative-stress induced brain sparing. We show that PHGPx is required in glia and in neuroblasts (NBs) for brain sparing but only when there is a high dietary ratio of PUFA to monounsaturated and saturated fatty acids. We also find that dietary supplementation with reinforced PUFA, which cannot be auto-oxidized, rescues the larval viability of glial PHGPx knockdowns but surprisingly not NB proliferation. We are currently investigating whether this selective rescue is due to tissue-specific transport of PUFA or to auto-oxidation independent functions of PHGPx during brain sparing.

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^{*}Speaker

Keywords: oxidative stress, hypoxia, brain sparing, lipid peroxidation, PHGPx, glutathione peroxidase, neuroblast, brain development

Transcriptional profiling of single neurons under specific nutrient deficiency: insights for nutrient homeostasis.

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To maintain health, longevity and reproductive success organisms must balance the intake of multiple key nutrients. Such nutrient homeostasis is demonstrated when organisms seek out and consume foods rich in specific nutrients, for example carbohydrates or amino acids, in response to a nutrient-specific physiological deficit. This behavior is well established in many organisms, yet its neuronal and molecular mechanisms remain unknown. Our investigation focuses on two neuronal populations key for state-dependent feeding behavior. Peptidergic neurons which secrete neuropeptides and peptide hormones that have been shown to have key roles in carbohydrate and amino acid metabolism, and dopaminergic neurons which are key in determining food choice for key nutrients under different internal states. We will use single cell transcriptomics to identify gene expression changes in these two neuronal populations across flies that were fully fed or deprived of either sucrose or amino acids using the chemically defined *Drosophila* diet. This analysis will provide a high-resolution cell atlas of molecular operations key to these different neuronal clusters. It also will give insight into gene expression signatures associated with nutrient-specific deprivation, providing a starting point in the determination of neuronal circuits that guide nutrient homeostasis behavior. I will present our latest results.

Keywords: nutrition, behaviour, neuroscience, brain, diet, transcriptomics, sequencing, dopamine, peptidergic

*Speaker

Unravelling the role of dopamine transport in the modulation of *Drosophila* learning and memory

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The dopamine transporter (DAT) is a target of both recreational and therapeutic psychostimulants, and is involved in conditions including depression and ADHD. Expressed primarily in dopaminergic neurons (DANs), DAT mediates dopamine reuptake from synapses, modulating extracellular dopamine levels. Recently, single-cell transcriptomics has uncovered unexpected DAT expression in a subset of Kenyon cells (KCs), which encode olfactory information and innervate the mushroom body, a structure in the fly brain essential for olfactory learning and memory. Although KCs are not dopaminergic, the synapses they form with mushroom body output neurons are modulated by subsets of reward or punishment DANs across individual mushroom body compartments. Here we show that knocking down DAT in reward DANs or in KCs increases appetitive memory, which suggests a joint action of both synaptic partners to modulate dopamine dynamics. Our results also indicate that post-synaptic DAT in KCs contributes to preventing dopamine spillover when functionally distinct dopaminergic synapses share the same mushroom body compartment. Interestingly, DAT knock down in punishment DANs had an adverse effect on aversive olfactory memory performance. We attribute this result to increased forgetting, which relies on the same dopaminergic neurons as aversive learning, highlighting the importance of dopamine transport to balance the acquisition and elimination of memories. We will present our latest work as we seek to elucidate how dopamine transport fine-tunes memory mechanisms.

Keywords: Memory, Dopamine Transporter

Wing flicking in immature *Drosophila* females: an acoustic rejection behavior?

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Living as gregarious insects, females of *Drosophila melanogaster* are courted by many different males throughout their lives, from pupae emergence to death. Their acceptance of copulation after male courtship depends on their internal state, mating history and maturation. Recently mated females reject male courtship via several behaviors, mainly through fleeing, kicking and ovipositor extrusion. In contrast, immature virgins flick their wings, causing, as previously called "rejection sounds". This behavior has been mentioned many times in review literature, but only few aspects of it have been briefly characterized (Paillette et al., 1991 Bioacoustics 3, 247).

Using audio recordings and video analysis we describe the sound properties of immature female wing flicks and assess the conditions for females to emit this behavior, in terms of age and social environment. We aim to understand which sensory stimuli are required, and to study the neuronal pathway involved in immature female wings flicking. If this is a rejection behavior used by the female to avoid disturbance or futile copulation, we expect to see an interdependence of wing flicks and male courtship and a possible adverse effect on females when flicks are inhibited.

The sound produced by immature female wing flicks has a higher frequency than male courtship song, (275Hz and 235Hz respectively) and a more irregular pulse structure. The interval between two wing flicks in immature female is greater and more variable than the interval between two male song pulses (30-250ms and 20-40ms respectively).

The occurrence of wings flicks is the highest around 5h after pupae emergence, followed by a decrease in older females, corresponding to the time females starts accepting copulation, around 10h old. Passed this age, we rarely see this behavior, regardless the mating status. Individually housed females produce more wing flicks than grouped females, indicating possible modulation from the social environment. Immature females predominantly produce wing flicks when tested with mature males, but remain silent when in company of immature males, wingless males, non-courting males or other females, implicating male courtship as an important factor to elicit wings flicking, notably through male song.

We are currently investigating other sensory cues from the male influencing wing flicks. We also test which wing motor neurons are responsible in eliciting female flicks and the involvement of female neurons known to control receptivity.

Keywords: Wing flicks, Rejection, Acoustic behavior, Immature females, Neuronal circuits

*Speaker

painless-mediated stiffness sensing of egg-laying substrates in *Drosophila melanogaster*

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For insects, the textural properties of egg-laying substrates are indicative of their suitability to harbor and nurture future offspring. In the fruit fly, females actively probe surfaces, gauge their respective properties, and choose to lay eggs on substrates of a specific stiffness. Evolutionarily, the distinct textural properties of fruits and the varying stages of ripening - present unique ecological niches that have shaped egg-laying decisions across various species of fruit flies. For instance, *D.melanogaster* choose to oviposit on soft, decaying fruits whereas *D.suzukii* prefer hard, ripe ones. In comparison to our understanding of the role of chemosensation in influencing egg-laying decisions, the contribution of mechanosensation and its underlying neuro-genetic architecture remains unknown. Here, we attempt to pinpoint oviposition substrate-stiffness sensors at genetic, neuronal, and tissue levels in *D.melanogaster*. Via genetic screens, we identified the *Drosophila* gene *painless*, a TRP ion channel, as a potential sensor of textural stiffness. Combining organ-ablation experiments and tissue-specific inactivation of *painless*-expressing neurons, we identified the leg tarsae as peripheral sense organs accommodating stiffness sensors. At the cellular levels, we visualized *painless* expression in the tarsae using the UAS-GAL4 system and identified *painless*-expressing neurons that innervate mechanosensory bristles, chemosensory bristles, and campaniform sensilla. To further gain genetic access to these neurons, we recognized a 4 kb-sized fragment within the *painless* genetic locus, which drives reporter expression in a tarsae-specific manner. The novelty of this study lies in elucidating the mechanosensory role of *painless* at the stimulus-reception level, identifying stimuli-specific afferent neurons, to interpret the stiffness-sensing function of *painless* in the context of egg-laying in *Drosophila melanogaster*.

Keywords: D.melanogaster, oviposition behavior, Decision making neurons, stiffness sensing

*Speaker

Morphogenesis & organogenesis

An In toto Imaging Study with Sub-Embryonic Resolution: Quantifying collective cell migration

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Collective cell movements are fundamental for tissue and organ formation during embryonic development. Among the first of these movements is the formation of the mesoderm layer during gastrulation. The genetic basis of gastrulation is particularly well studied in *Drosophila*, however, the collective movements of mesoderm cells within the embryo are yet to be analyzed quantitatively. In this study we present the first comprehensive, quantitative dynamic analyses of mesoderm spreading in the *Drosophila* embryo. We have constructed a multi-view tiling selective plane illumination microscopy (MT-SPIM) that captures *in-toto* images of the *Drosophila* embryo with subcellular resolution. The MT-SPIM provides data for a simultaneous analysis of the mesoderm and ectoderm to understand their coordination and differences in cell behavior along anterior-posterior axis. We present a detailed map of the entire mesoderm cell movement throughout their spreading underneath the ectoderm, including the less studied dorsal side. We demonstrate the spatio-temporal relationship of cellular events including mitoses, cell intercalations, and cell spreading behavior. Our dynamic image analysis of mesoderm spreading behavior sets a new standard for a detailed analysis of mutations in genes required for early *Drosophila* mesoderm morphogenesis.

Keywords: *Drosophila* development, Mesoderm morphogenesis, Collective cell migration, MT, SPIM microscopy, Machine learning

*Speaker

Analysis of the protein Sidekick

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Sidekick (Sdk) is a cell adhesion protein of the Immunoglobulin Superfamily highly conserved all over the animal reign with a unique localization at the tricellular Adherens Junctions (tAJ) in *Drosophila melanogaster*. This specific localization confers Sdk the ability to form a complex connected to the actin cytoskeleton to regulate the tension and rearrangements between cells within an epithelial tissue. However, less is known about how Sdk acts during a whole process of cell rearrangement (like cell intercalation) in trachea and epidermal tissue, and how Sdk interacts with other proteins of this complex to do its function. We are investigating different aspects of Sdk biology. On the one hand, we are analysing the localization and dynamics of Sdk using *In vivo* imaging and FRAP, showing us that Sdk is more stable in its localization than expected by its involvement in dynamic processes. On the other hand, we are analysing the possible interactions with other proteins at the tAJ complex. The protein Echinoid (Ed) is an excellent candidate to investigate for its similarities with Sdk in functions and pattern of accumulation. We found that Ed and Sdk in most cases are colocalizing or adjacent to each other and that Sdk regulates the accumulation levels of Ed in Adherens Junctions. Our results suggest that Sdk might regulate Ed by regulating its intracellular trafficking. Finally, we are investigating in detail the role of Sdk in tracheal cell intercalation at the cellular level, where it is known that myosin is not necessary for the process although myosin is required for most other cell intercalation events in other tissues.

Keywords: Sidekick, Tricellular Adherens Junction, Echinoid, Cell intercalation, Intracellular trafficking

*Speaker

Anterior-posterior axis establishment: the true contribution of follicle cells during oogenesis finally revealed

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In *Drosophila*, the anterior-posterior axis of the future embryo is set during oogenesis, by the polarized localization of cytoplasmic determinants, an process triggered by adjacent posterior follicle cells (PFCs). While the events occurring in the oocyte for *oskar* mRNA transport and anchoring to the posterior cortex have been extensively studied, the contribution of the overlying follicular epithelium remains elusive. Our work reveals a subpopulation of PFCs, which we named Posterior Anchoring Cells (PACs), since our results indicate that they precisely define the size of the *oskar* mRNA anchoring zone in the adjacent oocyte. We describe that the PACs act on *oskar* mRNA transport and anchoring by maintaining close contact with the posterior membrane of the oocyte. We unravel the mechanism behind the precise control of the number of PACs, which involve a crosstalk between E-Cadherin, the Gurken/EGFR pathway and JAK-STAT signaling. We describe that the differentiated PACs are the only PFCs maintaining high JAK-STAT activity, which is required for tight contact maintenance with the oocyte. This role is ensured by two JAK-STAT pathway targets, the genes encoding the adherens junction protein E-Cadherin, and Enabled, involved in filopodia formation. Accordingly, we uncover the presence of long filopodia at the PAC apical surface that penetrate the oocyte. Finally, this newly identified somatic contribution to *oskar* mRNA anchoring is crucial, as we report that setting proper PAC number is essential to establish the germline of the future embryo.

Keywords: JAK, STAT signaling, oogenesis, E, Cadherin, EGFR, morphogenesis, mRNA determinant, filopodia

*Speaker

Antp is an essential regulator of *Drosophila* thoracic adult muscle development

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Muscle development involves complex networks of transcriptional regulators that ensure the specification and diversification of different muscle types. The identification of these factors is essential for the establishment of muscle regenerative therapies that would be effective in curing myopathies. *Hox* transcription factors are long known for providing diversity along antero-posterior axis in all bilaterians. Recently much attention has been given to the role of Hox in early mesodermal patterning, muscle development regulation and regeneration in both *Drosophila* and vertebrates. During *Drosophila* larval muscle development, *Hox* genes were shown to provide identity to specific muscle progenitors, regulate muscle size and innervation. In contrast to larval myogenesis, much less is known about gene regulatory networks and Hox function underlying adult muscle specification and development. Using the powerful genetic model of *Drosophila*, we identify a novel essential regulator of *Drosophila* thoracic adult muscle development, the *Hox* gene *Antennapedia* (*Antp*). We show that Antp intervenes at several stages of adult flight muscle development, from the progenitor specification in the embryo to myoblast fusion and myofibrillogenesis in the pupa. Furthermore, *Antp* expression in myoblasts is driven by an alternative promoter named P2 that when mutated, abolishes adult muscle progenitor specification and leads to a complete absence of adult flight muscles. We also show that Antp is responsible for the choice between different adult muscle fibre fates, regulating the decision between fibrillar (such as flight) and tubular (such as leg) muscles. This work calls into question the long accepted postulate stipulating that the establishment of thoracic muscles would be independent of *Hox* genes and contributes to our understanding of the regulation of muscle development.

Keywords: Hox, muscle, flight, development

*Speaker

Cell and tissue polarization in the *Drosophila* embryonic midgut

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Endodermal cells twice change their cell character before forming the embryonic midgut epithelium. During or after invagination they lose their epithelial character and form a cluster of isotropic mesenchymal cells (epithelial-mesenchymal transition, EMT). Following spreading along the embryonic axis in two strands on the left and right side, they polarize, turn from a single layered arrangement and elongate to finally form the secondary midgut epithelium (mesenchymal-epithelial transition, MET). The secondary epithelium employs specific modes of polarity, junctions and cytoskeletal organization distinct from the typical epithelia of the epidermis. This process of epithelialisation and MET not only plays an essential role during organogenesis and cell differentiation but has also been implicated in tumorigenesis and formation of metastases. We focus on the initial polarization and cytoskeletal dynamics during MET which has received little attention, so far. MET requires a non-autonomous signal from underlying visceral mesoderm, whose molecular nature and targets are unknown.

We conducted a detailed phenotypic analysis of mutants with defects in formation of the secondary epithelium, including tinman, Laminin, Integrin and E-cadherin using cell shape, polarity proteins and markers for the cytoskeleton and extracellular matrix. Based on single cell transcriptomics (see accompanying poster by Abbaszadeh et al.) we have analyzed ligand-receptor pairs that are expressed in the transient cells, such as Robo-Slit and Sema5C-PlexA.

In parallel we are establishing a deep-tissue live-imaging assay for tracking cells during MET with confocal and light-sheet microscopy. To improve signal-noise ratio, we developed novel GAL4 driver lines and reporter lines with multiple copies of GFP/neogreen.

Keywords: embryonic midgut development, MET, secondary epithelium, cytoskeletal dynamics

*Speaker

Cell proliferation and Notch Signaling Coordinate the Formation of Epithelial Folds in the *Drosophila* leg

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Tissue folding is a recurrent morphogenetic process that plays a fundamental role in shaping three-dimensional organs from flat epithelial sheets. Like in origami, the precise location of the folds is dictated by the underlying patterning network, while changes in cell shape and mechanical forces direct the folding process. The mechanical forces driving tissue folding can occur at both local and global scales, with local forces involving cells within the fold and global forces acting on a tissue-wide level. It is becoming increasingly clear that a complex interplay must exist between these local and global forces to sculpt an organ in 3D. In this study, we investigated the role of cell division and its coordination with patterning mechanisms in epithelial morphogenesis, using *Drosophila* leg disc tarsal folds as a model system. Our findings demonstrate that tissue-wide cell proliferation generates compression forces that, in coordination with the Notch pathway, drive the formation of the epithelial folds that prefigure the formation of the adult leg joints. We show that cell proliferation generates compressive stresses, contributing to the buckling of the epithelium, while reinforcing the apical constriction of invaginating cells. Additionally, the Notch target *dysfusion* (*dysf*) plays a critical role in specifying the specific location of the folds, leading to the apical accumulation of F-actin and the apico-basal shortening of invaginating cells. Importantly, the formation of the tarsal folds is recreated by a simple computer-based simulation model that predicts the folding phenotypes and the behavior of the cells after reducing cell proliferation and in the absence of *dysf*. Our results provide new insights into the complex mechanisms underlying epithelial morphogenesis, highlighting the role of tissue-wide forces in shaping a three-dimensional organ in a reproducible manner.

Keywords: morphogenesis, tissue folding, cell proliferation, leg, Notch, *dysf*, apical constriction

*Speaker

Cephalic furrow formation prevents mechanical instability during gastrulation

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The cephalic furrow is a deep epithelial fold that demarcates the head–trunk boundary of fly embryos during gastrulation. It forms under strict genetic control using active cellular mechanisms and follows an invariant morphogenetic sequence. But unlike other embryonic invaginations, the cells that invaginate in the cephalic furrow do not give rise to any precursor tissues or larval traits. The cephalic furrow is transient and unfolds, leaving no trace. For these reasons, its function during development has remained elusive. Here, we show that the cephalic furrow plays a mechanical role during *Drosophila* gastrulation. By live-imaging mutant embryos, we find that without the cephalic furrow, ectopic folds appear around the head–trunk interface, indicating that the epithelial stability has been compromised. Using in vivo perturbations and in silico simulations, we demonstrate that ectopic folding in cephalic furrow mutants occurs due to the concomitant formation of mitotic domains and extension of the germ band. These events increase the tissue strain in the head–trunk interface, giving rise to mechanical instabilities. Further, we show by simulations that an early pre-patterned invagination can effectively prevent the build-up of compressive stresses by retaining epithelial tissue out-of-plane before other morphogenetic movements take place. Our findings suggest the cephalic furrow absorbs compressive stresses at the head–trunk boundary during fly gastrulation, and raise the hypothesis that mechanical forces may have played a role in the evolution of the cephalic furrow.

Keywords: tissue mechanics, cephalic furrow, morphogenesis, gastrulation, epithelial folding

*Speaker

Characterization of the Hedgehog protein in inter-organ communication

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Hedgehog (Hh) is a secreted protein that control tissue formation during development, and stem cell maintenance. However, while the morphogenetic action of Hh within a tissue is well known, its inter-organ function remains unclear. Importantly, Hh circulates in blood only as a lipoprotein-associated (Lpp) form to exert communication between different organs. In this project, we use the blood/haemolymph as a model in order (1) to determine the biochemical composition of circulating lipoprotein-associated Hh, (2) to identify the source tissue of Hh. We confirmed that the gut is the main source of circulatory Hh. We identified that the enterocytes (EC) and Intestinal Stem Cells (ISC) express Hh, and both cells participate in the secretion of Hh. Following an immunoaffinity purification and proteomics from haemolymph extracts, two proteins have been identified and associated with Lpp/Hh: Dally (a Glypican protein) and apolipoprotein Lipid Transfer Particle (LTP). By depleting LTP/Lpp in fat body or "Glypicans" in the gut by RNAi, we shown that Dally-like(Dlp) mutant gut secretes less Hh into haemolymph while LTP/Lpp RNAi induce an accumulation of Hh in gut. Likewise, by forming a protein complex, Dally, Dlp, Lpp and Hh proteins co-localize in the same regions of the intestine. Our discovery suggests a mechanism of secretion for Hh inter-organ function which is different from the one involved in regulating Hh morphogenetic function.

Keywords: Morphogene Hedgehog inter organ communication

*Speaker

Cling film – A new regulator of extracellular matrix remodeling during epithelial morphogenesis

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Precise regulation of epithelial morphogenesis is essential for the transformation of simple epithelial sheets into complex organs and its misregulation is associated with numerous diseases. Although the involved molecular signals are well understood, knowledge about the translation of early patterning information into morphogenetic changes is sparse. In *Drosophila*, external appendages like the wing develop from larval imaginal discs, sac-like epithelial structures. During metamorphosis, imaginal discs undergo drastic morphological changes and thus serve as a valuable model to address epithelial morphogenesis at tissue-, cellular- and subcellular level. Changes include proliferation, cell rearrangements, cell shape modulation, and extracellular matrix (ECM) remodeling, which are regulated by intrinsic and extrinsic cues and forces. We identified *cling film* (*cling*), a previously uncharacterized gene, as a new transcriptional target of larval BMP signaling in the wing disc. Adult *cling* mutant flies display strong wing malformations that manifest in early pupal stages. Mutant wings expand their surface normally but fail to stretch out along the proximo-distal wing axis due to incomplete apical ECM degradation. Further results indicate that Cling is critically involved in ECM remodeling by affecting the activity of apical ECM degrading proteases. Our current experiments aim at determining the exact molecular function of Cling and the mechanisms by which larval patterning cues affect *cling* transcription. Our studies on Cling provide a link between larval patterning and the subsequent events that drive wing development, thus contributing critical insights into ECM dynamics during epithelial remodeling and the regulation of organogenesis in general.

Keywords: extracellular matrix, ECM, epithelial morphogenesis, wing, pupa, BMP

*Speaker

Clustering of Rho1 activity localises and amplifies rhythmical actomyosin contractility during *Drosophila* adult abdominal morphogenesis

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During the shaping of tissues and organs, apical constriction is crucial to drive processes such as delamination, invagination and tissue bending. In many systems, apical constriction is driven by pulsed contractions of an apicomedial actomyosin network. These contractions are regulated by the small Rho GTPase Rho1, which is downstream of G protein-coupled receptor signalling. How this system generates rhythmical contractile activity remains unclear. Here, we use the larval epithelial cells (LECs) during *Drosophila* abdominal morphogenesis to study the mechanisms underlying rhythmical contractility. We show that active Rho1 localises to membrane microdomains in the apical membrane. Subsequently, Rho1 clusters in circular membrane-localised structures, correlating with strongest contraction of the actomyosin network. Eventually, Rho1 is inactivated, and the contraction ceases. We show that Rho1 clustering is mediated by septins and depends on the initiation of clathrin-mediated endocytosis. Like in the *Drosophila* embryo, Rho1 inactivation depends on β -arrestin activity. Interestingly, rhythmical contractions take place in the absence of Rho1 clusters, although less extensively, showing that clustering is important for localising and amplifying contraction but not for rhythmical contractility *per se*. Our data highlight that events at the apical membrane are crucial to regulate actomyosin contractility.

Keywords: morphogenesis, apical constriction, actomyosin contractility, Rho1

Control of *Drosophila* wing development by the stage-specific factors E93 and Broad-Complex

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Stage identity and progression in *Drosophila* is controlled by the regulatory activity of the temporal identity factors Chronologically inappropriate morphogenesis (Chinmo), Broad-complex (Br-C; also known as broad), and Ecdysone inducible protein 93F (E93). In addition to be regulated by main developmental hormones, ecdysone and Juvenile hormone, these three factors are also intimately connected to each other through a series of regulatory interactions that ensure their sequential expression to specify life stage identity. Whereas larval identity is maintained by the presence of Chinmo, Br-C functions as the pupal specifier, and E93 acts as the adult specifier. We present our new data on the regulation and function of these key temporal identity genes, particularly *E93* and *Br-C*, on the temporal control of wing development during *Drosophila* metamorphosis. For that, we have analyzed the role of E93 and Br-C in the regulation of the activity of well-characterized pupal-specific wing enhancers that are critical for adult differentiation. Interestingly, our results have shown that E93 binding is not required for the activation of these enhancers in pupal wings, and that the regulatory effect of this factor on the activity of the enhancers is channeled through the repression of the pupal specifier *Br-C*. Overall, these results will contribute to a better understanding of the molecular basis of insect development and the evolution of insect metamorphosis.

Keywords: ecdysone, temporal transcriptional programs, E93, broad, complex, hormones, wing development

*Speaker

Coordination of tissue invagination and flow during epithelial morphogenesis

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Epithelial folding involves inter-tissue interactions whereby tissue surrounding the folding region flows towards the invaginating region. Accordingly, during *Drosophila* adult neck formation, the invagination of the neck fold is accompanied by a large-scale flow of the dorsal notum. However, the mechanisms coordinating tissue flow and invagination remains poorly understood. Using large-scale laser ablations to uncouple the two processes, we established that tissue flow is not actively driven by neck invagination. Interestingly, we found that apical protrusions are formed in the direction of the movement in the dorsal notum. These protrusions are anchored to the apical extracellular matrix (aECM) and potentially generate migratory forces. In particular, the ZP proteins Dumpy, located within the aECM are required for the dorsal thorax flow. Finally, the transcription factor Stripe is required for Dumpy deposition and dorsal thorax tissue flow. Altogether, we propose cell migration enables the coordination of tissue flow with tissue invagination.

Keywords: Notum, Morphogenesis, Biophysics, Fold, ECM, Cell Migration

*Speaker

DAAM and FRL are redundantly required for sealing of the interommatidial lattice and patterning of the retinal floor

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Precise sight with the arthropod compound eye critically relies on perfect optical isolation of the individual light sensing units. Separation of the ommatidia is ensured by pigment cells that organize into a hexagonal lattice in the *Drosophila* eye, forming thin walls in between the facets. Cell adhesion, mediated by apically and latero-basally located junctional complexes, is crucial for stable attachment of these cells to each other and the basal lamina. Whereas former studies were focused on the formation and remodeling of the cellular connections at the apical region, here we report a specific alteration of the lateral adhesion of the lattice cells, leaving the apical junctions largely unaffected. We found that DAAM and FRL, two formin type of cytoskeleton regulatory proteins, play redundant roles in lateral adhesion of the interommatidial cells and patterning of the retinal floor. We show that formin dependent cortical actin assembly is critical for latero-basal sealing of the ommatidial lattice. We believe that the analysis of these formin dependent novel eye phenotypes will be highly beneficial as to a better understanding the three-dimensional aspects of pupal eye morphogenesis.

Keywords: formin, DAAM, FRL, eye development, ommatidia, pigment cell

*Speaker

Early interactions between muscle and tendon precursors: Roles of Amalgam and Neurotactin

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The coordinated development of muscle and tendon tissues is key to the development of a functional limb and to proper body locomotion. The appendicular myotendinous system of *Drosophila melanogaster* shares many similarities with that of vertebrate limbs. This system is made up of muscle fibers attached to long internal tendons (LTs) that transmit muscle contraction to the exoskeleton. The LTs are derived from subsets of epithelial cells of leg primordia called leg discs that develop during larval and pupal stages. They develop through a tubulogenic process to form long polarized tubes enclosing a central lumen. In addition, the leg muscle precursors (myoblasts) are ad epithelial cells laying on the internal surface of the leg disc. Myoblasts are maintained in an undifferentiated state and proliferate during the larval stages.

Muscle and tendon precursors interact with each other throughout the developmental stages. Thus, the main purpose of this work is to identify molecular elements involved in these interactions especially during early stages of development. I identified different candidate genes whose Amalgam (Ama) and its receptor Neurotactin (Nrt) as potential players of myoblast-tendon cells interactions.

My results show dual role for Ama: during early larval stages, Ama is implicated in the proliferation of myoblasts as well as their protection against apoptosis, independently of Nrt. During metamorphosis, myoblast proliferation ceases, tendons begin to elongate and to express Nrt. At this stage, reduced Ama expression affects myoblast-tendon interactions and in consequence tendon morphology. Interestingly, similar phenotype is observed in Nrt mutant context. Future works aim at confirming Nrt-Ama physical interaction and at identifying the molecular mechanisms involved downstream of this interaction.

Keywords: myoblast, tendon, leg, interactions

*Speaker

Ecdysone in Follicle Cell Morphogenesis: Notching Up the Game

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Epithelial morphogenesis generates diversity in tissue organization and aids in the formation of various organs in the metazoans. At the cellular level, this process mediates change in the shape and organization of the epithelial cells. Given the wide importance of this process in multicellular development, we still lack clear understanding as to how epithelial morphogenesis is regulated in the metazoans. Employing the *Drosophila* oogenesis model, we have examined the role of Ecdysone (EcR) pathway in mediating the shape transition of epithelial follicle cells. Typically, a previtellogenic fly egg is enveloped by a layer of 750 somatic epithelial cells called the follicle cells. As the developing egg enters the vitellogenic phase, approximately 50 anterior follicle cells (AFCs) undergo shape transition from cuboidal to squamous fate. We demonstrate that the activity of EcR pathway in the AFCs coincides with the timing of cuboidal-to-squamous shape transition. Satisfying, depletion of the Ecdysone Receptor function impedes cuboidal to squamous shape transition of anterior follicle cells (AFCs) without affecting the fate of AFCs. Employing genetic tools, we show that EcR function modulates Notch signaling to facilitate the shape change of the AFCs. We believe that EcR functions through Notch to assist the remodelling of cell junction proteins in the shape transitioning AFCs. Over all, our work provides novel molecular insight as to how Ecdysone signalling mediates shape change in the epithelial follicle cells. Results from the above will be presented.

Keywords: Drosophila Oogenesis, Epithelial Morphogenesis, Anterior Follicle Cells, Ecdysone Receptor, Notch

*Speaker

Exocytosis by large secretory vesicles: fusion pore formation, actomyosin assembly and vesicle membrane dynamics

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Secretion of adhesive ("glue") glycoproteins from the apical surface of *Drosophila* larval salivary gland cells to the gland lumen, serves as a prominent model for exocytosis via exceptionally large vesicles, a process that poses a variety of challenges to the secretory apparatus. Our studies address the cellular and molecular mechanisms underlying key junctures along this specialized route of secretion. We find that the fusion pore, through which vesicle content is released, displays a dynamic behavior, regulated by Bin-Amphiphysin-Rvs homology (BAR) domain proteins, most notably the fly homolog of the I-BAR domain protein MIM. Following fusion, a Rho GTPase-based pathway, employing balanced and regulated input from specific RhoGEF and RhoGAP elements, is activated, leading to coating of the vesicles by a contractile actomyosin network. Contraction of this network serves multiple roles: content release to the lumen, regulation of fusion pore dynamics, and notably, "crumpling" of the vesicle membrane, which keeps it insulated from the apical cell membrane, thereby maintaining membrane homeostasis.

Keywords: exocytosis, membranes, actomyosin, BAR domain, Rho GTPase, salivary gland

*Speaker

Flightless-I regulates radial growth of the sarcomeres

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Myofibrils are composed of serially organized contractile units, called sarcomeres. The first phase of myofibrillogenesis includes the formation of premyofibrils that contain immature sarcomeres. These proto-sarcomeres already exhibit a filamentous organization ensured by precise alignment of the actin-based thin filaments and the myosin-based thick filaments. Nevertheless, formation of a functional muscle requires a significant growth of the sarcomeres. Most notably, the sarcomeres grow in length and in the radial direction by adding new filaments to their periphery. Despite sarcomere development is extensively studied, the mechanisms of sarcomere elongation and radial growth remained largely unknown. Flightless-I (FliI) has been identified as a key factor of muscle development in *Drosophila* and in mammals as well. It's an evolutionarily conserved protein composed of leucine-rich repeats (LRR) and six gelsolin homology (GH) domains. Whereas the LRR domains are known to be involved in protein-protein and protein-lipid interactions, and the GH domains are implicated in actin-binding, the molecular mechanisms of FliI are largely unknown. We performed a detailed analysis of *Drosophila* FliI, using the indirect flight muscle as our major model system. In agreement with former data, we found that in the absence of FliI the myofibrils often look disorganized and they are composed of shorter and thinner sarcomeres than the wild type. Based on confocal and dSTORM measurements, the FliI protein is localized to the (+) end of the thin filaments in the Z disc, both in developing and mature sarcomeres, and it plays an important role in Z-disc formation and peripheral growth of the myofilaments. A structure-function analysis highlighted that the GH 1-3 domains of FliI are indispensable for FliI function, while the GH 4-6 domains are involved in the regulation of the activities provided by GH 1-3. Collectively, our observations further confirm that FliI is essential during muscle development, and we show that it is specifically required to promote myofilament incorporation at the Z-disc.

Keywords: Flightless, I, Indirect flight muscle, sarcomere, dSTORM

*Speaker

Genetic and mechanical regulation of symmetry breaking and polarized flow of the *Drosophila* posterior endoderm

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Tissue morphogenesis, one of the fundamental processes in biological systems is a complex interplay between genetics, mechanics, and geometry. The general paradigm in developmental biology has been that patterned gene expression and biochemical cues cause mechanical changes and in turn, affect geometry, but this hierarchy of functioning between genetics and mechanics varies depending on the tissue and organism. In the early *Drosophila* embryo, the posterior endoderm undergoes symmetry breaking characterized by an invagination and a polarized flow towards the anterior of the embryo, displaying a strong Dorsal- Ventral (DV) polarity. Apical constriction, induced by the activation of non-muscle Myosin-II (MyoII) via Fog signaling is responsible for the cells to invaginate. Our lab has recently shown that this polarized flow depends on an interaction between apical MyoII and the curvature of the eggshell. A mismatch between the peak of curvature and the domain of apical myosin specifically drives the onset of the flow. The factors that define the position of MyoII domain and set the DV polarity of the polarized flow remain unknown. The endodermal morphogenetic events are initiated by a transcriptional response under the control of the embryo's terminal patterning genes *hkb* and *tll*. Initial results suggest that these genes are present symmetrically along the DV axis of the endoderm. *Hkb* and *tll* activate *fog*, which we find to have an asymmetric transcriptional expression pattern that spans a broader dorsal domain. *Fog*, in turn, through the GPCR signaling, activates MyoII which shows a polarity. We characterize the onset of *fog* transcription and MyoII activation at the cellular level to understand the spatial and temporal dynamics of this genetic asymmetry. To further identify factors that cause this asymmetry, we perform a transcriptomic approach to screen for genetic determinants, using mutants of the terminal and DV patterning. We also characterize their cell mechanics, giving insight into how tissue autonomous and non-autonomous activities contribute to the morphogenetic events. This will give a holistic approach to understanding how genetics and cell mechanics function together to cause a polarity of endoderm morphogenesis.

Keywords: *Drosophila*, endoderm, morphogenesis, polarity

*Speaker

Genetic control of muscle fiber splitting during *Drosophila* myogenesis

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In *Drosophila* embryo and larvae, each muscle is composed of one syncytial muscle fiber with specific morphological features determined by the combinatorial expression of muscle identity transcription factors (iTFs). In certain genetic contexts morphological features of larval muscles are affected leading to split or branched muscle fibers, a phenotype also observed in patients with muscular dystrophies. We recently identified actin regulator Gelsolin, which acts as muscle identity effector downstream of iTFs and inhibits transversal muscles (LT) splitting, by negatively regulating fusion. Moreover, LT specific overexpression of the pro-fusion gene Dumbfounded leads to hypertrophy and occasional LT splitting. Intriguingly, splitting could also occur in a non-hypertrophic context, in a gain of function of Septin proteins, known for their implication in cytokinesis. We observed that one common feature of both hypertrophic and non-hypertrophic split fibers, is a mis-positioning of myonuclei. Further analyses are in progress to determine the impact of nuclear dynamics on muscle splitting.

Keywords: Myoblast fusion, splitting, nuclei positioning

^{*}Speaker

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How do the mechanical properties of surrounding tissues regulate epithelial cell shape?

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It is unknown how external forces and constraints impact morphogenesis in a neighboring tissue. To address this, we used the *Drosophila* ovarian follicle, where a cluster of 15 nurse cells and a posteriorly located oocyte are surrounded by a layer of epithelial cells, which are themselves resting on a basement membrane. As the nurse cells grow, the overlying epithelial cells flatten in a wave that begins in the anterior. We previously showed that this flattening depends on the TGF β signaling. We also demonstrated that an anterior to posterior gradient of decreasing cytoplasmic pressure is present across the nurse cells and that this gradient acts through TGF β to control both the triggering and the progression of the wave of cell flattening. We also prove that BM softens around the flattening cells and that this softening depends on TGF β pathway. Finally, we revealed that nurse cell pressure and BM softening combine to increase follicle elongation in the anterior, which is crucial for allowing nurse cell growth and pressure control. These results show that BM mechanical properties and the inner cytoplasmic pressure in the nurse cells have an important role in shaping cells and tissues. Now, we are investigating the cellular and subcellular mechanisms of the epithelial cells at play to respond to changes in the mechanical properties of the surrounding tissues.

Keywords: Mechanics, epithelial cells, organ shape

^{*}Speaker

Identification of Hox functions at high resolution during *Drosophila* embryonic myogenesis

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In *Drosophila melanogaster*, larval somatic muscles and adult muscle precursors (AMPs) are established during embryonic development according to a specific pattern. Each larval muscle acquires its own physical properties, such as orientation, attachment, length and number of nuclei, through different combinations of expression of various muscle identity transcription factors (mITFs). This larval muscle organization and the configuration of AMPs are repeated in a similar way in each embryonic segment. However, some differences are observed, particularly in the thoracic and posterior segments, suggesting an anterior-posterior (A-P) regulation of muscle specification. The architecture of the A-P axis is controlled by the highly conserved HOX transcription factors. However, while the role of HOX in the formation of ectoderm derivatives has been widely described, little is known about their influence on mesoderm specification and, in particular, myogenesis. Using single nuclei RNA-seq and tissue-specific cut&run-seq, we aim to characterize the molecular functions of HOX proteins in muscle diversification processes and describe their gene regulatory networks with a particular focus on their connections with mITFs.

Keywords: HOX, myogenesis, single nuclei RNA, seq

In vivo analysis of the cephalic endoskeleton development in *Drosophila melanogaster*

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Although insects possess an external cuticular exoskeleton secreted by the epithelial cells, they also form a well-conserved cephalic endoskeletal structure named "Tentorium" which serves as a major attachment site for mastication muscles.

In *Drosophila*, the study of the tentorium organogenesis has been diffculted by the fusion of the cephalic segments and the extreme reorganization occurring during head involution, which gives rise to an apparently acephalic larva with its head inside the body.

We have developed several reporter genes to study tentorium development *in vivo*. These reporters allowed us to follow tentorium morphogenesis from its early specification at stage 11, through its invagination and reorganization, until its final integration with other cephalic elements that form the cephalopharyngeal skeleton.

The tentorium originates from three lateral ectodermal primordia located at homologous positions to those forming the trachea in the trunk segments, and abutting the *corpora allata* and the prothoracic gland primordia in the maxilla and the labium. The three primordia, derived from the intercalary, the maxillary and the labial segments, invaginate without losing their epithelial character and fuse to form a rod structure that attaches anteriorly to the pharynx floor epithelium and muscles, and posteriorly to the dorsal pouch cavity created by the head's involution.

I will present experiments using *in vivo* and fixed embryos that allow understanding the complex development of this conserved apodeme during *Drosophila*'s embryogenesis, as well as the defects caused by mutations affecting different tentorial primordia.

Keywords: Embryogenesis/Morphogenesis/Head/Invivo/Genetics/Apodeme

*Speaker

Interplay between tissue flow and static anchors during *Drosophila* gastrulation

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Drosophila gastrulation is a complex set of synchronized morphogenetic events that transforms the monolayer of cells into a multilayered embryo. Despite extensive research on tissue flows during *Drosophila* gastrulation and their genetic regulation, little attention has been given to the existence of static regions with no cell movement in specific parts of the embryo. Recent reports suggest that integrin-mediated embryo-shell attachment plays a crucial role in shaping the *Drosophila* gastrulation movements in the hindgut region. Using in toto Light Sheet Microscopy, we mapped out tissue flows in relation to the static regions of the blastoderm in wild type and in integrin mutants. Additionally, we introduced artificial anchoring points by cauterizing the blastoderm to the vitelline envelope and examined the resulting flows. Our observations suggest that the integrin subunit *scab* acts in three distinct regions of the embryo in the vicinity of invagination sites as dynamic tissue anchor driving tissue flow directionality. Our findings underscore the importance of *scab* as a mediator of mechanical stability in the embryo and highlight the significance of static tissue in the morphogenesis of the gastrulating *Drosophila* embryo.

Keywords: morphogenesis, mechanobiology, gastrulation

*Speaker

Investigating *Drosophila* basement membrane formation and plasticity from inception to homeostasis

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Basement membranes (BMs) underlie nearly all epithelia where they act as extracellular scaffolds, providing structural integrity to tissues. Adult BMs are assumed to be stable and rigid structures due to a high degree of crosslinking, however, we hypothesise that during development BMs are highly labile and dynamic to enable tissue growth and morphogenesis. Here we exploit developing *Drosophila*, which undergo a 200-fold body mass increase between embryonic and pupal stages, to dissect the mechanisms regulating growth-related alterations in BM stability. We have adapted an extracellular matrix (ECM) enrichment protocol to characterise ECM components during *Drosophila* development, from embryonic to pupal stages. Proteins extracted from whole animals were biochemically fractionated, separating highly insoluble ECM from other more soluble proteins. Analysis of these fractions by Mass spectrometry revealed a dynamic developmental matrisome, in which core BM components shift from soluble to insoluble fractions at the end of larval development. This progressive decrease in BM solubility, which we believe is due to an increase in BM stability, coincided with the onset of signalling cascades triggering pupation, in which growth ceases and adult structures begin to form.

We hypothesise that changes in BM turnover may underlie this developmentally regulated increase in BM stability. We are therefore developing a novel fluorescent timer (FT) approach to measure turnover rates of the core BM component Collagen IV (ColIV) by ratiometric imaging. Preliminary characterisation revealed a higher turnover rate during rapid larval growth phases followed by slower turnover as growth slows and pupation occurs. Moreover, experimentally arresting larval growth by nutrient restriction decreased ColIV turnover rate, further indicating a relationship between growth rate and BM stability.

Together, these data highlight that fly BM stability increases during development, coinciding with formation of adult tissues. By exploiting our time-resolved analysis of the fly developmental matrisome, we are currently designing a targeted screen to pinpoint the molecular mechanisms controlling this developmentally triggered alteration in BM dynamics.

^{*}Speaker

Keywords: Extracellular matrix (ECM), Collagen, Mass spectrometry, turnover, fluorescent timer (FT), development

Investigating the developmental basis of eye size evolution and its functional implications for *Drosophila* vision.

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The striking diversity in eye shape and size among insects reflects adaptations to different habitats and behaviours. Eye size directly impacts the quality of vision, but how variation in eye size evolves, is specified during development, and affects vision is not well understood. We analysed multiple strains of two closely related *Drosophila* species, *D. mauritiana* and *D. simulans*, that showed significant differences in overall eye size. While variation in the number and size of ommatidia is strain specific, *D. mauritiana* compound eyes generally consist of more and larger ommatidia resulting in overall larger eyes. Species differences specifically in ommatidia size has previously been mapped to a small region on the X-chromosome where we identified *orthodenticle* (*otd*) as a candidate gene. We found that *otd* is expressed earlier in *D. mauritiana* eye disc development than in *D. simulans* and manipulation of this gene perturbs ommatidia development. To further elucidate how *otd* may regulate eye size we have performed CUT&RUN on eye discs with endogenously tagged Otd to find direct targets of this gene. We have identified several candidate genes downstream of Otd and we are currently testing their roles in eye development. To characterise the functional consequences of compound eye size variation, we modelled the impact of differences in eye morphology on vision using 3D ultrastructural information from synchrotron radiation microtomography and tested fly vision in vivo using behavioural assays. As predicted, *D. mauritiana* with their larger ommatidia demonstrated higher contrast sensitivity whereas *D. simulans* showed a higher spatial acuity consistent with the smaller interommatidial angles in the horizontal band. Taken together our work has provided new insights into the genetic basis for natural variation in compound eye size and the implications for vision.

Keywords: vision, eye, Otd, ommatidia, natural variation, evolution

*Speaker

Investigation of the role of the apical extracellular matrix in cell migration

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During *Drosophila* adult abdominal morphogenesis, larval epithelial cells (LECs) undergo directed migration before being replaced by histoblasts. While migrating, LECs form apical lamellipodia, suggesting that they crawl on their apical extracellular matrix (aECM). However, the role of the aECM in cell migration remains elusive. Using *in vivo* 4D microscopy, we characterised the dynamic behaviour of the LECs and their aECM over time using fluorescent markers. We found that Dumpy (Dpy), a major component of aECM, indeed localised apical to LECs. In early stage of morphogenesis, when LECs were stationary, Dpy was present in distinct foci. Once LECs started migrating, they secreted Dpy and produced an additional layer of Dpy, in which Dpy appeared fibrous. We observed deformation of the Dpy fibres coinciding with LEC lamellipodia, suggesting that cells interact with the aECM during migration. Inducing RNAi knockdown of Dpy in pupal stages impaired LEC migration, while earlier induction also affected LEC shape and LEC survival. We propose that Dpy has different roles in early and late morphogenesis, anchoring stationary LECs to the cuticle and, subsequently, serving as a substrate for migrating LECs. Our findings highlight that cells, following a change in behaviour, remodel their aECM to support this behavioural change.

Keywords: Cell migration, Apical extracellular matrix (aECM), Dumpy.

*Speaker

MYOTROPHIN-CP ANTAGONISM GOVERNS MUSCLE HYPERTROPHY BY REGULATING THE NUMBER OF MYOFILAMENTS

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During myofibril assembly, the number and length of thin and thick filaments increase significantly in a muscle type specific manner, which is critical for efficient activity. Mutations altering these processes are linked to nemaline myopathy and dilated cardiomyopathy. Despite a wealth of knowledge on the structure of myofilaments in mature sarcomeres, the molecular basis of their assembly and length specification remained poorly understood. To address this question we used the flight muscles of *Drosophila* as a model, and performed an RNAi screen to identify new factors that regulate the assembly, elongation and turnover of myofilaments. We found that silencing either subunit of the actin barbed-end binding capping protein (CP) significantly increased the diameter of myofibrils without affecting the overall organization of sarcomeres. Electron micrographs revealed that the loss of CP increases the number of myofilaments, while their organization remains intact. Conversely, the overexpression of CP in muscles significantly decreased the diameter of myofibrils. To explore the molecular function of CP in muscle cells in more detail, we looked at the proteins that were identified to regulate its capping activity in non-muscle cells. Of these, Myotrophin/V-1 is known to be a CP sequestering protein that binds to free CP molecules and prevents their interaction with F-actin barbed-ends. Myotrophin/V-1 has not been clearly identified in *Drosophila* yet, however, the gene CG7423 encodes a protein with 68% overall similarity to human Myotrophin. Also, the AlphaFold homology model of CG7423 superposes almost perfectly with the experimentally determined backbone structure of human Myotrophin/V-1. Accordingly, silencing of CG7423 decreased, while overexpression increased the diameter of myofibrils without altering other aspects of sarcomere organization. These findings suggest that CG7423 is the *Drosophila* homolog of Myotrophin/V-1, which is able to sequester CP and promote the formation of new myofilaments, eventually leading to muscle hypertrophy.

Keywords: actin cytoskeleton, myofibril, nanoscopy, IFM, dSTORM

*Speaker

Mechanosensing buffers rapid junction length changes in epithelial tissues.

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The regulation of cell-cell junction length is a critical aspect of epithelial tissue development, homeostasis, and proper organ function. Cell-cell junctions can undergo rapid changes in length over short periods of time, but over longer timescales, they tend to maintain a consistent length in developing tissues. In this study, we investigated the dynamics of cell junction length regulation and its relationship with E-Cadherin levels and cytoskeleton dynamics. Our findings reveal that rapid events of junction lengthening are associated with localized decreases in E-Cadherin levels, followed by a subsequent increase in MyoII. This increase in MyoII is controlled by the mechanosensing RhoGEF Cysts, as no MyoII increase is observed in the absence of Cysts. Consequently, rapid junction length changes are not buffered, E-Cadherin local decreases remain unresolved for longer times, and therefore junction lengths are not robustly maintained. Additionally, we discovered that this actomyosin-dependent mechanism works in conjunction with the Spectrin complex, which interacts with the actomyosin network and plays a crucial role in reducing the occurrence of localized E-Cadherin decreases. Taken together, our findings suggest a mechanosensing process driven by Cysts at the actomyosin cortex that, in combination with the Spectrin complex, contributes to the regulation of junction length in epithelial tissues.

Keywords: Cell Junction and Cortex, Mechanosensing, E, Cadherin, Cysts, Spectrin complex, Myosin

*Speaker

New sex pathways active in all somatic cells are essential to induce sex differences

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An individual can be characterized by the presence of particular sex organs: testes or ovaries. The formation of these reproductive organs is controlled by specific genetic elements: the sex chromosomes. However, sex differences encompass much more than sex organs. In *Drosophila*, the detection of sex chromosomes activates a splicing cascade that results in the production of the sex determinant TransformerF (TraF) only in females. Until now, researchers considered adult flies of both sexes to be mosaics of cells knowing their sexual identity (like the cells of the gonads) and cells not knowing their sexual identity (the majority of cells). Recent works demonstrated that the sexual identity of intestinal stem cells plays a key role in the adult gut for the sex-specific pre-disposition to tumours, highlighting the importance of a new cell-intrinsic mechanism outside the gonads. While these findings establish the proof-of-principle of the influence of sex chromosomes in adult cells, essential gaps remain to be filled. Indeed, the full range of phenotypic consequences of the presence of sex chromosomes in somatic cells, the genes, the mechanisms involved, and their sites of action remain entirely elusive. My project aims to understand where and how the intrinsic presence of sex chromosomes, the cellular sex, impacts physiology across the body. Surprisingly, I discovered that all the organs, from embryonic to adult stages, have an intrinsic sexual identity, which can be visualized by the expression of the female sex determinant TraF. I also identified all the new cellular contexts where the expression of traF is necessary and sufficient to drive sex differences in body size, weight, and fertility. I’m now characterizing the molecular mechanism(s) downstream of TraF.

Keywords: cellular sex, sex differentiation, sex differences, sexual identity

Nup107 controls developmental transitions in *Drosophila*

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Metamorphosis is integral to proper insect development where the juvenile transitions into an adult. In *Drosophila* this process is regulated by 20-hydroxyecdysone (20E) hormone. Production of 20E is regulated in response to neuropeptide signaling pathways. Nucleoporins (Nups) are proteins which constitute nuclear pore complexes (NPC), mediate nucleo-cytoplasmic transport, regulate gene expression programs and thus contribute to normal development of organisms. Involvement of Nups, more so the members of Nup107 complex in regulating metamorphosis and organismal development is poorly understood. Here, we report that when Nup107 was depleted ubiquitously, larval development was arrested at the third instar stage. To dissect the underlying molecular mechanisms, we tested and found reduced levels of developmentally induced gene EcR, Eip75, and Eip74EF in Nup107 knockdown organisms. Quantification of ecdysone biosynthesis genes also established involvement of Nup107 in inducing delay in metamorphosis. Our observations suggest that Nup107 plays an important regulatory role in *Drosophila* metamorphosis.

Keywords: Nucleoporin, Metamorphosis

*Speaker

Pleiotropic roles of Rbfox1 in early adult myogenesis via regulation of JAK/STAT signalling in *Drosophila*

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Striated muscles are the primary effectors of motor activity, and defects in their structure and function result in myopathies. Although it has been demonstrated that the number of fibres in each muscle is mainly determined prenatally in vertebrates, the molecular players that control this phenomenon remain largely unknown. The dorsal longitudinal muscles (DLMs) of *Drosophila melanogaster*, the largest muscles in the fly, develop and function similarly to the vertebrate skeletal muscles, and, thereby, serve as a model to identify new factors in muscle patterning. In this study, we identify RNA-binding Fox protein 1 (Rbfox1) functions as a transcriptional co-activator of *Signal-transducer and activator of transcription protein at 92E* (*Stat92E*), the sole *Drosophila* orthologue of *STAT* genes, a regulation required for proper splitting of the DLMs. Other members of the JAK/STAT signalling pathway, and some of its targets, are also involved in this process. Moreover, results suggest that other signalling pathways may be in dialogue with JAK/STAT signalling during adult myogenesis in *Drosophila*. Also, Rbfox1, in its role as a splicing regulator, may directly dictate the conserved switch between the long and short isoforms of *Zn finger homeodomain 1* (*Zfh1*), a *Stat92E* target, highlighting the robustness of the regulatory mechanisms. Together, our findings reveal the distinct facets of Rbfox1 function during early adult myogenesis, including, via JAK/STAT signalling, maintenance of stemness, mediation of F-actin dynamics, and inhibition of apoptosis in myoblasts.

Keywords: dorsal longitudinal muscles, myogenesis, Rbfox1, JAK/STAT signalling

*Speaker

Shaping epithelial tubules during embryogenesis

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The Malpighian (renal) tubules form during embryogenesis and persist into adulthood. During their development, they undergo a 4-fold elongation step, solely via cell rearrangements, to traverse the embryo allowing efficient filtration of the haemolymph. Current research has characterised the signalling processes controlling tubule elongation, but tubule mechanics is yet to be fully explored. This project investigates the mechanics of Malpighian tubule elongation by using two photon microscopy to capture live tubulogenesis. These movies show a range of dynamic processes including the folding of the anterior tubules to form a tight kink. Once formed, the kink leads the tubules anteriorly causing the tubules to stretch. Additionally, movies have shown haemocytes (macrophages) to cluster around the Malpighian tubules depositing a sheath of collagen IV. Perturbing macrophage migration removes the collagen sheath causing the tubules to misform and misroute into the posterior of the embryo. This suggests that collagen may provide mechanical restrictions guiding the tubules into the appropriate shape. Finally, movies have revealed that macrophage protrusions interact with the deposited collagen which may alter the collagen structure to allow for tubule elongation. Using a combination of biophysical and genetic techniques, this project aims to elucidate the physical role collagen plays in tubule development and the importance of the macrophage collagen deposition. Together, this will develop our understanding on collagen's contribution to tissue dynamics and tension, potentially aiding the research of morphology-related diseases.

Keywords: Malpighian tubules, biomechanics, collagen IV, tubulogenesis, macrophages

*Speaker

Teaching an old hog new tricks - An updated model system to decipher Hedgehog morphogen transport in vivo

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Most recently, we published that direct Hedgehog (Hh) morphogen switching between cell-surface HSPGs (Heparan Sulfate Proteoglycans) is essential for Hh signaling (Gude et al 2023). To this end, we used an attP modified *hh* locus (Alexandre et al 2014) and reintroduced engineered *hh* alleles via a reintegration vector. For the lethal *hh* alleles *hhR238/239E* and *hhR238/239A* we used clonal analysis with the Minute technique to generate homogeneous mutant tissue to address HSPG function in hh transport, mainly analysing endpoint phenotypes. We also generated a Gal4-independent FLP source for the fly wing by CRISPR mediated Gal4 to FLP transgene conversion applying the HACK technique (Lin & Potter 2016). The resulting nubbin-FLP is highly specific for the wing blade. We also observed that large homogeneous clones expressing eyFlp3.5 yielded head phenotypes with affected complex eye and ocelli, and affected head appendages such as maxillary palps, which are part of the olfactory system. We have now started to analyze the phenotypes in the olfactory system in more detail. We are also extending our clonal analysis of HSPG function in *hh* transport and signaling into more tissues, using our existing *hhR238/239E* and *hhR238/239A* alleles. An initial analysis of unpublished phenotypes will be presented and their relation to previous findings discussed.

Keywords: morphogen, hedgehog signaling

*Speaker

The Origin of the *Drosophila* Malpighian Tubule

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The developmental origin of the insect renal or Malpighian tubule has been contested for over a century. Fate-mapping studies have revealed the approximate position of the cells which adopt tubule identity within the blastoderm embryo in *Drosophila*, however the precise location, timing and mechanism by which their identity is established are not known. Here we show *Drosophila* Malpighian tubule identity is established in the posterior terminal region of the blastoderm embryo (at a time several hours earlier than appreciated before) as part of the posterior terminal patterning system. We present evidence that the tubules form from cells traditionally considered as hailing from both ectoderm and endoderm, calling into question the usefulness of the germ layer distinction for this organ. Surprisingly, we also show that the future proximo-distal (P-D) axis of the tubule-which underpins its mature physiological functions-is established at the same time, and by the same terminal patterning system. Together, this work provides definitive evidence for the developmental origin of the *Drosophila* Malpighian tubule, resolves age-old debates about its germ-layer origins, and reveals that P-D axis formation in this organ can be traced back to a system that patterns the primary body axis.

Keywords: Organogenesis, Malpighian tubule, tissue identity, terminal patterning system, germ layer origins, proximodistal axis

*Speaker

Toll-like Receptors and the *Drosophila* neck fold – a morphogenetic process controlled by the interplay of mechanical forces and geometry

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Forming the 3D shape of an organism relies on large scale changes in epithelial sheets, such as epithelial folds. The mechanical forces driving these changes are generated by accurately positioned and timed changes in collective cell behavior. Thus, the 3D geometry of a tissue is defined by the interplay between genetically controlled cell behavior and mechanical forces. Interestingly tissue geometry itself can play a role in mechanical force generation. In this work, we show that the large scale epithelial fold that forms the *Drosophila* neck is controlled by the concerted action of patterned gene expression and tissue curvature. The neck is defined by the homeotic gene *Deformed* (*Dfd*), which controls a regional increase in in-plane tension. This tension is converted to an orthogonal force driving folding when combined with the curved geometry of the tissue. We also show that *Dfd* is important for the characteristic actomyosin organization of the neck, necessary for promoting the tension build up. This structural organization is formed over several hours prior to neck invagination, in which cell behavior and actomyosin distribution is dynamic, while tissue tension is gradually increasing. Aiming to understand the factors that regulate actomyosin dynamics within the neck, we have identified Tollo, a Toll-like receptor, to be enriched in the neck under *Dfd* control and to regulate actomyosin contractility. Here we further characterize the role of Toll-like receptors in the dynamic control of actomyosin and explore the mechanisms that generate and respond to increasing tension in the developing neck. Collectively, our work provides important insights on the interplay of gene expression patterns, mechanical force generation and tissue geometry.

Keywords: morphogenesis, tissue geometry, Toll, like receptors, epithelial folding, tissue mechanics, actin, myosin

^{*}Speaker

Tricellular junction recruitment of Wave regulatory complex by Sidekick and Lar induces protrusive activity resolving cell intercalation

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Cell intercalation is a key morphogenetic process during which cells exchange neighbors. How cells initiate the lengthening of new junctions is not yet well understood, although a role for tricellular junction actors is proposed. Here, we identified that the WAVE regulatory complex (WRC), involved in branched F-Actin generation, is required for tissue elongation and cell intercalation in the *Drosophila* ovarian follicular epithelium. WRC is localized at tricellular junctions where it generates very dynamic protrusions emanating from one cell and extending between the bicellular junction of the two others. This protrusive activity is required in the cells at the extremities of a new junction for its initiation. Finally, we show that WRC is redundantly recruited at tricellular junction by Sidekick and Lar, and that blocking this recruitment affects protrusive activity, cell intercalation resolution and tissue elongation, thereby mechanistically bridging molecular, cellular and tissular scales. Altogether, our data decipher a critical mechanism for epithelium morphogenesis based on actin polymerization from tricellular junctions.

Keywords: tricellular junction, follicle cells, branched actin, lamellipodia, rearrangements

*Speaker

Tuning cytoskeletal and mechanical polarity from the cell to the embryo scale to trigger gastrulation movements

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During embryo development, tissues remodel their shape under the action of biomechanical forces. Contractile networks of F-actin and non-muscle myosin II (MyoII) constitute a primary force-generating machinery in epithelial cells. Embryo-scale polarized force patterns are necessary to initiate coordinated epithelial movements and shape changes. How actomyosin cytoskeleton polarity is tuned at the cell scale to ultimately result in the emergence of embryo-scale polarized force patterns is still poorly understood. To investigate this, we use the early developing *Drosophila* model system. During the blastula-to-gastrula transition (i.e., during end of cellularization), the F-actin network and the MyoII distribution is spatio-temporally remodeled and tuned at both the basal and apical sides of epithelial cells establishing a polarized pattern along the embryo dorsal-ventral axis (1). For instance, basal MyoII accumulation in ventral cells rapidly vanishes to then reappear apically. This eventually results in a polarized force field driving tissue coordinated movements initiating embryo gastrulation. Here we investigate the cellular mechanisms responsible for fine tuning the F-actin network and the MyoII distribution at basal and apical cell sides. In addition, we investigate how these mechanisms are regulated with high spatio-temporal specificity across the embryo. Finally, by employing advanced light sheet imaging, quantitative live image analysis, optogenetics, and laser manipulation, our research will shed new light on the mechanisms and regulatory factors driving actomyosin polarity from the cell to the embryo scale, ultimately initiating embryo gastrulation. (1) Rauzi, M. et al. Embryo-scale tissue mechanics during *Drosophila* gastrulation movements. Nat Commun 6, 8677, doi:10.1038/ncomms9677 (2015).

Keywords: Gastrulation, epithelial polarity, tissue mechanics, actomyosin networks

*Speaker

Transcription & chromatin

An inter-chromosomal kiss: Decoding mechanisms of pairing-dependent interallelic interactions

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Transvection is an interesting case of interchromosomal interaction in which the physical proximity of the two alleles of a gene alters their regulation and thus gene expression. To understand the mechanisms that regulate the genomic distance between the participating alleles and translate proximity into functional interaction, we use the spotted-wing fly species *Drosophila biarmipes* with a sexually dimorphic expression pattern of the X-linked gene *yellow* on male wings. Female flies (XX) show pairing-dependent silencing of the *spot* enhancers of the two *yellow* alleles, while in hemizygous males (XY) the *spot* enhancer of the unpaired yellow allele is strongly active, giving rise to the characteristic male wing pigmentation spot. Our approach to understanding the relationship between the structure of the *yellow* alleles and their function uses genetics, biochemistry, and high-resolution DNA FISH. Sequential mutagenesis of the *yellow* locus has revealed short regions within the *spot* enhancer and the intron that are required for the pairing-dependent silencing of the *spot* enhancers. These DNA regions are used as baits for in vitro pull-down assays using staged pupal extracts to identify binding factors. In parallel, we are using high-resolution DNA FISH to test the hypothesis that the physical distance between the *spot* enhancer and the intronic silencer is affected when the *yellow* alleles are paired, reflecting a potential pairing-dependent structural modification of the locus. Results from this approach, coupled with the quantitative visual output of the *spot* enhancer, would help to identify key players that regulate inter-allelic distances and functional interactions, making *yellow* a promising system for furthering our understanding of the mechanisms that regulate the structure-function relationship of the diploid genome.

Keywords: Transvection, interchromosomal interaction, gene regulation, structure function relationship

*Speaker

Characterization of spatiotemporal roles of Slbo during border cells migration by optogenetics

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The development of multicellular organisms is controlled by highly dynamic molecular and cellular processes organized in spatially restricted patterns. Therefore, perturbation methods that match these dynamics are needed. But traditional methods based on genetic backgrounds, such as gene mutants, often have limitations in studying the dynamics and spatiotemporal roles of gene expression. As an alternative tool, optogenetics approaches make it possible to precisely monitor and manipulate protein functions with high temporal and spatial resolution. The C/EBP transcription factor, Slbo, (*slow border cells*), is a key transcriptional factor that controls border cell fate and migration ability in *Drosophila* egg chamber. Genetic inhibition of Slbo strongly blocks border cell detachment process so that precise dynamics and roles of Slbo during border cell migration are poorly known. To overcome the genetic limitation, we tagged Slbo with GFP and iLexy (improved light-inducible nuclear export system) by CRISPR/Cas9 knock-in technique in *Drosophila*, which can allow us to monitor and manipulate dynamics and roles of Slbo protein during border cell migration. Firstly, we demonstrated that Slbo protein levels are not the same during different migratory stages. Secondly, we noticed that Slbo protein levels are also different in individual border cells within the group, and unexpectedly lower in the leader cell than in follower cells. This indicates that a moderate Slbo protein level may determine the cell fate of being the leader cell. Besides, by acutely manipulating Slbo protein from the nucleus to the cytoplasm via iLEXY, we found that border cells display multiple protrusions and minimally dynamic migration, and F-actin and Myosin-II in these border cells are enriched at protrusion leading edge. Currently, we focus on the precise roles of strong to mild Slbo photoinhibition on border cell migration. Altogether, our studies reveal unknown dynamics and spatiotemporal roles of Slbo protein during border cells migration.

Keywords: *Drosophila*, C/EBP, border cells migration, optogenetics, spatiotemporal control

*Speaker

Chromatin dynamics during gliogenesis

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During development, distinct cell types acquire their properties under the control of transcription factors called cell fate determinants. These factors act on genes necessary for the progression of cell differentiation and this is accompanied by chromatin reorganisation. The acquisition of a specific chromatin conformation is required for cell differentiation and function. These two phenomena, gene regulation and chromatin reorganisation, have been well studied independently in numerous systems however their interdependency is poorly understood.

One of our main research axes focus on the differentiation of glia in the developing *Drosophila* embryo. Our current challenge is to understand the interplay between the differentiation program and chromatin modulation during gliogenesis. In the nervous system of *Drosophila*, glia and neurons are produced by common neural stem cell-like precursors. Thus in early embryonic stage, they share the same chromatin background, which is progressively modified during development to adopt the final conformation necessary for their functions. This provides an ideal platform to understand the mechanisms behind the acquisition of distinct chromatin landscapes.

Our laboratory has characterised the glia’ differentiation program in the *Drosophila* embryo. Our past work revealed notably the role of the glial cell fate determinant Gcm in gliogenesis. In addition, we have shown that Gcm impacts chromatin landmarks, interacts with the histone acetyl-transferase CBP and regulates the expression of chromatin modifying enzymes. We recently assessed the impact of Gcm on chromatin conformation using Cut & Run, revealing strong modifications of the histone mark profiles and transcriptomes, suggesting that Gcm recruits histone modifiers for its function.

Keywords: dCBP, Gcm, gliogenesis, Cut & Run

^{*}Speaker

Contribution of TE phenotype and plasticity in *Drosophila melanogaster*

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Phenotypic plasticity is the ability for one genotype to yield different phenotypes in response to environmental changes. This process may be adaptive and allow organisms to adapt to environment changes. Most of the molecular mechanisms underlying phenotypic plasticity are epigenetic mechanisms, such as histone marks, DNA methylation or small RNAs, allowing differential expression of genes, and eventually result in phenotypic changes. Not only these mechanisms regulate gene expression, but they are also involved in the repression of transposable elements (TEs). TEs are repeated DNA sequences potentially capable of moving within genomes. Thanks to their transposition mechanisms, but also to their inherent regulatory sequences, TEs are known to impact gene expression, genome architecture and integrity. We hypothesize that TEs' regulatory mechanisms, namely epigenetic mechanisms, might also vary with the environment and potentially lead to changes in TE expression and transposition rate, and in turn alter gene expression. To test this hypothesis, we first investigated the impact of different TE content on life history traits in *Drosophila melanogaster*. We then evaluated the contribution to phenotypic plasticity. We took advantage of genetically engineered fruit flies that bear different TE content but identical genetic background, to tackle this question in a controlled manner.

Keywords: plasticity, transposable elements, *Drosophila*

*Speaker

Crosstalk between chromatin and the transcription factor Shavenbaby defines transcriptional output along the *Drosophila* intestinal stem cell lineage.

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The transcription factor Shavenbaby (Svb/ovo), the only member of the OvoL family in *Drosophila*, controls the fate of various epithelial embryonic cells and adult stem cells. Svb is central to adult intestinal stem cells homeostasis. Post-translational modification of Svb produces two protein isoforms, Svb-ACT and Svb-REP, which promote adult intestinal stem cell renewal or differentiation, respectively. To define Svb mode of action, we used engineered cell lines and develop an unbiased method to identify Svb target genes across different contexts. Within a given cell type, Svb-ACT and Svb-REP antagonistically regulate the expression of a set of target genes, binding specific enhancers whose accessibility is constrained by chromatin landscape. Then, Svb modifies local and long-range chromatin landscape in an isoform-specific manner. Along the differentiation of intestinal stem cells into enteroblasts and enterocytes, the isoform of Svb changes, concomitant with changes in chromatin accessibility at its target genes, and a progressive change of the set of target genes Svb regulates along the lineage. We propose that Svb-ACT-to-REP transition promotes enterocyte differentiation of intestinal stem cells through direct gene regulation and chromatin remodeling. In addition to mechanistical aspects, this work shows that genes regulated by Svb in intestinal stem cells are typically key players of stemness. The unique dual regulation by Svb-ACT and Svb-REP isoforms highlights how Svb controls the subtle balance between proliferation and differentiation of stem cells. Finally, this suggests a window of plastic gene regulation in enteroblasts that might regulate their commitment into terminal differentiation or re-entry into mitosis.

Keywords: Transcription factor, chromatin, gene regulation, intestinal stem cells, Svb/Ovo

*Speaker

Histone removal in sperm protects paternal chromosomes from premature division at fertilization

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In many animals, including insects, the differentiation of the haploid spermatids in sperm cells is characterized by a global replacement of histones with sperm nuclear basic proteins such as protamines. This results in the ultracompaction of the paternal genome but the function of this peculiar chromatin organization remains largely enigmatic. We show that in the *Drosophila* paternal effect mutant *paternal loss (pal)*, sperm chromatin massively retains histones H3 and H4 -but not H2A, H2B and H1- without impairing sperm viability and fecundity. Strikingly however, after fertilization, *pal* sperm chromosomes are aberrantly targeted by the egg Chromosomal Passenger Complex, which is required for meiotic acentrosomal spindle assembly. Paternal chromosomes are forced to engage into a catastrophic premature division in synchrony with female meiosis II, leading to male pronucleus fragmentation and paternal chromosome losses. We find that *pal* encodes a transition protein which is expressed transiently during spermiogenesis and is required to complete H3-H4 histone removal in spermatids. Our study thus reveals an unsuspected role of histone eviction from insect sperm chromatin: safeguarding the epigenetic identity of paternal chromosomes at fertilization.

Keywords: protamines, histones, sperm, fertilization

*Speaker

In vivo contribution of a single DPE core promoter motif to transcriptional regulation in developing *Drosophila melanogaster* embryos

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Regulation of transcription is critical for most biological processes, including embryonic development. Transcription initiation occurs at the core promoter, frequently referred to as "the gateway to transcription". Core promoters are diverse in their architecture and function, and can contain distinct DNA motifs termed core promoter elements. Interestingly, downstream core promoter element (DPE)-containing genes are highly enriched for heart-related and mesoderm development GO terms. To study the *in vivo* function of the DPE during *Drosophila* embryonic development, we focused on the *tinman* gene, which encodes a transcription factor that orchestrates the formation of the dorsal musculature and heart. Using a co-CRISPR approach, we show that a 7bp mutation of the DPE motif, located within the 5' UTR of the *tinman* gene, is sufficient to reduce *tinman* expression at both the RNA and protein levels. Remarkably, this mutation results in significantly reduced viability. Nascent transcriptomics analysis shows that this substitution results in a massive perturbation of Tinman's regulatory network orchestrating dorsal musculature and heart formation. Our findings demonstrate the feasibility and importance of characterizing DNA sequence elements *in vivo* in their natural context, and accentuates the critical impact a single core promoter element can have during *Drosophila* embryogenesis.

Keywords: Core Promoter, RNA Polymerase II transcription, DPE, Gene Expression, nascent transcription, *Drosophila*, heart development

*Speaker

In-cellulo approach to study the effect of Hox proteins and their interacting partners on chromatin dynamics

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For several decades, the context-dependent functions of transcription factors have been under scrutiny. Among them, Homeodomain transcription factors (Hox TFs), first identified in *Drosophila*, regulate transcription during embryogenesis through their ability to bind specific DNA/chromatin sequences via their homeodomain. This binding orchestrates a myriad of developmental trajectories. While certain operational mechanisms of Hox TFs have been elucidated, others remain ambiguous. Recent findings from our laboratory suggest that the Hox transcription factor Ultrabithorax (Ubx) interacts with lineage-specific transcription factors across various cell lineages, hinting at a fundamental role for Hox TFs in lineage commitment. Furthermore, evidence suggests that certain Hox TFs can modulate chromatin states by interacting with multiple co-factors, facilitated by the non-homeodomain regions of the Hox TFs. Building on these insights, our research seeks to delve into the interactions of Hox transcription factors with diverse co-factors and their consequent impact on chromatin dynamics. To this end, we have standardized an expression system within *Drosophila* cell lines, allowing the controlled co-expression of multiple transcription factors to discern their combinatorial downstream effects. To realize this cellular framework, we employ a dual-pronged strategy. The first approach leverages the expression of transcription factors controlled by inducible promoters, specifically the metallothionein (MT) and Tetracycline (TetON) responsive promoters, which can be activated or deactivated in the presence of Copper Sulphate and Doxycycline (a Tetracycline analog), respectively. The second part utilizes the established SiMPI system (Split Intein-mediated enzyme reconstitution) to engineer a double transgenic cell line bearing the plasmids encoding our transcription factors of interest. A selectable marker is partitioned into two discrete components that reassemble post-translation through the Split-INTEIN system, facilitating the selection of double transgenic cell lines. Successfully implemented, this system has enabled us to generate stable double transgenic S2R+ cell lines, providing an optimal platform for the controlled induction of specific transcription factor combinations. Utilizing this system, our goal is to investigate the downstream ramifications of interactions between paired transcription factors, with a focus on their influence on chromatin dynamics.

Keywords: Development, embryogenesis, Homeodomain Transcription Factors, Chromatin

*Speaker

Investigating the role of redundant regulatory elements in mutational robustness in *Drosophila melanogaster*

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The regulation of gene expression is highly dynamic and context dependent. This entails the need for continuous adaptation of expression levels for some sets of genes while others – in the same cell - need to remain stably expressed. We have previously shown that one can employ population-scale data for individual cell types to measure the expression variability genes can exhibit. The extent to which expression levels for the same gene can vary across individuals depends on a trade-off between stable and responsive gene expression. This variability is largely determined by sequence features in the promoter. It remains however unclear which role additional parameters of gene regulation play and how they influence gene expression variability in more complex settings such as tissues, primarily due to limitations in current assays for characterizing regulatory elements. Hence, to study the involvement of enhancers, the overall architecture of promoters and the interaction of both - enhancers and promoters - in gene regulation, we devised a more sensitive method to detect their location and expression, nucleiCAGE. This method maps the 5'-ends of transcribed nuclear RNA species with a cap. We applied nucleiCAGE to wing discs extracted in bulk from 49 inbred lines of the DGRP panel. This allowed us to precisely map transcription start sites (TSSs) on a genome-wide scale, aggregate them to decipher promoter architectures, identify promoter upstream transcripts (PROMPTs) and bidirectionally transcribed enhancers. Based on the expression levels and variability of these regulatory elements, we characterize mechanisms of gene regulation that determine whether and to what degree genes are stably or adaptably expressed. This project provides insights into the role of enhancers, promoter architecture and promoter-enhancer interactions in regulation of gene expression variability and robustness in *Drosophila*.

Keywords: gene expression, robustness, variability, CAGE, gene regulation, promoters, enhancers, wing discs, DGRP

^{*}Speaker

Kdm3 acts as a bookend to limit the spreading of heterochromatin

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To preserve genome integrity of the animal's germline, transposable element activity is regulated by small non-coding RNAs called PIWI interacting RNA (piRNA). In *Drosophila melanogaster* ovaries, piRNAs arise from heterochromatic regions called piRNA clusters. These regions are both enriched for incomplete TE sequences and epigenetic marks such as the trimethylation of lysine 9 of histone 3 (H3K9me3). Although the chromatin is compacted, these regions are transcribed thanks to the Rhino protein which binds to certain regions enriched in H3K9me3 marks and recruits the transcriptional machinery via specific partners. Rhino is a member of the Heterochromatic Protein 1a (HP1a) family and plays a critical role in piRNA clusters biology but its binding specificity remains to be elucidated: not all regions enriched in H3K9me3 recruit Rhino. We recently showed that the knock down of Kdm3, a histone demethylase targeting methylated H3K9, nearly doubles H3K9me2 level on the whole genome in the *Drosophila* ovary. Among the newly enriched domains, some gene-containing regions were also found enriched in Rhino, leading to the formation of de novo piRNA clusters and the production of auto-immune piRNAs. Here, we will present a deeper analysis of the chromatin landscape modifications following Kdm3 knock down. Our results show that new H3K9me2-enriched domains are also globally newly enriched in H3K9me3 and strongly suggest that Kdm3 acts as a bookend to limit the spreading of heterochromatin.

Keywords: Chromatin, piRNA clusters, heterochromatin, epigenetics, histone demethylase

*Speaker

Measuring the impact of m6A methyltransferase complex on R-loops dynamics at paused promoters in *Drosophila*

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RNA modifications, known collectively as the epitranscriptome, have emerged over the past decade as an important additional regulatory layer of gene expression. RNA modifications can control many aspects of RNA metabolism including pre-mRNA splicing, degradation, and translation, and their disruption has been associated with a wide range of physiological alterations, neurological diseases, heart failure as well as various cancers. The modification of N6-methyladenosine (m6A) is of particular interest because of its high conservation during evolution, its asymmetric distribution along mRNAs in correlation with the corresponding functions and its involvement in a wide range of developmental processes. Although the role of the m6A methyltransferase complex (m6A MTC) in transcriptional regulation has recently been described in many organisms, the context-dependent molecular mechanisms by which m6A deposited on RNA modulate RNA polymerase II (RNAPII) activity remain incomplete. We recently demonstrated that m6A MTC complex stimulates the release of RNAPII from its paused state. Our new project aims to specifically assess in this context (i) how m6A affects the stability of RNA/DNA hybrids called R-loops at promoter proximal regions (ii) what are the novel players linking m6A marks to R-loop resolution (iii) how this mechanism can be correlated to RNAPII pausing and to a particular epigenetic context (iv) how affecting the regulation of R-loops by m6A can lead to developmental defects. We believe our results will shed new light on the molecular understanding of m6A RNA function in transcriptional regulation.

Keywords: m6A RNA, RNAPII pausing, R loops, Transcription

*Speaker

Phylogenomic instructed target analysis reveals ELAV complex binding to multiple optimally spaced U-rich motifs

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The RNA binding protein ELAV is a gene-specific regulator of alternative pre-mRNA processing in *Drosophila* neurons. ELAV/Hu family proteins bind to short U-rich motifs which are found in most pre-mRNAs, making it unclear how they achieve gene specificity. ELAV/Hu proteins multimerise and ELAV forms a dodecameric complex on *ewg* RNA. However, it is unclear how ELAV recognises degenerate sequence and whether multimerisation is a mechanism for gene specificity. Here we show that ELAV forms a saturable complex with extended RNA and nucleates from a single binding element. We reveal that the *ewg* binding site forms a stemloop secondary structure that is unwound upon ELAV binding at three distal U4 motifs. Further, we indicate that ELAV polyadenylation sites are enriched in stemloops. We probe the *ewg* site and show that high-affinity ELAV binding requires spaced U-rich motifs that cannot be completely base-paired in a stem-loop context. Enrichment of N6-methyladenosine (m6A) methylation in spacer sequences improves ELAV binding minimally. Furthermore, through cross-species analysis and binding assays we describe a minimal ELAV binding element and that optimal spacing between binding motifs is a decisive mechanism for ELAV complex formation. Our findings address a crucial gap in how ELAV/Hu family proteins recognise degenerate sequences for gene-specific mRNA processing.

Keywords: ELAV, multimerisation, RNA binding, motif spacing, nucleation, minimal binding element, alternative polyadenylation, alternative splicing

*Speaker

Playing both sides: Hox function in co-transcriptional splicing

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Transcription factors (TFs) are central players in gene expression acting on DNA-regulatory sequences to coordinate genetic programs. Beyond this DNA-centric viewpoint, many TFs also bind RNA and/or modulate mRNA splicing and yet, the importance of these RNA-regulatory functions in cell fate decisions remains elusive. We previously identified an interplay between the *Drosophila* Hox TF Ultrabithorax (Ubx) and splicing factors which ensures proper muscle development in embryos. Our results revealed that Ubx regulates co-transcriptional splicing via its DNA- and newly uncovered RNA-binding properties. Based on these findings, we are currently investigating the molecular basis of Ubx function in splicing. Using muscle patterning as a readout, we performed rescue experiments that revealed an unexpected functional interplay between Ubx wild-type (WT) and a Ubx mutant version unable to bind DNA (N51A). Combining molecular and imaging approaches in *Drosophila* cells, our work demonstrates an interplay between Ubx WT and the mutant N51A in regulating splicing. From these results, we proposed a refined model for Ubx function in splicing, in which TF dimerization is an essential feature for Ubx to spatially connect transcription and splicing in the nucleus. Overall, our work shed new light on the function of Ubx in splicing thereby providing entry points to elucidate the RNA-regulatory function of key developmental TFs in tissue patterning and morphogenesis.

Keywords: Transcription, Splicing, Hox, Transcription Factor, RNA

*Speaker

Ploidy levels regulate cell fate during *Drosophila* metamorphosis

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During *Drosophila* metamorphosis most larval tissues are eliminated, whereas imaginal cells survive and remodeled to meet the needs of the adult fly. Remarkably, we found that both processes (death and differentiation) depend on the presence of the stage-specific ecdysone-dependent transcription factor E93 in larval and imaginal cells. Thus, the presence of E93 in the prothoracic gland (PG), the main endocrine larval organ, induces its degeneration by apoptosis and autophagy during the pupal stage. In contrast, in wing imaginal cells, E93 promotes terminal differentiation. However, the molecular context that determines the fate of larval versus adult precursor cells upon activation of E93 remains unknown. In this context, it is worth noting that one of the main features that distinguish these two cell populations is their DNA content. Whereas imaginal cells are mainly diploid, larval cells, as those of the PG, shows a high grade of polyploidy. Interestingly, conversion of PG polyploids cells into diploid by depleting *fizzy-related* impairs degeneration of the organ. In contrast, polyploidization of wing imaginal cells resulted in an increase in cell death during metamorphosis with dramatic morphogenetic defects observed in the adult wing. Altogether, our results indicate that E93 may induce either cell death or differentiation depending on the DNA content of larval and imaginal cells.

Keywords: ecdysone, polyploidy, prothoracic gland, autophagy, apoptosis, E93.

*Speaker

Tardigrade intrinsically disorder protein Dsup (damage suppressor) provides DNA protection and induces transcriptional repression in *D. melanogaster*

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Studies of the unique tardigrade proteins uncover principles of extreme resistance and can serve a basis for applications in medicine, biotechnology, pharmacy and space research. The nuclear-localized DNA-binding Dsup protein was discovered in *Ramazzottius varieornatus* in 2016 by Hashimoto et al. Dsup directly involved in the suppression of DNA damage by reactive oxygen species (ROS) and enhances resistance to ionizing radiation and oxidative stress in Dsup-expressing cell culture HEK293. However, the mechanism of Dsup action and its effect on other processes in complex animal organisms remain unclear. In this work, for the first time we reveal an ability of Dsup to enhance radiation and oxidative stresses resistance in *Drosophila melanogaster*. In addition, transcriptome analysis revealed that Dsup protein can be considered as nonspecific repressor of transcription and among enriched biological processes for list of down-regulated DEGs were regulation of transcription, nucleosome assembly, chromatin condensation, DNA folding, neuroregulation and locomotion behavior. To better explanation of Dsup action, *in vitro* studies of the protein structure and characteristics of Dsup-DNA complex were conducted. The combined use of small-angle X-ray scattering (SAXS), circular dichroism spectroscopy and a set of computational methods allowed to determine intrinsically disordered nature of Dsup protein with highly flexible structure. Based on SAXS data obtained for Dsup-DNA complex, we demonstrated increased DNA compaction in Dsup-DNA complex. Obtained results enable the development of new protective principles for multicellular organisms.

Keywords: DNA protection, tardigrade, tardigrade disordered proteins, TDPs, intrinsically disordered proteins, radioprotector, ROS

*Speaker

The Groucho co-repressor can inhibit progression through the early transcription elongation checkpoint to repress gene expression

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The *Drosophila* Groucho (Gro) protein was the founding member of the family of transcriptional co-repressor proteins that includes the Transducin-like enhancer of split (TLE) and Gro related gene (Grg) proteins in vertebrates. Gro does not interact directly with DNA but is recruited to downregulate target genes by specific DNA binding transcription factors that include Brinker, Capicua, Hairless, Hairy and the Enhancer of split bHLH proteins in *Drosophila*. Consequently, Gro plays a key role in many developmental contexts including segmentation, neurogenesis, and patterning of the wing veins. Despite being established as acting as a co-repressor protein nigh on thirty years ago, the molecular mechanisms through which Gro acts to repress transcription are not well understood. Recent studies revealed that Gro is enriched at a subset of genes that exhibit RNA polymerase II (RNAP II) pausing in *Drosophila melanogaster* cells and that depletion of Gro can lead to pause release. Here we present evidence from bioinformatic and genetic analyses that supports a model in which Gro can promote RNAP II pausing to repress gene expression. Bioinformatic analyses revealed that Gro recruitment frequently overlaps that of general factors which promote RNAP II pausing including NELF and GAGA Factor. However, Gro recruitment does not preclude recruitment of factors that mediate release of paused RNAP II (Cdk9 and Cyclin T), indicating that Gro restricts the activity of pause release factors rather than their recruitment. Genetic interaction analyses reveal that the association of Gro with factors known to promote RNAP II pausing in *Drosophila* cell lines reflects a functional relationship in vivo. Knock-down of factors that promote RNAP II pausing enhance *gro* knock-down phenotypes during wing vein patterning. However, knocking down these factors in the eye concurrently with *gro* had different effects on patterning (enhancement) and growth (rescue), indicating that the molecular mechanisms underlying Gro function differ in these two processes. Our results identify a mechanism through which developmentally regulated transcription factors can establish RNAP II pausing to modulate transcription.

Keywords: Groucho, repression, gene regulation, co, repressor, transcription factors, genetic interactions, wing vein development, eye development

*Speaker

The role of HP1-associated repressive chromatin on adult intestinal stem cell regulation

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Chromatin organization and remodeling hold a crucial role in adult stem cell regulation. However, the way repressive chromatin could serve as a dual regulator of gene expression in stem cells remains unknown. In our previous studies, we have demonstrated the role of different chromatin modifying factors, such as Kismet/CHD7 and Trr/MLL3-4, as regulators of adult intestinal stem cell proliferation. More recently, we characterized chromatin organization in the intestinal lineage of *Drosophila* under homeostatic conditions *in vivo* (Josserand et al, in revision). In that study, the genome-wide binding profiles of 5 chromatin-associated proteins were used in a modeling approach where their combinatorial binding along the different genomic regions was employed to identify different active or repressive chromatin states. One of the repressive chromatin states was found to be associated with heterochromatin protein 1a (HP1a), a key heterochromatin component primarily known as a transcriptional repressor, also related with positive transcriptional regulation in a context-dependent manner. Examining chromatin state transitions during stem cell differentiation revealed an enrichment of stem cell-specific genes in HP1a-associated chromatin, suggesting an implication of HP1a chromatin in stem cell functions. However, we still lack a proper understanding of the role of HP1a chromatin in intestinal stem cells (ISC) regulation and its implications for tissue homeostasis. Here, we report that HP1a not only is important for heterochromatin maintenance, but also for the expression of genes with metabolic and translation functions on adult ISCs. We found that when we knocked down HP1a in the ISCs and their enteroblast progenitors (EBs), there was a decrease on stem cell proliferation accompanied by a gradual ISC loss. We generated ISC-specific transcriptomes upon the same conditions and observed a strong deregulation on genes associated with metabolic processes and translation, suggesting a role of HP1a in their expression. ATAC-seq analysis on ISCs and EBs under HP1a knockdown demonstrated an increased chromatin accessibility in pericentromeric regions that are normally marked by repressive chromatin, highlighting the role of HP1a in maintaining condensed chromatin. Collectively, our results help us gain further insights on the HP1a functions as both a heterochromatin factor and a positive transcriptional regulator, important for ISC homeostasis. Nevertheless, the processes through which HP1a positively regulates gene expression, and whether it acts as a direct or indirect transcriptional regulator remain to be identified.

^{*}Speaker

Keywords: adult intestinal stem cells, chromatin remodelling, transcription regulation, tissue homeostasis

The somatic piRNA pathway is progressively activated during embryonic development to effectively repress transposable elements in the adult stage of *Drosophila melanogaster*.

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Metazoan genomes are largely composed of repeated sequences, including transposable elements (TEs). TEs are DNA sequences that can move from one genomic locus to another. Their mobilization causes genomic instabilities, which can lead to pathologies. In animal gonads, TEs expression is restricted by a specific pathway involving small non-coding RNAs called piRNAs (PIWI-interacting RNAs). These piRNAs target TEs by sequence complementarity, preventing their expression and mobilization.

In *Drosophila melanogaster*, the piRNA pathway is active in adult female gonads: in the germ cells (GCs), but also in somatic gonadal cells (SGCs) surrounding GCs. Some TEs which are abnormally reactivated in SGCs can infect and invade the germline genome. Therefore, activation of the somatic piRNA pathway is also essential for genome protection of the offspring.

The formation of the gonads starts at the embryonic stage. Little is known about the activation of the piRNA pathway and the establishment of TEs repression during development. In our study, we investigated the developmental window during which the somatic piRNA pathway becomes active in the gonads. We monitored the establishment of the repression of TEs, such as *412*, known to be silenced by the *flamenco* piRNA cluster, the major locus involved in piRNA production in SGCs. Our results reveal that *flamenco* and the piRNA pathway actors start to be expressed in SGCs during embryonic gonad formation. Surprisingly TEs are expressed in embryonic SGCs, and progressively repressed later in development, from the larval stage onwards. These results suggest that the somatic piRNA pathway is initiated during embryonic gonad formation and becomes functional during larval stage.

Keywords: Transposable element, small RNA, piRNA, ovary, development

^{*}Speaker

Transcriptional Regulation of Intestinal Stem Cell Ageing

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Ageing is associated with a reduction in physiological functions and increased incidence of several chronic diseases. Many tissues are constantly turned over throughout adult life and the balance of cell loss and replacement is often perturbed in ageing. The *Drosophila* intestine has emerged as an excellent model for epithelial ageing. In normal homeostasis, the tissue is constantly turned over as differentiated enterocytes are lost and replaced by the proliferation of stem cells. In ageing, the stem cells become misregulated, initially overproliferating and eventually failing to maintain the tissue. This occurs in parallel to decline in epithelial barrier function and dysbiosis of the intestinal microbiota. Epigenetic changes have been described as a hallmark of ageing and we are exploring how transcriptional regulation and chromatin states change with age in the intestinal stem cells. Based on published transcriptomic data we have identified a number of transcription factors whose expression changes in ageing intestine. We have used enhancer traps to characterise the expression patterns of these transcription factors in normal homeostasis and in the ageing intestine, validating age-related changes in expression. We are currently using stem/progenitor-specific knockdown and overexpression to explore their functions in normal homeostasis. Future work will use targeted DamID to identify the downstream transcriptional targets of these factors in intestinal stem cells.

Keywords: Intestinal stem cells, Chromatin, Transcription factors, drosophila

*Speaker

Two distinct waves of transcriptome and translome remodelling drive germline stem cell differentiation

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Adult stem cells divide repeatedly, replenishing the stem cell population while producing daughter cells that undergo a change in fate and differentiate to specific cell types. The process of stem cell differentiation is complex and yet reproducible, relying on the tight control of gene expression. However, a full understanding of the gene expression changes driving fate transitions has been hindered because stem cells are sparsely distributed in tissues. Here, we have overcome these technical limitations by establishing a protocol to synchronise *Drosophila* female germline stem cell differentiation *in vivo*, using mutation of *bag-of-marbles* (*bam*) to accumulate undifferentiated GSC-like cells followed by a pulse of Bam protein driving synchronous entry into differentiation. We performed RNA-seq, Ribo-seq and mass spectrometry at high temporal resolution to produce an unbiased, genome-wide road map of gene expression during differentiation. While the data uncovers the extensive remodelling of both the transcriptome and translome, we observed 3-fold more changes in translation compared to mRNA level. Furthermore, changes in mRNA level were frequently buffered by changing translation efficiency to stabilise the final rate of translation. Contrary to the expected cumulative changes, following gene expression throughout differentiation reveals two distinct waves of remodelling at both the transcriptome and translome level. The early wave upregulates cell cycle genes and down-regulates translation-related genes, while the late wave upregulates genes involved in terminal differentiation (oogenesis and meiosis). Altogether, our data provide a systematic and genome-wide roadmap of the changes in gene expression occurring during the differentiation process *in vivo*.

Keywords: Differentiation, germline, stem cell, translation efficiency, ovary, transcriptomics

*Speaker

Stem cells & regeneration

A stem cell activation state coupling social interactions with spermatogenesis in *Drosophila* males

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Reproduction is paramount for organisms. To be successful, animals need to coordinate the production of gametes, the integration of social cues, triggering specific behaviors and mobilizing energy reserves. How social interactions impact gametogenesis? Does the activity of the gonads modify the animal behavior and physiology? How is this inter-organ communication achieved? While we have insights about this coordination in females, its existence in males remains largely unknown. By using *Drosophila* as a model and new approaches for behavioral study, we are uncovering a novel mechanism by which the presence of females triggers an activation state on both the germline stem cells (GSCs) and the cyst stem cells (CySCs) of the testis, boosting the spermatogenesis. This activation does not require physical interaction with the female – it relies on pheromonal communication. We have identified the inter-organ signaling network responsible of it, based on TNF α /Eiger secreted by the visceral muscle of the testis, as well as neuronal-secreted Octopamine. These signals trigger respectively the JNK pathway activation and Calcium signaling on the CySCs. Overall, our work represents a prime example of how social behaviors affect animal physiology and, to my knowledge, it is the first description of the behavior-gametogenesis coordination in male reproductive biology.

Keywords: Stem cells, germline stem cells, cyst stem cells, spermatogenesis, testis, reproduction, social behavior, interorgan communication, JNK, Octopamine

*Speaker

Caspar is a maternal effect gene that regulates primordial germ cell fate

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Early embryonic development in metazoans is regulated by maternal factors deposited in the embryo during oogenesis. In this study, we explore maternal roles for Caspar (Casp), the *Drosophila* orthologue of human Fas-associated factor-1 (FAF1), and previously implicated in host-defense as a negative regulator of IMD/ NFB signaling. We discover that Casp activity regulates primordial germ cell (PGC) fate during blastula formation, with the number of PGCs correlating with Casplevels. Interestingly, maternal loss of function of Transitional endoplasmic reticulum 94 (TER94), a physical interactor of Casp (Tendulkar et al., 2022), and a hexameric AAA-ATPase protein involved in the proteasomal degradation of embryonic proteins, also leads to the reduction of PGCs. Casp contains the following interaction domains, the N-terminal Ubiquitin-associated (UBA), the central lipid binding UAS domain, the TER94 interacting C-terminal Ubiquitin-like regulatory X (UBX), and a short FFAT (two phenylalanine's in an acidic tract) motif that interacts with the ER-resident VAMP associated protein B (VAPB). Maternal expression of Casp with domain deletion, namely Casp(delUBA), Casp(delUBX), Casp(delUAS), and Casp(delUASdelUBX) have proven informative in terms of Caspar function in the determination of cellular identity in PGCs. Based on our data from the embryo and the previously uncovered VAPB:Casp:TER94 complex (Tendulkar et al., 2022) that includes components of the ER-associated degradation complex, we hypothesize that Casp and TER94 work in the PGCs to clear maternal proteins in the PGCs, as part of the Maternal to Zygotic transition.

Keywords: Caspar, Primordial germ cell, TER94

^{*}Speaker

Characterizing the role of Jelly Belly in the gut

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Organ size homeostasis is a balance between cell loss and renewal occurring throughout the life of an organism. In adult tissues, stem cell proliferation is tightly controlled and coordinated with cell fate choices to promote tissue homeostasis. The adult gut is a suitable model to study the mechanisms regulating epithelial homeostasis due to its high turnover rate. In the *Drosophila* model, intestinal stem cell (ISC) activity is controlled by local signals released from the ISC niche composed of enteroblasts (EBs), enterocytes (ECs), enteroendocrine cells (EECs), and visceral muscles (VM). To identify niche-secreted peptides involved in gut homeostasis, a cell-specific loss-of-function screen was performed. Among the candidate genes, Jelly belly (Jeb), a ligand known to interact with Anaplastic lymphoma kinase (Alk), was identified as required in ECs to maintain epithelial integrity. Here, we show that Jeb restricts ISC divisions and EEC numbers in homeostatic conditions. Furthermore, EC-specific knockdown of Jeb in ECs causes defective CCR anatomy and physiological function. All together, these results support the hypothesis that Jeb promotes gut homeostasis by maintaining EE numbers and CCR function.

Keywords: *Drosophila*, gut homeostasis, stem cells, proliferation

*Speaker

Choices between symmetric and asymmetric cell division of progenitors contribute to the regional diversity of enteroendocrine cells in *Drosophila*

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Enteroendocrine cells (EEs) in metazoans are scattered along the digestive tract, where they sense various environmental stimuli and secrete neuropeptides to regulate diverse physiological processes. To fulfil these functions, EEs are specified into multiple subtypes that occupy specific gut regions, which is frequently reflective of regional adaptability to specific biological functions. Thus, exploring how the regional diversity of EE subtypes is established will provide insight into the understanding of gut segmentation and functional specialization. EEs in adult *Drosophila* midgut are scattered along the length of midgut, which is morphologically and functionally divided into R1-R5 subregions along the anterior-posterior axis. They are generated from intestinal stem cells (ISCs) via a transient progenitor stage termed EE-progenitors (EEPs). Each EEP undergoes exactly one round of mitosis before terminal differentiation to yield a pair of EEs. Previous studies revealed that the division of EEPs is asymmetric and the resultant two EE daughter cells are commonly found to respectively express AstC and Tk, which mark class I and class II EEs, respectively. We have recently identified a group of class III EEs, characterized by lacking both Tk or AstC expression, which are distributed specifically in the R2 region. To understand how these three major EE classes are derived from progenitor cells, here we carefully analysed their regional distribution pattern and determined how they are specified in the EE pairs. We found that the class III EEs are paired with TK+ class II EEs during their differentiation from EEPs. Surprisingly, in some specific gut regions including R1 and R4b, EEPs appear to undergo symmetric cell division to yield identical EE pairs. Although Notch is essential for the specification of Tk+ class II EEs but not AstC+ class I or class III EEs, the involvement of Notch or not, could not fully explain the decision of symmetric or asymmetric EEP division. Therefore, we concluded that EEPs could divide symmetrically or asymmetrically, and there are Notch-dependent and independent mechanisms to guide the decision between symmetric versus asymmetric cell divisions, which contribute to EE regional diversity along the length of the midgut.

Keywords: enteroendocrine cell, regional diversity, intestine, asymmetric cell division, symmetric cell division, Notch

*Speaker

Deciphering Wnt-EGFR signalling network: Tissue specific active Wnt response in adult Malpighian tubules

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Adult tissues are exposed to constant internal and external insults which lead to loss of cells. Stem cells in such tissues play a critical role in replacing these lost cells through division and differentiation to maintain homeostasis. This process of division and differentiation is governed by a complex yet delicate signalling network, which, when disrupted, can lead to aberrant stem cell phenotypes such as cancer.

In humans, colorectal cancer is initiated by loss of function mutation in the APC gene (Wnt signalling), followed by secondary mutations in genes such as K-Ras (EGFR signalling). Active Wnt and EGFR signalling pathways interact and fuel carcinoma formation, leading to a poor prognosis. While both Wnt and EGFR pathways are well studied individually, little is known about the factors involved in the Wnt-EGFR signalling network.

To study the factors involved in the Wnt-EGFR signalling network, we generated a single fly line that contains: APC RNAi, RasV12 and a Wnt reporter together with the stem cell driver. We observed that driving APC RNAi and RasV12 in esg positive intestinal (ISC) and renal stem cells (RNSC) leads to massive tumor formation. Interestingly, tumors in the malpighian tubules but not the midgut showed high wnt reporter activity, indicating a tissue specific Wnt signalling response. By applying a sequencing and screening strategy, our aim is to identify genes that influence the Wnt-EGFR signalling network in the Malpighian tubules.

Keywords: Wnt signaling, EGFR/Ras signaling, Malpighian tubule, Renal stem cells, Midgut, Intestinal stem cells, Cancer

*Speaker

Decoding the transcriptional dynamics of muscle stem cells response to Notch signaling

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The regeneration of skeletal muscles relies on muscle stem cells (MuSCs). Under normal circumstances, these cells remain quiescent. However, in response to muscle injury, they are activated, proliferate, and migrate to the damaged muscle. Throughout this intricate process, the fate of MuSCs is regulated by the Notch signalling pathway. Despite being a seemingly simple pathway, it is intriguing to consider how Notch regulates and coordinates all these steps. Our hypothesis is that the transcriptional response to Notch might dictate the different cell fate decisions. To address this hypothesis, we aim to analyze the *Drosophila* MuSCs transcriptional response to Notch pathway. Specifically, we aim to monitor and quantify the transcriptional response to Notch, in real time, using the MS2-MCP method. We generated new reporter fly line in which the MS2 stem-loops are inserted downstream of the Notch responsive element. We first showed, in fixed tissues, by single molecule FISH that the NRE-MS2 reporter are specifically expressed in the MuSCs. We also validated that the NRE-MS2 reporter responds correctly to Notch over-activation. Finally, we expressed a MCP-GFP in the MuSCs population and showed that the MCP protein localizes at the transcriptional foci. Together these data validated that our newly generated transcriptional reporter is expressed in the MuSCs and respond to an increased activity of the Notch signaling pathway. We will use this reporter to decipher whether and how the transcriptional response to Notch guides the cell fate decision of the MuSCs during muscle development and repair.

Keywords: Muscle Stem Cells, Notch, Regeneration

^{*}Speaker

Dual role for the orthologue of HECA, Headcase, in blood cell progenitor maintenance in the *Drosophila* lymph gland

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Since signaling pathways controlling blood cell differentiation show high levels of conservation, *Drosophila melanogaster* is a widely used model to research hematopoiesis. In the primary lobes of the hematopoietic organ of the *Drosophila* larva, the lymph gland, cells are classified into 3 main zones based on cell-surface markers and function. This includes the medullary zone (MZ), containing hemocyte (blood cell) progenitors, the cortical zone (CZ), harboring differentiated hemocytes, and the posterior signaling centre (PSC), a stem cell niche that maintains the undifferentiated state of the progenitors through its regulatory function. We previously described Headcase (Hdc), the orthologue of the HECA human tumor suppressor as a factor that plays a non-cell-autonomous role in the niche to suppress premature differentiation of the progenitors. *hdc* loss-of-function in the niche causes the differentiation of special effector blood cells, lamellocytes, which normally appear only after immune challenges. We found that lamellocyte differentiation in response to *hdc* depletion is caused by continuous activation of the insulin/mTOR pathway, which in turn results in higher levels of reactive oxygen species (ROS) in the hematopoietic niche that triggers premature progenitor differentiation. In addition, we also found that Hdc functions cell-autonomously in the MZ progenitors to limit their differentiation by acting upstream to JNK, Toll and EGFR pathways. Interestingly, similar to the niche, silencing *hdc* in the MZ leads to the elevation of ROS levels, further highlighting the importance of ROS as a stem cell maintenance regulator. We hope our findings will lead to a better understanding of how hemocyte progenitor differentiation is regulated in *Drosophila*, which in turn will give us more insight into how hematopoietic stem cells (HSCs) are maintained in mammals. Funding: This work was supported by the National Research, Development

*Speaker

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Keywords: Drosophila, Headcase, HSCs, lymph gland, ROS

Epigenetic control of muscle homeostasis and repair through the histone demethylase Lsd1

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Skeletal muscles are responsible for body motion and are composed of syncytial myofibers that can be damaged at various degree during exercising or upon severe injuries. However, myofibers are terminally differentiated cells that cannot self-renew. The maintenance of muscle homeostasis relies on a population of muscle stem cells (MuSCs) also called satellite cells that are essentially quiescent. Upon tissue damages, MuSCs get activated and engage in a wave of cell proliferation by symmetric divisions, followed by asymmetric divisions to give rise to differentiating myoblasts that will ultimately fuse and repair damaged fibers. Many studies have established the role of signaling pathways and transcription factors in the control of MuSC quiescence, proliferation and self-renewing properties. However, a current challenge is to gain further insights into how epigenetic programs act to control MuSC activation. We address this question making use of *Drosophila* that has been recently proven to offer unprecedented in vivo access to study MuSCs regulation. Starting from a candidate-based screen approach, we identified the histone demethylase Lsd1, also known as Su(var)3-3, as a promising hit. We found that Lsd1 is expressed in the flight muscle progenitors during larval wing imaginal disc development while its expression is restricted to MuSCs in adult indirect flight muscles (IFM). Detailed analysis of *lsd1* mutant animals showed a premature differentiation of larval muscle progenitors. Consistently, the adult flies display a penetrant flight phenotype, suggesting a role of Lsd1 in IFM function. Thus, we coupled lineage tracing experiments (ReDDM) and MuSC specific *lsd1* loss-of-function at adult stage and found that Lsd1 regulates both MuSC activation and contribution to the muscle. Altogether, our results reveal a key role for Lsd1 in muscle development and homeostasis and help to gain insight in epigenetic regulation of muscle biology.

Keywords: Muscle biology, stem cell, tissue homeostasis, regeneration

*Speaker

FSC-EC stem cell-niche dynamic and the importance of cell-cell adhesion in this process in the *Drosophila* ovary

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The follicle stem cells (FSCs), and their niche which is provided by escort cells (ECs) in the *Drosophila* ovary are a useful model to study epithelial stem cell biology. Traditionally, the interaction of stem cells and their niche was viewed as static. However, our live imaging data suggests that the stem cell-niche interaction of FSCs and ECs is highly dynamic. Cells located at the FSC niche region are the most motile cells of the germarium. We found that the movement of FSCs and ECs depends on actin cytoskeleton, as the inhibition of actin polymerization by Latrunculin A treatment leads to a loss of FSCs and ECs motility. Further, the downregulation of actin and actin-interacting genes leads to severe phenotypes with loss of the follicle epithelium and fusion of several egg chambers. FSCs display a lateral movement, where one FSC moves from one side of the germarium towards the other side. Our data suggests that this lateral movement plays a role in separating and budding of cysts in the germarium. Through an RNAi screen, we found that knock-down of several septate junction components in the FSCs leads to severe phenotypes with cysts accumulation before the 2a/b boarder and stalk formation defects. Together, our data suggest that stem cell dynamics plays important biological roles and cell adhesion components may be vital to allow niche adhesion to cope with continued positional changes of the stem cells.

Keywords: stem cell, niche, actin cytoskeleton, cell, cell adhesion, septate junctions

*Speaker

Integrated stress response in intestinal stem cells – molecular mechanisms and role in intestinal homeostasis

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The intestinal epithelium provides a selective boundary between the animal and its environment, allowing absorption of nutrients while protecting other tissues from pathogens and toxins. It consists of differentiated cell types, such as absorptive enterocytes and hormone-secreting enteroendocrine cells, and their self-renewal is orchestrated by intestinal stem cells (ISCs). ISC functions are regulated by environmental factors, such as nutrition, as well as intrinsic signaling networks that aim to keep intestinal epithelium under homeostasis. How externally and intrinsically induced molecular responses influence ISCs, remain incompletely understood.

Cellular protein homeostasis, proteostasis, can be lost under several conditions, such as upon amino acid imbalance or aging. Loss of cellular proteostasis activates stress response pathways, including the integrated stress response (ISR). Activation of ISR leads to phosphorylation of eukaryotic initiation factor 2- α (eIF2 α), which inhibits translation of most proteins, thus relieving the risk of impaired proteostasis. ISR also activates transcription factor ATF4, which restores the cellular homeostasis activating stress response genes. Since the activity of translation is a key driver of cell growth, activation of ISR in somatic cells leads to inhibition of growth.

Unexpectedly, we have discovered that phosphorylation of eIF2 α levels is elevated during normal ISC differentiation into absorptive enterocyte fate. Furthermore, loss of proteostasis in ISCs, either genetically or pharmacologically, leads to dysplastic phenotype with hyperproliferation and increased enterocyte differentiated of ISCs. We hypothesize that this ISR mediated signaling network would be a double-edged sword, allowing maintenance of ISC homeostasis when the frequency of impaired proteostasis is low, but leading to uncontrolled differentiation and dysplasia when too prevalent.

Collectively, this work is expected to uncover a new mechanism involved in dynamic control of intestinal turnover and homeostasis.

Keywords: stem cell, intestinal stem cell, metabolism, regeneration, cell differentiation, homeosta-

*Speaker

sis, proteostasis, integrated stress response

Mitochondrial and metabolic adaptations of stem cells and their niche during intestinal regeneration

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Stem cell proliferation represents an indispensable process to rebuild intestinal homeostasis following damage. However, little is known about how intestinal stem cells (ISC) adapt and respond to the substantial metabolic needs for acute cell proliferation and differentiation in the regenerating intestine. We have used RNA sequencing and metabolomics of FACS-sorted *Drosophila* ISCs and enteroblasts (EBs), coupled with live imaging and functional genetics studies, to identify metabolic adaptations necessary to drive regenerative stem cell proliferation in the adult intestine. Interestingly, we have identified mitochondrial and metabolic adaptations of EBs -a stem cell progeny and essential component of the ISC niche- that are non-autonomously required for ISC proliferation and tissue repair following damage. We are currently performing further studies to fully uncover the mechanisms underlying this metabolically supportive function of EBs in intestinal regeneration. Our study highlights the potential of targeting the epithelial stem cell niche metabolism for regenerative and anti-tumour purposes.

Keywords: metabolic adaptations, mitochondria, stem cell, EB, regeneration, gut

*Speaker

RNA Methyltransferase complex, Wdr4-Mettl1, Maintains Steady State Ribosomal Biogenesis in Intestinal Stem Cells via let-7 miRNA-TOR regulatory axis

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The WD repeat protein 4 (Wdr4) is highly conserved and known to control the self-renewal and differentiation capacity of mouse embryonic and fly germline stem cells via protein interactions. However, its role in somatic stem cells remains unknown. Here, we report that Wdr4 maintains the expression of *let-7* miRNA, which maintains steady-state ribosomal biogenesis in *Drosophila* intestinal stem cells (ISCs) via suppression of TOR signaling. Depletion of Wdr4 leads to reduced expression of *let-7*, resulting in the activation of TOR, which overrides ribosomal biogenesis and elevates ROS-JNK signaling. Consequently, high JNK signaling promotes ISC proliferation and ISC misdifferentiation, mimicking aging gut phenotypes, and shortening lifespan. We also report that the depletion of Wdr4 partner, Methyltransferase-like protein 1 (Mettl1, an RNA methyltransferase) phenocopies Wdr4-depleted intestine. In addition, both Wdr4 and Mettl1 depletion reduce the level of m7G modification, which has previously been shown to enhance *let-7* stability *in vitro*. Furthermore, human WDR4 is able to replace fly Wdr4 to maintain ISC homeostasis. Our findings shed light on the novel and conserved role of the RNA methyltransferase complex in miRNA regulation for intestinal integrity.

Keywords: WDR4, tRNA methyltransferase, ISC, Ribosomal Biogenesis

*Speaker

Regulation of adult muscle progenitor fate by the epigenetic enzyme Tet

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The development and the function of the muscular system require a tight regulation of gene expression, which notably involves several epigenetic actors. Enzymes of the TET family are key players in the epigenetic regulation of gene expression but their role in myogenesis is poorly characterized. In mammals, TET enzymes catalyze the oxidation of methylated cytosines on DNA (5mC). This process of active demethylation has been shown to regulate gene expression during muscle differentiation in mice. In *Drosophila* there is only one *Tet* gene but its genome does not code for DNA methyltransferases and lacks 5mC modifications. Hence, *Drosophila* represents a unique model to investigate Tet non-canonical mode of action notably during muscle development.

Using a *Tet-GFP* knock-in line, we showed that Tet is expressed in adult muscle precursors (AMPs) and in their epithelial niche in the larval wing disc. We also found that *tet null/null* flies exhibit a reduced number of AMPs in the larvae. In contrast, in a *tet* mutant lacking catalytic activity context, the number of AMPs was normal, suggesting that Tet controls AMP development in an enzymatic-independent manner. Interestingly too, knocking-down *tet* expression in the AMPs or in their epithelial niche was sufficient to cause flight defects in the adult, suggesting that Tet expression is required in both tissues to control muscle development.

To better understand the role and mode of action of Tet in the epithelial niche and AMPs, we profiled Tet chromatin binding localisation in both cell types using the NanoDam technique. In parallel, to define the impact of Tet on gene expression in the AMPs and the niche, a scRNA-seq has been performed in *tet null/null* context. Our preliminary results show that in the AMPs, Tet binds in majority to intronic regions and is recruited to 40% of AMP identity genes, suggesting that it plays a role in controlling AMP fate. The results of these ongoing analyses will be presented and discussed.

This project will allow to understand the molecular function(s) of Tet in muscle development and bring further information concerning the regulatory interactions between the AMPs and their developmental niche.

Keywords: Epigenetic enzyme, Tet, Myogenesis, Adult muscle precursor, Epithelial niche

*Speaker

Senescence Behaviors in *Drosophila* tissue repair

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The exposed position of epithelia makes them susceptible to injury and damage. Around damaged tissue, different cell populations with their distinct cell behaviors arise. These cell populations coordinate their cell behaviors by interacting with stress signaling pathways. Eventually, spatial and temporal control of these interactions restores tissue integrity. In *Drosophila* wing imaginal discs, the stress response pathway JNK coordinates all these processes. Upon tissue damage, high JNK signaling activity at the wound edge induces a wound organizer cell population characterized by a transient cell cycle arrest at the G2 phase, further triggering senescent behaviors. This population also exhibits striking changes in its nuclear dynamics. It is unclear, however, how the cells in this population adjust these dynamics to respond to the tissue damage and whether senescent behaviors directly mediate these necessary responses. Therefore, we initially described the nuclear features in the wound organizer cell population by using the single-cell RNA-sequencing on TNF- α /eiger expressing *Drosophila* wing imaginal discs. Our results suggest that wound organizer cell populations have differential expression patterns of the genes encoding nuclear envelope components, High mobility group (Hmg) proteins, and histone acetyltransferases *nejire* and *Gcn5*. Interestingly, we also observed some of these features in G2-arrested cells during normal wing disc development. In light of these observations, we now characterize nuclear features that may underlie the reprogramming of senescent cell populations but may arise either from cell cycle arrest or from stress signaling. Our findings will provide a molecular characterization and tissue-level integration of nuclear adaptations in tissue repair and regeneration.

Keywords: tissue repair, regeneration, senescence, G2 arrest, JNK

*Speaker

Signalling Dynamics in Intestinal Stem Cell Fate

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Precise regulation of cell fate in time and space is critical for normal development and adult homeostasis. Genetic, developmental biology and biochemical approaches have identified many of the signalling pathways involved in regulating specific processes and revealed that the same pathways are used repeatedly to generate different outcomes in different contexts. Differential chromatin states, expression of pathway regulators, crosstalk with other pathways and signal strength have all been shown to play important roles in outcomes. However, the importance of temporal properties of a signal have been much less well studied in *in vivo* contexts. *Drosophila* intestinal stem cells are an excellent model system for exploring dynamics as they are amenable to live intravital imaging followed by long-term cell fate tracking, and are known to be regulated by conserved developmental signalling pathways including Ras/MAPK, JAK/STAT and Notch. We are using intravital imaging of fluorescent signalling reporters to explore the importance of signalling dynamics in normal stem cell homeostasis and in response to challenges such as damage and ageing.

Keywords: stem cells, niche, signalling, dynamics

*Speaker

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Spermatogenesis and phagoptosis are both mediated by one cyst cell

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In flies and mammals, somatic and germline cells co-differentiate from the stem cell stage throughout spermatogenesis within ‘cyst’ units. While these units are essential for proper stem cell maintenance and germ cell differentiation, their intra-cyst organization and interactions with neighboring cysts, are unknown. We report that in the *Drosophila* testis, the intra-cyst organization involves only one somatic cyst cell with remarkable stretching capabilities that enable it to wrap around the dividing interconnected germ cell progenitors. Furthermore, this single cyst cell has a dual function in supporting intra-cyst progenitors and extending a noose-like projection to kill distant cysts. Our findings resolve the conflicting function of somatic cells as supporters required for germline differentiation and as phagocytes that induce germ cell death. In addition, we show for the first time a 3D structure of a dual stem cell niche, presenting a new model of 1:1 ratio between the soma and the germline.

Keywords: Germline stem cells, spermatogenesis, cyst cells, phagoptosis

*Speaker

The RNA binding protein Brat regulates intestinal stem cell proliferation and differentiation in *Drosophila*

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The intestinal epithelium is a crucial barrier that enables individuals to respond to the external environment. Intestinal stem cells (ISCs) renew themselves and give rise to terminally differentiated cells to maintain the basic functions of the gut. It is of great significance to understand the regulatory mechanisms underlying the proliferation and differentiation of ISCs. Despite the important tumor suppressor role of the RNA binding protein Brat (Brain tumor) in many developmental stages, we know little about its function in *Drosophila* intestinal stem cell lineage. Here, we conducted screening and genetic experiments and discovered that *Brat* is a positive regulator of proliferation in ISC lineage. We found that *Brat* is expressed in ISCs, progenitor cells along with EEs (Enteroendocrines). Depletion of *Brat* in stem cells dampened their proliferation in both normal and injury/stressed conditions, while overexpression of *Brat* induced stem cell hyper-proliferation and generated EEs. These findings indicate that *Brat* act as a positive regulator of both ISC proliferation and EE differentiation from ISCs. In addition, we have demonstrated that the function of Brat in the ISC is dependent on its NHL domain, which can directly bind to target mRNAs. Mechanistically, our preliminary results suggest that *Brat* promotes ISC proliferation by regulating the JAK/STAT signaling pathway. In summary, we have discovered a novel role of *Brat* that promotes the proliferation of ISCs in *Drosophila*. Our ongoing investigation of the underlying mechanisms will provide new insights into understanding the process of intestinal stem cell self-renewal and differentiation in *Drosophila*, and potentially in mammals as well.

Keywords: Intestinal stem cell, Brat, Translational regulator, JAK/STAT

*Speaker

The role of Toll signalling in midgut epithelial stem cell proliferation

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The *Drosophila* midgut is known as the functional equivalent of the mammalian small intestine. It is composed of intestinal stem cells (ISC), enteroblasts (EB), hormone-producing enteroendocrine cells (EE) and enterocytes (EC). The latter forms the absorbent cells of the midgut. Previous studies from the lab have shown that loss of signalling from the immune pathway Toll, reduces the density of cultivable commensal bacteria, leading to a loss of *Lactobacteriaceae*. This was due to the loss of lipid catabolism that Toll regulated through the 4EBP regulator of translation. Here we further study the contribution of Toll in integrating host defence with intestinal metabolism and epithelial renewal. We report that knock-down of the Toll receptor or the NF-B homologue *dif* downstream of Toll in progenitor cells (ISCs and EBs), results in a significant reduction of ISC proliferation. Conversely, constitutively active Toll signalling in progenitor cells results in an increase of progenitor cells, which is accompanied by more densely populated cultivable commensal intestinal bacteria. Increase in progenitor cells resulted in more EEs and ECs, indicating that ISC proliferation went "all the way" and was not blocked at the EB stage. Toll activity triggered JNK-dependent transcription. This was verified by using the JNK transcriptional target *puckered* and a dominant negative form of JNK (*bskDN*), which blocked progenitor cell expansion. Upstream of Toll, we found that Notch and Toll have antagonistic activities. RNAi of Notch in progenitor cells leads to an increase in ISC, which is blocked by Dif RNAi. Our results place Toll as the signalling element to integrate epithelial renewal, immune response and commensal bacteriome preservation at the intestinal interphase.

Keywords: Toll, Immunity, gut, stem cell, epithelial renewal, bacteriome, JNK, Notch

*Speaker

The roles of SPARC and PLOD in *Drosophila* intestinal stem cell homeostasis

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Epithelia are constantly turned over as cells are lost from the surface and replaced by the proliferation of stem cells. Epithelial stem cells must be tightly regulated to maintain homeostasis and prevent over-proliferation. The intestinal stem cells of the *Drosophila* midgut are an ideal model system to identify regulators of intestinal stem fate, and study their function and regulation. We have identified PLOD and SPARC, regulators of collagen IV secretion and extracellular distribution respectively, as candidate regulators of the *Drosophila* intestinal stem cells. We are using RNAi knockdown and overexpression to explore their effects on intestinal stem cell proliferation and homeostasis. Initial results suggest that changes in SPARC and PLOD expression affect intestinal stem cell regulation and tissue structure in the *Drosophila* intestine. Both SPARC and PLOD are highly conserved in metazoans, raising the possibility of a conserved role in regulating intestinal stem cells.

Keywords: intestinal stem cells, homeostasis, SPARC, PLOD, collagen

*Speaker

The transcription factor Shavenbaby/OvoL ; a new regulator of adult muscle stem cells

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To compensate for organs damage and cell death, adult homeostasis requires organ regeneration mediated by long-lived stem cells. In young healthy individual, skeletal muscles are regenerated by the activity of resident muscle stem cells (MuSCs), which are quiescent and, upon injury, become activated and differentiate into myoblasts to repair the muscle. While MuSC differentiation starts to be understood, little is known about the molecular mechanisms that regulate the early steps of their activation. To gain further insight into the regulatory networks controlling MuSCs activation, we performed a gene candidate screen, based on a transcriptomic dataset of developing *Drosophila* flight muscles (*Spletter et al., 2018*). We identified the transcription factor Shavenbaby (TF Svb/OvoL) as a new potential MuSCs regulator. We found that Svb is expressed in larval muscle progenitors, and maintained in adult MuSCs. Consistently, we identified a new *svb(MuSC)-Gal4* enhancer that controls its expression in the muscle progenitors and adult MuSCs. Using this enhancer, we performed lineage tracing experiments (ReDDM, *Antonello et al., 2015*) and found that the adult Svb-positive MuSCs are able to proliferate and generate differentiated myoblasts. Importantly, we found that *svb* loss of function in adult MuSCs, leads to a significant increase in both the number of MuSCs and the rate of myoblasts production, indicating that *svb* controls MuSCs activation. Moreover, we observed that the high number of MuSCs and their progeny's location coincides with a muscle deformation at the attachment sites. Based on these findings, we hypothesize that the high production of muscle progeny leads to a muscle deformation characterized by a muscle detachment from the myotendinous junctions. We performed viability tests and found a significant decreases of fly survival in *svb* loss of function condition, and suggest that the muscle detachment might lead to premature adult lethality. Altogether, these data indicate that Svb controls MuSCs activation to finely regulate muscle homeostasis. However, how and when Svb acts to adjust MuSCs activation is currently under investigation. At the 27th EDRC we will present our recent unpublished data on the functional analysis of Svb/OvoL in MuSCs activation and muscle repair.

Keywords: Muscle, Stem cell, Activation, Regeneration, Transcription factor

*Speaker

The transmembrane proteoglycan Syndecan is essential for intestinal stem cell maintenance and division

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The stem cell niche is a complex microenvironment which regulates stem cell behaviour through secreted, cell-cell contact and mechanical cues. Understanding how these signals are integrated *in vivo* is imperative for elucidating how stem cell division is exquisitely tuned to meet tissue demand during homeostasis and regeneration, and how this goes awry in disease states. Using the adult *Drosophila* midgut as a model system to investigate stem cell behaviour *in vivo*, we have newly identified Syndecan as an essential regulator of intestinal tissue renewal and stem cell maintenance. Syndecans are transmembrane heparan sulfate proteoglycans, which can act as growth factor and extracellular matrix receptors, and whose dysregulation is implicated in multiple human diseases, including cancer. We have found that Syndecan is enriched in intestinal stem cells and when depleted of Syndecan, these cells are lost from the epithelium, with a corresponding failure in new cell production. Our results suggest that Syndecan has a negligible contribution to growth factor signalling pathway regulation. Instead, multiple lines of evidence suggest that Syndecan is required for correct cell division, in this and other stem cell contexts, and that loss of Syndecan leads to defects in nuclear shape and nuclear envelop/lamina remodelling. Our ongoing work seeks to establish whether this role of Syndecan depends on adhesion to the niche and/or on intracellular signalling via protein interactions with Syndecan's highly conserved cytoplasmic domain. Our work enhances understanding of stem cell behaviour, and uncovering Syndecan's precise mode of action and its molecular partners may help provide a new framework to delineate its function in human disease.

Keywords: Stem cells, Intestine/midgut, Cell division, Proliferation, Nuclear lamina, Nuclear shape

*Speaker

Unraveling heterogeneity and dynamics of muscle stem cell niches through integration of single-cell transcriptomics and advanced imaging

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The size and heterogeneity of the muscle stem cell (MuSC) pool have a significant impact on tissue homeostasis, regenerative capacity, and the resistance of muscle tissue to external environmental factors. For healthy and disease-free muscle tissues throughout life, it is critical to understand the mechanisms that determine the size of the MuSC pool during development and its maintenance in adulthood, as well as the factors that contribute to its change. To this end, we combine single-cell and spatial transcriptomic methods with cell type-specific CRISPR gene editing and imaging approaches to assess both molecular and cellular phenotypes using *Drosophila* as powerful in vivo model. We have recently integrated available single-cell RNAseq datasets from the larval muscle stem cell niche of three different laboratories into a unified dataset, providing us with a spatially resolved gene expression map of muscle stem cell populations and their niche with greatly improved resolution. To enable rigorous quantitative analysis of MuSC populations, we have developed a sophisticated image-processing pipeline, encompassing a deep-learning based approach for precise cell segmentation and a state-of-the-art random-forest algorithm for accurate MuSC subpopulation classification. Leveraging these advancements, we have successfully quantified the entire pool sizes of MuSC subpopulations and extracted intricate cellular features from diverse MuSC subpopulations.

Together our data provides new insights into the biology of muscle stem cell niches during development resolving MuSC heterogeneity and pool dynamics. This research paves the way for future investigations aimed at deciphering the complex relationship between developmental processes and the functioning of adult MuSCs.

Keywords: muscle stem cell niche, single, cell transcriptomics, advanced imaging

*Speaker

Signalling

A novel fly model to measure hydrogen peroxide signalling during ageing

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As we continue to live longer, age-related diseases have become one of the greatest public health threats of this century. To combat these diseases, we need to better understand the mechanisms driving healthy ageing. Hydrogen peroxide (H₂O₂), which is produced in various cellular compartments, has emerged as a key player in the ageing process. When present in excess, H₂O₂ can cause damage to DNA, lipids and proteins. At lower levels, however, H₂O₂ is an important signalling molecule that can modulate protein functioning by reversibly modifying cysteine residues. This dual nature of H₂O₂ and the lack of precision tools in ageing organisms have complicated the interpretation of its role in the ageing process. While H₂O₂ dynamics indisputably change during ageing, we need a better mechanistic insight into its contribution to the ageing process. The fruit fly *Drosophila melanogaster* has proven to be an invaluable and popular model in ageing research. Many ground-breaking discoveries regarding genetic and metabolic drivers of longevity have been done in fruit flies. Interestingly, a new ultrafast and ultrasensitive genetic H₂O₂ probe, called HyPer7, was recently developed. This development has prompted us to generate a novel redox fly model by genetically integrating HyPer7. We will use this fly model to measure H₂O₂ signalling across specific tissues and cellular compartments of ageing flies. Ultimately, this could offer valuable insights into the underlying molecular processes of ageing and potentially identify novel targets to promote healthy ageing.

Keywords: Ageing, Hydrogen peroxide, H₂O₂, HyPer7, Redox, Redox signalling, Longevity, Genetically encoded probes

*Speaker

A novel mechanosensor signaling pathway during *Drosophila* cellularization: Filamin and Drak kinase

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Cytoskeletal regulation is fundamental to eukaryotic cell shape changes and tissue morphogenesis. The basis of the actin cytoskeletal network is formed by actin and myosin, which together can relay forces to the cell and the surrounding tissues. In non-muscle tissues myosin is regulated by phosphorylation via various kinases such as Rho-associated protein kinases (ROCKs) and death-associated protein kinases (DAPKs). It is known that actomyosin network organization and contraction is mechanically regulated. This means that the network can sense forces generated by itself or by the extracellular structures and adjust itself based on these forces. This phenomenon is called mechanosensor signaling. The molecular details of mechanosensor signaling, meaning how mechanical forces are converted to biochemical changes, are not completely understood. To study mechanosensor signaling, we have focused on the actin cross-linking protein Filamin, which is encoded by the *cheerio* in *Drosophila melanogaster*. Filamin has a mechanosensor region with force-exposed protein-binding sites. We find that the *Drosophila* DAPK homologue Drak binds to the open version of Filamin mechanosensor region. Drak is known to affect the actomyosin organization and myosin contraction during cellularization of *Drosophila*. We show that Drak localizes at the actin front during cellularization. Furthermore, we find that actomyosin ring perimeter contraction during cellularization is impaired in Filamin-closed mutants, similarly to Drak knockout flies. Our findings support Filamin's role in a new mechanosensor signaling pathway: We propose that forces within the actomyosin network expose the binding site in Filamin, facilitating Drak binding, and local phosphorylation of myosin regulatory light chain Spaghetti squash (Sqh). This provides a feed-forward loop to further actomyosin contraction. Clarifying the mechanosensory role of Filamin in tissue development offers new perspectives on cellular responses to mechanical stimuli and contributes to our understanding of mechanisms of human congenital filaminopathies.

Keywords: Filamin, Cheerio, Drak, Myosin, Sqh, Mechanosensor, Cellularization

*Speaker

Baiser/TMED10 regulates the early steps of Hedgehog secretion

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The final Hedgehog (Hh) gradient, and consequently its signaling strength depends not only on the level of the Hh receptor Patched on the surface of receiving cells, but also on additional factors. In Hh producing cells there are intracellular trafficking regulatory processes to ensure the place and amount of Hh secreted both apically and basally. These include the dynamin-dependent endocytosis of Hh, followed by apical or basal rerouting in a Rab4/Rab8-dependent manner. Finally, once Hh is extracted from the plasma membrane in a Dispatched-dependent manner, it can use various means of transportation, including multimers, cytonemes, lipoprotein particles and extracellular vesicles. How such mechanisms can work together, is still enigmatic and poorly understood. Our work, presented here, aims to understand the first steps of Hh/Shh secretion dynamics following Hh maturation in the endoplasmic reticulum. We show that inhibiting the function of Baiser/TMED10 of the p24 family of cargo receptors specifically affects the dynamics of Hh/Shh secretion from endoplasmic reticulum exit sites (ERES) to the cis-Golgi compartment. This leads to a temporal delay and consequently a depletion of Hh/Shh at the plasma membrane of producing cells and ultimately decreased Hh target gene expression levels in receiving tissue. Our data argues that in Hh primary secretion this specific Baiser/TMED10 function is one of the earliest step, when morphogen level can be regulated, with direct consequence on the establishment of the final morphogenetic gradient.

Keywords: Hedgehog, morphogen, trafficking, TMED10

*Speaker

Characterizing new mediators of cell competition

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Cell Competition is a conserved cellular phenomenon contributing to tissue development, homeostasis, and disease. Individual cells within a tissue or organ compete for survival and resources by constantly checking their status regarding metabolism, growth, or genetic identity to favour the strongest cells at the expense of the weakest. Interestingly, the deletion of tumour suppressor genes such as *scribble* or *discs large 1* makes the cells behave as losers in a heterotypic context. These data suggest that cell competition may be involved at multiple levels during tumorigenesis. Comparing mRNA levels in wild-type flies and flies inactivated for *scrib*, in which cell competition is activated, highlighted genes potentially involved in cell competition. We selected the most modulated genes in *scrib* mutant cells and performed a small-scale genetic screen through tissue-specific RNAi-mediated invalidation or overexpression to unveil new players of polarity loss cell competition. Surprisingly, one of our candidates, an uncharacterized factor up-regulated in *scrib* mutant cells, resembles the protein sequence of an already known cell competition mediator. The lab demonstrated that while our candidate cannot promote cell competition alone and is dispensable for normal development, it is indeed required for polarity-loss-mediated cell competition to implement the loser program. I further characterized this protein via mass spectrometry and biochemistry as a general stress protein.

Keywords: cell competition, stress response, cell death, cellular interactions

*Speaker

Dense-core granule biogenesis is regulated by a Rab6 to Rab11 transition, intraluminal vesicles and amyloidogenic proteins

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Dense-core granules (DCGs) are compact stores of signalling proteins that play an important role in cell-cell communication in neurons and specialised secretory cells such as insulin-secreting β -cells. Formed within dedicated secretory compartments, DCGs allow large numbers of signalling proteins to reversibly aggregate, often within amyloid-like structures which then dissipate following secretion. Notably however, aggregates fail to disassemble normally in diseases such as type 2 diabetes, a defect that may also be relevant to neurodegenerative diseases like Alzheimer's Disease, where secreted β -amyloid forms cytotoxic extracellular plaques.

Despite its fundamental importance, DCG biogenesis remains relatively poorly understood due to the difficulty of studying the nanoscale events involved. To address this, we utilised the recently developed *Drosophila* secondary cell (SC) system, which features highly enlarged secretory compartments labelled by Rab11, the conserved recycling endosome marker. These compartments produce enormous DCGs as well as clustered intraluminal vesicles (ILVs), which are later secreted as exosomes.

Using SC-specific knockdowns and ex vivo imaging, we demonstrate that SC DCGs are regulated similarly to mammalian DCGs, being controlled by the conserved DCG regulators Arf1 and the AP-1 complex. We also show that Arf1 and AP1 control a novel Rab6 to Rab11 transition at DCG precursor compartment membranes which triggers both DCG and ILV biogenesis. Uniquely, we follow this event with real-time imaging, finding that ILV biogenesis initiates just before DCG assembly and that the two processes are functionally linked. Furthermore, DCG aggregation is absolutely dependent on *mfas*, encoding the *Drosophila* homologue of TGF β -induced (TGFBI), the major human amyloid protein involved in corneal dystrophy. Other experiments reveal that proteins involved in familial Alzheimer's Disease also regulate DCG biogenesis in SCs, demonstrating a physiological link between secretory compartment membranes, ILVs and protein aggregation.

Our results provide novel insights into the fundamental biology underpinning DCG compartment biogenesis, and highlight the involvement of recycling endosomes, ILVs and amyloidogenic proteins in compartment maturation. They also showcase how secondary cells are uniquely suited

*Speaker

to model and investigate the highly conserved subcellular mechanisms underpinning regulated secretion in both health and disease.

Keywords: Dense, core granules, Amyloid, Rab GTPases, Endosomes, Secondary cells, Secretion, Exosomes

Drosophila macrophages control systemic cytokine levels in response to oxidative stress via a non-canonical DNA damage repair signaling cascade

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Environmental factors, infection, or injury, cause oxidative stress in diverse tissues, resulting in immune activation and loss of tissue homeostasis. Effective stress response cascades, conserved from invertebrates to mammals, ensure reestablishment of homeostasis and tissue repair. Plasmacytes, the *Drosophila* macrophage-like cells, are thought to respond to oxidative stress by immune activation; however, the signaling cascades involved in oxidative stress sensing and subsequent immune activation are yet to be defined. Furthermore, their role in modulating and controlling oxidative stress response to facilitate tissue repair and survival of the organism is not resolved. Here, we describe the responses of hemocytes in adult *Drosophila* to oxidative stress and the essential role of non-canonical DNA damage repair activity in direct "responder" hemocytes to control JNK-mediated stress signaling, systemic levels of the cytokine upd3 and subsequently susceptibility to oxidative stress. Our results point to an essential systemic role of hemocytes in controlling systemic oxidative stress response in *Drosophila*, including energy mobilization for potential tissue repair.

Keywords: Hemocytes, oxidative Stress, JNK signaling, single cell RNA, seq

*Speaker

Exploring Molecular Mechanisms of Premalignant Lesion Expansion and Transformation

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Accumulation of mutations can originate developmental defects and cancer. Normally, healthy normal cells identify and eliminate mutated cells. However, some dangerous and potentially cancerous cells can escape this control, survive and grow, giving rise to tumours. How premalignant cells manage to evade this surveillance mechanism is still poorly understood. Activating mutations in Ras genes are an early event in various cancers and are found in 25% of human cancers. Cells expressing oncogenic *Ras* (*Ras85DV12*) have been shown to behave differently depending on clonal size. In *Drosophila*, we have demonstrated that conditional activation of *Ras85DV12* in expanded clones leads to further clonal expansion by compressing and eliminating surrounding healthy cells. Alternatively, healthy cells can eliminate transformed cells when they are isolated. This differential cell behaviour resulting from *Ras85DV12*:healthy cells interaction makes it an excellent paradigm to identify molecular mechanisms controlling cell-cell communication in a premalignant microenvironment.

Our data confirm that changes in actomyosin cytoskeleton are detected in clones that grow from isolated *Ras85DV12* cells in *Drosophila* wing epithelium. Additionally, we show that alterations in extracellular matrix (ECM) and activation of epithelial-to-mesenchymal transition (EMT) factors are associated with those cells. These changes may contribute to *Ras85DV12* cells' survival by enabling them to escape from an unfavourable environment.

Our work will contribute to a broader understanding of the relevance of the epithelial microenvironment in tumour development and the quality control mechanisms used by healthy cells to maintain epithelial homeostasis, preventing premalignant lesions expansion and transformation.

Keywords: cell:cell communication, premalignant microenvironment, actomyosin cytoskeleton, extracellular matrix, epithelial to mesenchymal transition

*Speaker

Fat body glycolysis defects inhibit mTOR and promote distant muscle disorganization through TNF-alpha/egr and ImpL2 signaling in *Drosophila* larvae

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The fat body in *Drosophila* larvae serves as a reserve tissue and participates, through its endocrine function, in the regulation of organismal growth and homeostasis. To better understand its role in growth coordination, we induced severe fat body atrophy by knocking down in adipose cells several key enzymes of the glycolytic pathway. Our results show that impairing the last steps of glycolysis led to a drastic shrinkage in adipose cell size and lipid droplets content, and a downregulation of the mTOR pathway. Strikingly, fat body atrophy resulted in the distant disorganization of body wall muscles and the release of muscle-specific proteins in the hemolymph. Molecularly we showed that REPTOR activity was required for fat body atrophy downstream of glycolysis inhibition, and that the effect of fat body atrophy on muscles did not require upd3 secretion, but depended the production of egr/TNF- and of the insulin pathway inhibitor ImpL2.

Keywords: fat body, muscle, interorgan communication, mTOR, Egr signalling, Insulin signalling

*Speaker

Function and specification of macrophage subpopulations in *Drosophila*

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Macrophage heterogeneity is well-established in higher vertebrates but until recently the evidence for this phenomenon in *Drosophila melanogaster* was less clear. We have demonstrated molecularly and functionally-distinct macrophage subpopulations within the developing embryo that exhibit subsequent plasticity across the lifecourse. These subpopulation macrophages exhibited enhanced wound responses and decreased rates of efferocytosis compared to the total macrophage population, potentially representing more pro-inflammatory subsets of cells. Increasing the apoptotic cell burden upon macrophages decreased the number of subpopulation macrophages that could be found in the embryonic microenvironment.

We are currently investigating the function of these subpopulations across the lifecourse in regards immunity and ageing and are also seeking to understand the signalling pathways and molecules determining their specification *in vivo*.

We have developed a range of transgenic reporters enabling imaging and manipulation of *Drosophila* macrophages and their subpopulations *in vivo*. Reporters were crossed into mutant backgrounds lacking genes encoding apoptotic cell receptors and other genes related to efferocytosis. Numbers of macrophage subpopulation cells within the embryo were then quantified following confocal imaging. Macrophages were also challenged with increased amounts of developmental apoptosis through phenotypic analysis in a *repo* mutant background, which impairs specification of other professional phagocytes in the developing *Drosophila* embryo.

We show that loss of the phosphatidylserine receptor Simu increases numbers of macrophages within specific macrophage subpopulations. Loss of *simu* prevented decreases in numbers of subpopulation cells that are normally observed in the presence of large amounts of apoptotic cell death. Analysis of other apoptotic cell clearance receptors did not alter numbers of subpopulation cells in the developing embryo. Consistently, mutations in *amo* (the *Drosophila* homolog of *PKD2*), a calcium-permeable channel that operates downstream of Simu, phenocopy *simu* mutants, retaining numbers within specific macrophage subpopulations in the presence of an increased apoptotic cell burden. Analysis of phagosome maturation, reveals that Amo is involved in acidification of the apoptotic cell-containing phagosomes, suggesting that this process is associated with macrophage reprogramming. We also find that Ecdysone, a steroid hormone, influences numbers of specific subpopulations *in vivo*.

We conclude that a specific apoptotic cell clearance receptor has a role in macrophage specification *in vivo* with this receptor, Simu, required to shift macrophage subpopulations away from

^{*}Speaker

putative pro-inflammatory states. Overall, these results further validate macrophage heterogeneity in *Drosophila* and implicate Pkd2/Amo and ecdysone in modulation of subpopulation fate, while also hinting that polarisation-like reprogramming of macrophages may be conserved in *Drosophila*.

Keywords: hemocyte, macrophage, immunity, signalling, development, apoptosis

Identification of Mon1 as a regulator of anterior-posterior patterning in the oocyte.

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The anterior-posterior axis of the embryo is established in the oocyte by localization of mRNAs *bicoid* and *oskar* at the anterior and posterior poles respectively. These transcripts are synthesized in the nurse cells and transported to their respective locations in the oocyte by Staufen in a microtubule dependent manner. Here, we describe a novel role for *mon1* in anterior-posterior (A-P) patterning of early embryos. Mon1 (Monensin sensitivity 1) is a conserved protein which complexes with CCZ1 to function as a GEF for Rab7. Embryos lacking maternal *mon1* show absence of head structures. Consistent with this, we observe defects in anchoring and localization of both, *bicoid* and *oskar* mRNAs at the anterior and posterior poles respectively. In the mutant oocyte, Staufen appears ‘clumped’ and exhibits a delay in localizing to the posterior. In addition, the oocytes also show reduced levels of translated Oskar. An analysis of the expression pattern of *mon1* reveals enriched expression in posterior follicle cells with temporal regulation leading to strong expression at stage 8 which declines by late stage 9. Interestingly, knockdown of *mon1* in these cells, recapitulates the phenotypes observed in the mutants. This indicates that *mon1* functions non-autonomously to regulate Staufen organization with downstream consequences on the patterning RNAs. Its effect on the localization of both, *bicoid* and *oskar* indicates that it is likely to play a pivotal role in regulating RNA localization and anchoring.

Keywords: Mon1, Endosome, Rab7, oocyte, signaling

*Speaker

Investigating the role of Frizzled2-mediated ligand-independent canonical Wnt signaling in the development of *Drosophila* wing epithelium

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Wnts are a class of highly conserved secreted lipid-modified glycoproteins known to regulate embryonic development and adult tissue homeostasis. Secreted Wnts form a concentration gradient at a long-range and short-range to elicit a differential response in the receiving cells. However, the mechanisms regulating the range of gradient signaling remain sparsely understood and debated. *Drosophila* Wg (Wnt1) in wing epithelium is one of the best models to study the Wnt gradient signaling. Wg is secreted from two cell-thick layers along the dorsoventral boundary of the wing disc to form a concentration gradient and activates signaling in cells away from the source. However, perturbing the Wg gradient does not abolish target gene expression altogether. These reports suggest an additional ligand-independent mechanism to maintain the Wg signaling, which remained unknown. Our study shows that the positive regulator and receptor Frizzled 2 (DFz2) maintains ligand-independent canonical Wnt signaling in the absence of the Wg gradient. We show that DFz2 is necessary for target gene expression in cells beyond the reach of the Wg protein. Also, the survival of cells, along with adult patterning and growth, requires Fz2 in the absence of the Wg gradient. We further show that other Wnts are not involved in the DFz2-mediated maintenance mechanism. DFz2 acts redundantly with DFz1 to activate Wg-dependent canonical signaling. In contrast, we found that the maintenance mechanism depends on Dfz2, not DFz1. Thus, ligand-independent signaling is a novel non-redundant function of Dfz2. We also show that cells lacking Dfz2-mediated maintenance are less fit and are eliminated via cell competition. Our study shows that canonical Wnt signaling in *Drosophila* is an amalgamation of active ligand-dependent and ligand-independent maintenance. Cells lacking both are eliminated for tissue homeostasis.

Keywords: Wingless, Frizzled2, canonical Wnt signaling, ligand independent signaling, cell competition

*Speaker

Lipophorin receptors and Drospondin participate in Mushroom Bodies development and function.

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The Lipophorin receptors (LpRs) are members of the Low-density lipoprotein receptor family that mediate lipid uptake in insects. In *Drosophila melanogaster* two LpRs have been described, LpR1 and LpR2, which are orthologs of vertebrate ApoER2 and VLDL-R. ApoER2 and VLDL-R are associated with the development of structures like the hippocampus and cerebral cortex, and also linked to synaptic function. For doing so, ApoER2 and VLDL-R bind the signaling molecule Reelin. It is currently unknown whether LpRs play similar roles in the fly brain, though Reelin is not present in the *Drosophila* genome. Herein we show that LpR-deficient flies exhibit impaired olfactory memory and sleep patterns, function that relays on the Mushroom Bodies (MB) in fly brain. The LpR-deficient animals also present anatomical defects in this critical association area. We also present evidence that LpRs function through Dab, the fly homologue of Dab1 in mammalian cells and first mediator of Reelin signaling. In the search for a protein with similar functions to Reelin in *Drosophila*, we described the previously uncharacterized protein we called "Drospondin". The exposure of primary cultures of MB neurons to Drospondin and to mammalian Reelin, results in augmented neurite growth and complexity, similar to what is observed in hippocampal neuronal cultures treated with Reelin. This is not observed in neurons with deficient expression of LpRs. Our studies show MB development defects similar to those found in LpRs deficient flies, when Drospondin expression is downregulated. Our data show that Drospondin and LpRs genetically interact to modulate these anatomical phenotypes. Thus, these results support of the idea of a new signaling cascade gated by the Drospondin protein, that depends on LpRs and Dab, and whose actions define the mature structure of the fly brain and its operation. FONDECYT grant 1220393 to MPM, FONDECYT grant 1231556 to JMC, FONDECYT grant 1231685 to CO, ANID Doctoral fellowship N°21180582 to FR-C.

Keywords: Lipophorin receptor, Lipoprotein receptor, Mushroom bodies, Reelin, Dab, LpR1, LpR2

*Speaker

Neuropeptide signaling in the male reproductive system of *Drosophila*

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Myosuppressin peptides (Ms) are well-conserved insect brain-gut peptides and has roles in regulating muscle activity of the gut, heart and female reproductive tissues. The *Drosophila* genome has two Ms GPCR type receptor genes (*MsR1* and *MsR2*) whose pharmacology appears to be very similar when heterologously expressed in cell lines. We have been investigating a role for Ms signaling in the male reproductive tract of *D. melanogaster* and its possible involvement in regulating ejaculation. Both sperm and non-sperm components (seminal fluid, SF) of the male ejaculate are important for reproductive success in insects. In *Drosophila*, mature sperm is released from the seminal vesicles (SV) and mixed with seminal fluid prior to ejaculation. The paired male accessory glands (MAG), the ejaculatory duct (ED) and the ejaculatory bulb, all contribute to the seminal fluid with the MAG providing the bulk of the material. Initiation and termination of ejaculation will rely on the coordinated activity of the muscle layers surrounding the male reproductive tissues. Four corazonin/cholinergic interneurons of the male abdominal ganglion promote transfer of sperm/SF by activating 5-HT neurons that project directly to the MAG, ED and SV. We find distinctive Ms neurons from the abdominal ganglion, two rectal cells and a single cell positioned close to the ED innervating the male reproductive tract, suggesting a role for Ms in muscle inhibition. In Schneider's cell culture medium the MAGs display bursts (~25 s) of strong muscular contractions that are asynchronous with the sustained peristaltic contractions of the ED. The MAG contractions are strongly inhibited by exogenous Ms, with full inhibition at 10 μ M. Null mutant alleles of both Ms receptors (*MsR11* and *MsR21*) were generated by targeted gene-editing. Ms inhibited *MsR11* MAG contractions, but no Ms-induced suppression was observed with *MsR21* glands, demonstrating a specific role for the latter receptor in transduction of the Ms signal. Strong expression of MsR2-GAL4, but not of MsR1-GAL4, in the MAG muscle is consistent with this conclusion. Direct evidence of the importance of male Ms signaling in reproduction was obtained by silencing male Ms-neurons and by phenotypic analysis of the performance of *MsR21* males. We conclude that Ms plays an important role in male reproductive physiology and that this is dependent on MsR2.

Keywords: neuropeptide, myosuppressin, male accessory glands, seminal fluid

*Speaker

Novel players involved in Flower-mediated cell competition – A whole-genome RNAi screen

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Accumulation of viable yet suboptimal cells may lead to developmental malformations, accelerated ageing and disease. Suboptimal cells (losers) can be identified and eliminated when in presence of fitter cells (winners), through a process called cell competition. This ensures optimal tissue fitness and homeostasis, being relevant for lifespan and several disease contexts.

Fitness fingerprints-mediated cell competition relies on direct cell-cell contact. The expression of different isoforms of the transmembrane protein Flower (Fwe) labels cells according to their fitness status. Despite its importance in lifespan extension, tumorigenesis and Alzheimer's disease, this conserved cell selection mechanism is still poorly understood.

To gain a better insight into this mechanism, we designed a highly innovative assay. The Easy Win Assay allows us to identify genes functionally required in cell competition triggered by the expression of the conserved loser isoform, *human fwe1*. In this blind whole-genome RNAi screen, we categorized candidates according to the rescue or enhanced elimination of loser cells in the adult *Drosophila* eye.

Following the validation of top candidate genes, and since the initial screen does not discriminate in which cell population the candidates play a role, we will clarify if the candidates are required by the loser or winner cell population. Then, we will evaluate their place in the Fwe pathway, and determine their requirement in different Fwe-dependent contexts, including development, ageing, cancer and Alzheimer's disease.

A better understanding of cell-cell communication between winners and losers will be essential to developing new approaches to promote tissue homeostasis and, overall, increased healthspan.

Keywords: Cell competition, flower gene, Cell fitness, Easy Win Assay, Whole genome RNAi screen

*Speaker

Rab35 GTPase functions as a tumor suppressor and modulate Wnt signaling and differentiation when APC is lost in intestinal stem cells

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Subcellular processes are intimately linked with regulating signalling activity which in turn is important for maintaining healthy adult organs. During cancer, these subcellular pathways are hijacked to facilitate tumour progression, however, we currently lack a detailed understanding of how these events are involved in intestinal tumours. Here, using a conditional *in vivo* modifier screen in fly intestinal progenitor cells, we identify Rab35 GTPase as a tumour suppressor which modulates Wnt signalling activity when Adenomatous Polyposis Coli (APC) is lost. We find that active Rab35 co-localises with Armadillo (Arm) at cell junctions, while inactive Rab35 is predominantly found in intracellular puncta located at the Golgi. Our genetic, lineage tracing and localization experiments support a role for Rab35 in regulating Golgi structure, ISC proliferation and differentiation. Mechanistically, we show that Rab35 controls the activity and localization of the Rho GTPase, CDC42, which in turn positively regulates JNK signalling. Indeed, forced activation of JNK signalling or CDC42 potentiates ISC proliferation and Wnt signalling. Our findings reveal a subcellular circuitry that is initiated when APC is lost which sustains intestinal Wnt signalling and highlights a key pathway that could be therapeutically targeted.

Keywords: Apc, Wnt signalling, colorectal cancer, Drosophila, Rab35, Cdc42, JNK signaling, scRNA seq

Reduction of nucleolar NOC1 induces MYC-dependent nucleolar stress and p53 upregulation: a novel response to ribosomal biogenesis impairments

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Ribosome biogenesis is a complex cellular process that relies on the coordinated action of numerous nucleolar proteins and RNAs. Defects in this mechanism result in a condition known as nucleolar stress, and many details about this response and how it affects cell behavior are currently a matter of intensive studies.

The NOC family of genes, including NOC1, NOC2, and NOC3, is essential for correct ribosome synthesis and cell growth in *S.cerevisiae*. Our group showed that NOC1 controls rRNA maturation and ribosomal function in *Drosophila*, and that it is required for proper larval and organ development. In addition, reduction of NOC1 in cells of the wing imaginal disc results in MYC-dependent nucleolar stress, and our transcriptomic analysis reveals the activation of genes involved in DNA damage and proteotoxic stress. These events are accompanied by the transcriptional upregulation of p53. Cells with reduced levels of NOC1 are also subjected to cell competition, a mechanism induced by different MYC levels and mutations in ribosomal proteins, where robust protein synthesis allows the best-fitted cells (winner) to expand at the expense of less fitted (loser), that die by apoptosis. Cell competition is conserved also in mammalian development and is considered relevant for the initial step of tumorigenesis.

Our findings reveal a novel nucleolar stress response caused by impairments in ribosome biogenesis. Further analysis is ongoing to better understand the mechanisms linking NOC1 function in the nucleolus with MYC and p53 upregulation. Since dysregulation of ribosomal activity is implicated in various pathologies, including ribosomopathies and cancer, these results may also provide new insights in the mechanisms that lead to disease development after ribosomal defects.

Keywords: Ribosomal biogenesis, nucleolar stress, apoptosis, Myc, p53, cell competition

*Speaker

The peroxisome assembly factor Pex19 is required for insulin-like peptide release

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Peroxisomes are vital organelles whose relevance for cellular and organismal physiology are underestimated. They form various contact sites with other organelles (mitochondria, endoplasmic reticulum, lysosomes, and lipid droplets). Mutation of the peroxisomal biogenesis factor Peroxin 19 (Pex19) leads to loss of peroxisomes, which in *D. melanogaster* causes mitochondrial damage, delayed development, early lethality and lipotoxicity (Bülow et al., 2018). We show that Pex19, a cytosolic protein required for peroxisomal membrane protein import, is required for the secretion of insulin-like peptide 2 (dilp2) and is trafficked in insulin-producing cells (IPCs) along with DILP2-containing vesicles. Pex19 mutants show impaired secretion of dilps from the IPCs, a set of neuroendocrine cells, upon a nutrient stimulus. This leads to reduced levels of dilp2 in the hemolymph, and impairs insulin signaling in peripheral tissues. Surprisingly, re-introduction of Pex19 in IPCs rescues the lethality of Pex19 mutants. By contrast, entire peroxisomes are not trafficked in IPCs upon a nutrient stimulus, but peroxisomal lipid metabolism and interaction with other organelles could also contribute to dilp release. Peroxisome loss in *D. melanogaster* leads to depletion of medium-chain fatty acids (MCFA, Sellin et al., 2018). We use alkyne-labeled fatty acids and click chemistry to show that MCFA depletion in Pex19 mutants leads to altered incorporation of fatty acids into membrane phospholipids such as phosphatidylinositol (PI). The altered lipid profile of phospholipids affects membrane contact sites and organelle interaction and might contribute to the impaired secretion of dilp2 in Pex19 mutants. By analysis of a broad spectrum of organelle contacts and its nutrient-dependent dynamics using super-resolution and expansion microscopy, we untangle the role of peroxisomes and their assembly factors in insulin signaling.

Bülow MH et al. Unbalanced lipolysis resulting in lipotoxicity provokes mitochondrial damage in peroxisome-deficient Pex19 mutants. *Mol Biol Cell*. 2018 Feb 15;29(4):396-407

Sellin J, (...) Teleman AA and Bülow MH. Dietary rescue of lipotoxicity-induced mitochondrial damage in Peroxin19 mutants. *PLOS Biol* 16(6): e2004893 June 19, 2018

Keywords: Organelles, Peroxisomes, insulin like peptides, Peroxisomal Biogenesis Disorders, Membrane contact sites

*Speaker

The serotonin signaling pathway regulates *Drosophila* hematopoiesis in response to an immune stress

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In mammals, Hematopoietic Stem and Progenitor Cells (HSPCs) in the bone marrow ensure continuous blood cell production. Self-renewal, proliferation and differentiation of HSPCs are controlled by a specialized microenvironment called the "niche". Sympathetic nerve fibers innervate the bone marrow and are part of the niche. The crucial role of neural innervation in the regulation of hematopoiesis has only recently emerged, and the underlying mechanisms remain largely unknown. The *Drosophila* (fruit fly) hematopoietic organ called the lymph gland (LG) has become an important model to study how hematopoiesis is controlled. The LG is aligned along the cardiac/vascular system, which acts as a niche to control LG hematopoiesis under homeostatic conditions. In response to an immune challenge, stress hematopoiesis is induced in the LG, but the role of the cardiac/vascular niche remains unknown. I identified the serotonin receptor 1B (5-HT1B) as being expressed in the cardiac/vascular niche and required to regulate LG stress hematopoiesis. I also established that serotonin (5-HT) produced by a subset of brain neurons which extend axons towards cardiac cells is required to regulate LG stress hematopoiesis. I am currently i) investigating whether the serotonergic neurons involved are activated in response to parasitism and whether this activation controls 5-HT1B activation in cardiac cells, and ii) characterizing synapses and their temporal activation in response to an immune stress. Understanding the role of neurons in the regulation of stem cell activity is only at its beginning in vertebrates. The conservation of this regulation in *Drosophila* opens new perspectives in the exploration and understanding of the regulation of the hematopoietic niche by neurons in humans.

Keywords: *Drosophila*, hematopoiesis, vascular niche, neurons, immune stress

*Speaker

Ubiquitin-conjugating E2-enzyme Effete controls hemocyte activation in *Drosophila melanogaster*

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The fruit fly *Drosophila melanogaster* is a valuable model organism for studying many biological processes relevant to human health. *Drosophila* has robust, evolutionarily conserved innate immunity including antimicrobial peptide production, phagocytosis, and encapsulation of pathogens. The main fly immune tissues are the fat body, functional equivalent of mammalian liver and adipose tissue, and hemocytes, i.e. fly blood cells. *Drosophila* larval hemocytes include three main types: plasmatocytes, lamellocytes and crystal cells. Plasmatocytes are phagocytic cells, often compared to mammalian macrophages, and they can differentiate into either lamellocytes or crystal cells on demand, after getting an appropriate signal. Lamellocytes are produced in response to parasitization, and they form a melanized capsule around an intruder. Crystal cells produce melanin to seal wounds and kill bacteria by trapping them inside a melanized mass. Several signaling pathways function in hemocyte differentiation and activation, including the Toll pathway. We have identified a Ubiquitin-conjugating enzyme Effete (Eff) as negatively regulating Toll signaling *in vitro* in *Drosophila* S2 cells, and we also show that RNAi-mediated silencing of *eff* causes hemocyte differentiation *in vivo*. To study the role of Eff in fly immune tissues and the relationship between Eff and the Toll pathway, we carried out transcriptome analysis from fly hemocytes and fat body tissue, with *eff* knockdown and/or ectopic Toll pathway activation and controls. Our results so far indicate that while Toll activation in either of the tissues induces hemocyte differentiation, Eff is an important regulator of hemocyte differentiation in hemocytes but not in the fat body.

Keywords: hemocyte differentiation, cellular immunity, Toll pathway, immune regulation

*Speaker

Uncovering a novel Groucho phosphatase using an RNAi screen

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Groucho is an evolutionary conserved transcriptional co-repressor that has been implicated in numerous cellular processes during embryogenesis and adult patterning, particularly when acting downstream of signaling pathways such as the Wingleless/Wnt and the Ras/Erk cascades. Various studies indicate that post-translational modifications modulate Groucho's ability to function as a co-repressor. Phosphorylation by Erk or by Cdk1, for example, inactivates the repressor function of Groucho. However, whether Groucho also undergoes dephosphorylation and reactivation remains unknown. We have, accordingly, conducted an RNAi-based genetic screen that has uncovered, for the first time, a candidate phosphatase that affects the phosphorylation state of Groucho. The existence of such a phosphatase is consistent with a rapid and reversible ON/OFF post-transcriptional switch regulating Groucho-dependent repression.

Keywords: Groucho, phosphorylation, phosphatase

*Speaker

RNA biology

Characterization of a cluster of three snoRNAs, including *jouvence*, involved in Lifespan, Neurodegeneration, and Metabolism, in *Drosophila*.

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In our society, aging, longevity, and metabolic disorders are major concerns of public health. They result of complex biological processes of accumulation of different types of damage at molecular, cellular, tissue and organ levels. In the last few years, in *Drosophila*, we have identified a new small nucleolar RNA (**snoRNA**) (*jouvence*) and showed that its mutation (**deletion-F4**) reduces lifespan, while its overexpression increases it (Soulé et al., *Nat. Comm.*, 2020). At the organismal level, the old mutants (**deletion-F4**) flies show more neurodegenerative lesions than the controls (Soulé and Martin, *bioRxiv*, 2020), while the flies overexpressing *jouvence* show only few lesions, suggesting a neuroprotection. The **deletion-F4 (mutants)** present also a hypertrophy of the fat body, suggesting a perturbation of the lipids metabolism (triglycerides and cholesterol). *Jouvence* is expressed in nurse cells (ovary), and in the epithelium of the gut (enterocytes). Since, the re-expression (rescue) of *jouvence* in the gut is sufficient to increase lifespan, we hypothesize that the brain lesions in old flies might be a consequence of the perturbation of the gut homeostasis. In addition, as revealed by a RNA-Seq analysis performed on the gut, several genes involved in the lipids metabolism, and in the cholesterol pathway, are deregulated, suggesting a gut-brain axis. However, recently, a deeper characterization of this genomic locus has shown the presence of **two other snoRNAs**, revealing rather a cluster of three snoRNAs. The aim of this project is to evaluate the role of each of them in longevity, neurodegenerative lesions and lipids metabolism. To assess the role of each snoRNA, we express each of them either in the gut or in the fat body. Then we are analyzing the lifespan, neurodegenerative lesions, as well as metabolic parameters in order to trace a link between the lesions of the brain, the metabolic disorders and the lifespan.

Keywords: longevity, neurodegeneration, metabolism, cholesterol, triglycerides, snoRNA

*Speaker

Characterization of the function of Hecw in TFEB/Mitf-mediated autophagy

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Autophagy is a crucial catabolic process aiming to recycle cytosolic material to maintain cellular homeostasis. During autophagy, dysfunctional organelles and macromolecules are captured by newly formed double-membrane organelles, named autophagosomes, and delivered to lysosomes for degradation. Impairments in the autophagic pathway are associated with a wide number of pathologies, including cancer and neurodegeneration. Nonetheless, despite the wide effort in finding new components of this pathway, we are far from fully elucidating the molecular mechanisms behind autophagy. Recent studies support a role for E3 ubiquitin ligases in autophagy, highlighting the importance of ubiquitination in tightly controlling the progression of this pathway. We recently characterized the function of Hecw, the *Drosophila melanogaster* ortholog of human HECW1. Hecw is essential to maintain the liquid-like state of ribonucleoprotein particles (RNPs). In its absence, flies show defective oogenesis as well as neurodegenerative-like phenotypes. While it is possible that the function of Hecw in RNPs biology might support both egg development and prevent neurodegeneration, the onset of the neurodegeneration-like phenotypes in flies lacking Hecw remains unexplained. Our data indicates Hecw may act in TFEB/Mitf mediated-autophagy as we find that Hecw is essential for the correct engagement of starvation-induced autophagy in larval and adult tissues. Moreover, aging Hecw mutant flies present a strong reduction of TFEB/Mitf protein levels, possibly leading to impaired autophagy as shown by reduced levels of the autophagic marker Ref2P. Since neuronal cells strongly rely on autophagy for their survival, these results might be important to find new actors in the autophagic process and elucidate the genetics of neurodegeneration.

Keywords: Autophagy, Protein clearance, Ubiquitination, Neurodegeneration, Development

*Speaker

Elucidating putative RNA binding protein interactions and RNAi knockdown phenotypes in *Drosophila melanogaster* sperm development.

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Sperm development progresses as a tightly regulated program; with each stage defined by cell-type-specific changes to shape, volume and expression profiles. After meiosis, the spermatids undergo synchronised differentiation, elongation and maturation, transforming from round cells to highly specialised and polarised, needle-like cells that extend to 1.8mm in length. Individualisation separates these interconnected spermatids to herald mature, coiling sperm.

While most transcription in the germline occurs before meiosis, a set of post-meiotically transcribed genes have been identified. mRNA transcripts from these genes undergo asymmetrical, subcellular localisation; they are transported away from the nucleus and accumulate at the tail-ends of growing spermatid bundles in patterns resembling shooting speckled "comets" or U-shaped acorn "cups". Fluorescently tagged reporter constructs reveal that these generate distinct protein gradients. In addition to the localised mRNAs, we have also identified several RNA binding proteins (RBPs) enriched at the ends of spermatid tails. How and why do these non-uniform mRNA distributions arise? What role do these RBPs play in ensuring the active localisation, anchoring and translation of comet and cup mRNAs in spermatids?

We used RNA-affinity pull-down assays to extract out bound fractions of comet and cup mRNAs with the endogenous RBP interactors Bruno 1 (Bru1), Alan Shepard (Shep), Polypyrimidine-Tract-Binding Protein (dPTB) and IGF-II mRNA-binding protein (Imp). This revealed direct and indirect differential binding between these candidate RBPs and 11 comet or cup mRNAs in vitro – with binding affinities varying depending on the mRNA of interest. We plan to use a modified CIAP and RIP assay to precipitate whole, multi-RBP complexes (and their respective interacting RNAs) to re-verify these binding interactions in a more appropriate testis-exclusive context, and to identify further co-localised mRNAs.

We have used RNAi screens to investigate the functional roles of specific RBPs in spermatids. For example, we have found that a knockdown of imp leads to a variable spectrum of abnormal testis phenotypes, including considerable disruption to spermatid elongation. We are currently investigating the effects of RNAi expression on the localisation of mRNAs that bound to Imp in the pull-down assay. We are also studying the translation of localised mRNAs in these RNAi lines. By conducting HCR RNA-FISH and Lightsheet fluorescence microscopy, we will determine

*Speaker

whether these RNAi-induced defects are attributed to translational disruption alone, or whether this dysregulation is detectable at an earlier point of mRNA production, stability and/or localisation.

Keywords: Sperm development, Post meiotic transcription, RNA localisation, RNA binding proteins, Comet and cup mRNAs.

Exploring the function and structure of heterogeneous ribosomes in the gonads of *Drosophila melanogaster*

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The cellular pool of ribosomes is highly diverse in rRNA and ribosomal protein (RP) content. The biological significance of ribosome heterogeneity remains to be fully understood. However, disrupting specific ribosomal genes can produce tissue-specific phenotypes, implying such ribosomes may have specialised function. For example, ribosomal mutations in *Drosophila melanogaster* often result in the *minute* phenotype, which can impact fertility.

We have previously profiled the composition monosomes and polysomes from different *Drosophila* tissues (Hopes et al., 2021). We discovered the enrichment of specific RP paralogs within the ribosomes of *Drosophila* gonads, with 4 paralogs enriched in ovary ribosomes and 6 in testis ribosomes. One testis-specific paralog, RpL22-like, is incorporated into ~50% of testis ribosomes and shares 45% aa sequence identity with the canonical paralog, RpL22. Interestingly, *Drosophila* and mammalian RpL22-like/RpL22L1 are both testis specific, and their expression can be directly controlled by RpL22. However, RpL22-like and RpL22L1 evolved independently suggesting an evolutionary pressure for a conserved function.

To assess the impact of these paralog switching events, RNAi of RpL22 and RpL22-like paralogs was performed. RpL22 knockdown in the ovary impacted germ cell maintenance. Rescue experiments expressing RpL22-like failed to fully rescue the phenotype, leading to underdeveloped ovaries which impacted fertility. RpL22 knockdown in the testis had no observable effect. Moreover, RpL22-like RNAi targeted to the gonads did not impact oogenesis or spermatogenesis. Intriguingly, immunostaining with paralog specific antibodies revealed RpL22 and RpL22-like localise to different cellular subtypes and different sub-cellular compartments within the testis. RpL22 localises to somatic cells, late spermatids and the nucleus of germline cells, while RpL22-like localises to germline cytoplasm. The differential localisation of each paralog suggests that they may have different roles to play during spermatogenesis.

To dissect the contribution of RpL22-like to mRNA translation in the testis, quantitative mass spectrometry was performed on RNAi testes. The levels of 18 proteins change upon RpL22-like knockdown, suggesting that the incorporation of RpL22-like may alter the ribosome's translational preference. Levels of Dgp-1, an ortholog of mammalian ribosome rescue factor, GTPBP1, were reduced ~50% upon RpL22-like RNAi, while mRNA levels were unaffected. This suggests that RpL22-like may play a role in the translational control of Dgp-1. Work is underway to assess

*Speaker

the impact of these translational changes to spermatogenesis. Together, these analyses will allow us to decipher the mechanisms by which RpL22 and RpL22-like containing ribosomes regulate the translation of specific mRNAs, and how specialised ribosomes contribute to gametogenesis.

Keywords: Ribosomes, Translations, Spermatogenesis, Oogenesis, Gametogenesis

Impact of the viral RNA epitranscriptome during infection in insects

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To date, more than 150 post-transcriptional modifications of RNA have been identified. These RNA modifications are made by a class of enzymes called "writers" and can be detected by "readers". The study of these RNA modifications has given rise to the field of epitranscriptomics, whose emergence has been enabled by recent technological advances in the detection of these chemical modifications. While some RNA modifications are known to play a key role in cellular RNA biogenesis, the role of many of them remains unknown. In particular, viral RNA modifications are still poorly studied and represent an emerging area of research. During my PhD, I focus on the epitranscriptomic marks of Flock House virus (FHV) genomic RNAs during infection in *Drosophila melanogaster*. The position of 2'-O-methylated nucleotides in FHV's genome have been determined and host writer enzymes responsible for these modifications are still to be identified. My current goal is to identify writer enzymes and to get a better understanding of the role of these methylations on the viral replication cycle and/or the virus' ability to escape the innate immune system. For this purpose, I use a knock-down (KD) screening approach in S2 cells of RNA methyltransferases in the context of FHV infection. The impact of the KD on the viral RNA level is assessed by RT-qPCR and the methylation profile of the viral RNAs will then be mapped by RiboMeth-Seq.

Keywords: epitranscriptomic, RNA methylation, virus, 2', O, methylation, innate immunity

*Speaker

Molecular dissection of the RNP granule regulator Ataxin2

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The RNA binding protein Ataxin2 has been linked to the formation of RNA granules in different animal models. An expansion of a poly-Q domain in the human Atxn2 protein is associated with the neurodegenerative diseases spinocerebellar ataxia type 2 (SCA2) and amyotrophic lateral sclerosis (ALS) and antisense oligonucleotides against human Ataxin-2 are currently in clinical trials for ALS. Like its mammalian homologs, *Drosophila* Ataxin-2 has an LSM and LSMAD domain, a PABP-binding PAM2 motif and extended intrinsically disordered regions (IDRs), which for many RNA binding proteins have been shown to support assembly of dynamic membrane-less complexes termed biomolecular condensates. While *Drosophila* Ataxin-2 is essential for survival, we have previously shown that flies lacking the Atx2 IDRs are viable, but show fewer neuronal RNP granules as well as resistance to cytotoxicity observed in fly models of ALS, FTD and Huntington's disease.

To understand mechanisms of Atx2 function in more detail, we performed multiple structure function analysis to investigate the various roles of structured and unstructured protein domains of Atx2. Through a series of experiments on wild-type and mutant forms of *Drosophila* Atx2, using genetics, TRIBE ('Targets of RNA-Binding Proteins Identified by Editing'), co-localization and immunoprecipitation experiments we propose a model where the structured LSM domain and PAM2 motif of Atx2 may be a major determinant of the mRNA and protein content of Atx2 mRNP granules and the unstructured IDRs regulate the aggregation of RNPs.

Further we investigated the role of Atx2 in the formation of mRNPs in other tissues. We show that Atx2 is required for the formation of germline granules in nurse cells and the oocyte. Further, depletion of Atx2 effects the regulation of important maternally deposited proteins. Like previous results this role of Atx2 is as well dependent on its cIDR domain. This establishes Atx2 as a general regulator of RNP granules in different cell types and tissues highlighting the fundamental importance of Atx2.

*Speaker

Keywords: RNA granules, RNPs, Neurodegeneration, Intrinsically disordered regions, RNA binding proteins

Optimising RNA Trans-Splicing for Targeted Gene Transcript Reprogramming

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Optimising RNA *Trans*-Splicing Technology for Targeted Gene Transcript Reprogramming
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Spliceosome-mediated RNA *trans*-splicing, or ‘SMaRT’, is a promising method for tagging or modifying RNA transcripts whilst they remain under endogenous regulation of target gene expression. Altering specific precursor messenger RNA can be achieved through the sole delivery of an exogenous pre-*trans*-splicing molecule, known as the PTM, which contains the replacement coding sequence and other elements to facilitate desired splicing events. Upon binding to a target pre-mRNA intron, this artificially designed RNA sequence would exploit a cell’s spliceosome for exon exchange after it assembles onto the PTM splicing domain. From previous demonstrations of the SMaRT system, *trans*-splicing efficiency appears to be limited by outperforming *cis*-splicing events, off-target effects and self-translation of the PTM.

We identified various characteristics to consider when designing a PTM binding domain, from its hybridisation site, for optimal reprogramming of targeted pre-mRNA exons. For more high-throughput selection of potent PTMs, we also developed an *in vitro* fluorescence-based *trans*-splicing reporter system. Random libraries of PTMs with split GFP tags and unique binding domains were screened during fluorescence-activated sorting of *Drosophila* Schneider 2 cells. Finally, we assessed PTM sequence elements serving to improve *trans*-splicing efficiency by facilitating antisense inhibition of *cis*-splicing or accelerating spliceosome recruitment. Ultimately, we hope to establish a robust and versatile PTM architecture that can be easily tailored to different mRNA reprogramming strategies. Bolstering the SMaRT system would provide a transient and more scalable technique for molecular imaging of gene expression, interrogation of splice-variant functions and various therapeutic approaches.

Keywords: Splicing, mRNA, reprogramming, neuronal

*Speaker

The role of Adar in regulation of innate immunity in *Drosophila*

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Adenosine deaminases acting on RNA (ADARs) are enzymes that deaminate adenosine (A) to inosine (I) in double-stranded RNA (dsRNA) structures. In vertebrates, loss of ADAR1 RNA editing leads to aberrant activation of interferon and interferon-stimulated genes by activation of antiviral cytoplasmic dsRNA sensors which recognize endogenous dsRNA as virus-derived. *Drosophila* lacks these cytoplasmic antiviral dsRNA sensors and their closest relative in *Drosophila* is Dicer2. There is only one *Adar* gene in *Drosophila melanogaster* and *Adar5G1* null mutants show aberrant innate immune induction of antimicrobial peptide (AMP) transcripts that requires the dsRNA sensor Dicer-2 (1). *Drosophila* Adar also carries out efficient site-specific RNA editing in CNS transcripts to recode over six hundred proteins; this is unrelated to innate immunity.

The aim of this study is investigation of the pathway leading to induction of AMP transcripts in *Adar5G1* null mutants by identifying further mutations that can suppress the aberrant innate immune induction. To achieve this we use the *UAS/Gal4* binary system, specifically a weak ubiquitous (*Arm-GAL4*) driver, to knock-down different components of the Dcr-2 innate immune signaling pathway. Our preliminary results show rescue of AMPs levels in *Adar5G1* null mutants by knocking down *Dcr-2* or *r2d2*, one of Dcr-2's main cofactors. In addition, it seems that overexpression of human ADAR1 p150 isoform is sufficient to prevent aberrant AMP transcript induction in *Adar5G1* null mutants.

In parallel, we knocked out *Adar* in Schneider S2 cells, derived from larval hemocytes, and checked levels of immune induction in comparison to wild-type S2 cells. *Adar* knock-out lines do not show an increased immune induction compared to wild type in normal conditions. Also, neither *Adar* knock-out nor normal S2 cells show increased immune induction in response to synthetic dsRNA transfected into the cytoplasm. We observe a complete lack of expression in S2 cells of the *cGRL1* transcript encoding the main sensor of dsRNA (2). A related poster by Khadija Hajji, shows that cGlr1/Sting signaling is involved in aberrant innate immune induction in *Adar5G1* mutant flies, in addition to the Dcr-2 involvement reported previously and studied here. The two signaling pathways appear to be required to act together.

1. Deng, P., et al., *Adar RNA editing-dependent and -independent effects are required for brain and innate immune functions in Drosophila*. Nat Commun, 2020. **11**(1)
2. Slavik, K. M., et al., *cGAS-like receptors sense RNA and control 3'2'-cGAMP signalling in Drosophila*. Nature, 2021. **597**(7874)

*Speaker

Keywords: Adar, Dcr, 2, Immunity, RNA editing, dsRNA

Understanding RNA Binding Protein modularity: function of the different RNA binding domains of Imp

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The spatiotemporal control of subcellular RNA distribution has recently emerged as a fundamental post-transcriptional mechanism involved in both physiological processes and the progression of various diseases such as cancer or neurodegenerative diseases. Long-distance transport and more local concentration of RNA molecules in ribonucleoprotein (RNP) granules have been shown to be crucial for the tight and dynamic compartmentalization of RNA translation in both polarized and non-polarized cells. These granules are composed of numerous RNAs bound to RNA binding proteins (RBPs) that promote their segregation and transport within the cell. RBPs play a key role in the establishment of dynamic interaction networks, interacting with multiple molecules of RNAs and proteins *via* their different RNA binding domains. To address the molecular and functional importance of RBP multiple RNA binding domains, we are using as a model the conserved Imp protein. In *Drosophila*, Imp is composed of four RNA binding domains called hnRNP-K Homology domain 1 through 4 (KH domains). These domains function in pairs (or di-domains): KH1-KH2 and KH3- KH4, each having the capacity to bind RNAs. To date, the populations of RNAs bound to these two di-domains and the respective role of these domains *in vivo* are unclear.

To address these questions, we designed Imp variants with point mutations in KH GxxG loop, which inhibits the capacity of KH domains to bind RNA without modifying their folding. To explore the respective role of KH di-domains in the recognition of Imp RNA target *profilin*, we produced wild-type and mutant recombinant proteins and performed *in vitro* EMSA experiments, thus revealing that both KH1-KH2 and KH3-KH4 are involved in binding to *profilin* 3’UTR. In parallel to this transcript-specific approach, we are currently performing *in vitro* RIP-seq experiments to get a wider, transcriptome-wide view on KH di-domain binding preferences.

Using the CRISPR/Cas9 technique, we constructed *Drosophila* strains expressing the mutant forms of Imp from the endogenous locus and showed that both di-domains are functionally required for fly viability. In contrast, KH3-KH4 is dispensable for the developmental remodeling of Mushroom Body neurons in the fly brain, suggesting tissue-dependent requirements for KH3-KH4. To identify the specific tissues in which KH3-KH4 function is required, we are launching a systematic screen combining RNAi and rescue constructs.

Altogether, this work will help understand the advantages of RBP modularity in a physiological multi-cellular context.

*Speaker

Keywords: RNA, RiboNucleoProtein (RNP) granules, RNA Binding Proteins (RBPs), Imp, KH domains, Mushroom Body (MB), Rip, Seq

Xrp1 governs the stress response program to spliceosome dysfunction

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Co-transcriptional processing of nascent pre-mRNAs by the spliceosome is vital to regulating gene expression and maintaining genome integrity. Here, we show that the deficiency of functional U5 snRNPs in *Drosophila* imaginal cells causes extensive transcriptome remodeling and accumulation of highly mutagenic R-loops, triggering a robust stress response and cell cycle arrest. Despite compromised proliferative capacity, the U5 snRNP deficient cells increased protein translation and cell size, causing intra-organ growth disbalance before being gradually eliminated via apoptosis. We identify the Xrp1-Irbp18 heterodimer as the primary driver of transcriptional and cellular stress program downstream of U5 snRNP malfunction. Knockdown of *Xrp1* or *Irbp18* in U5 snRNP deficient cells attenuated JNK and p53 activity, restored normal cell cycle progression and growth, and inhibited cell death. Reducing Xrp1-Irbp18, however, did not rescue the splicing defects, highlighting the requirement of accurate splicing for cellular and tissue homeostasis. Our work provides novel insights into the crosstalk between splicing and the DNA damage response and defines the Xrp1-Irbp18 heterodimer as a critical sensor of spliceosome malfunction and mediator of the stress-induced cellular senescence program.

Keywords: pre, mRNA splicing, U5 snRNP, Ecd, R, loops, DNA damage, Xrp1, Irbp18, Prp8, cellular senescence

*Speaker

Physiology & metabolism

A role for Ceramide synthase Schlank in gut-brain communication

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Ceramide synthases (CerS) are central enzymes required for the de-novo synthesis of ceramides, a class of bioactive sphingolipids with cell signalling and second messenger capabilities. Ceramides are particularly important contributors to insulin resistance. However, the link between CerSs and the ceramides produced thereof with insulin signalling (IS) is not yet entirely understood. Whereas mammals express six CerS orthologs, *Drosophila* encodes for only one, called "Schlank". Schlank is prominently expressed in the gut, fat body, CNS and PNS. We already showed that loss of Schlank affects growth and lipid metabolism, and we asked which tissue is required for the observed phenotypes and how IS is affected. To this end, we expressed a *schlank*-RNAi construct in different tissues. Knockdown (KD) in the gastric caeca (GC) and midgut (MG) resulted in severe growth defects in larval and pupal development. In addition, we observed morphological changes of GC and MG, and increased lipid droplet area in the fat body (FB). Strikingly, we found an accumulation of dILP2 (Drosophila Insulin-like peptide) in the brain IPCs (Insulin-producing cells) in these animals. This would explain the observed growth phenotypes. Furthermore, rescue experiments using the *Drosophila* CerS or mammalian CerSs (CerS1-2 and CerS4-5), led to a complete rescue indicating a conserved function of CerSs. These data suggest an essential function of CerS Schlank in the gut in controlling the secretion of dILPs and imply a role of Schlank in gut-brain communication.

Keywords: Ceramide synthase, Schlank, ceramide, insulin, producing cells, gut brain axis, interorgan communication

*Speaker

A switch in intestinal lipid metabolism coincides with juvenile growth spurt in *Drosophila*

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Animal development includes a period of juvenile growth before the onset of adulthood and sexual maturity. The gut plays a crucial role in metabolic homeostasis by regulating nutrients and energy uptake from the diet. Surprisingly, whether intestinal function is regulated during development to support the nutritional and metabolic needs of juvenile growth remains largely unexplored. In *Drosophila*, embryonic development is followed by 3 larval stages that are dedicated to growth. During larval life, body volume increases 70-fold with a dramatic accumulation of metabolic stores. Our morphometric and metabolic analyses show that growth is mostly restricted to the 3rd larval instar, which lasts only 48 hours. We performed temporal transcriptomic analyses in larval guts to determine whether changes in the function of this organ support this growth spurt. Our data show a dramatic increase in transcripts related to lipid digestion, metabolism and transport shortly after the entry into the 3rd larval instar. This transcriptional signature translates into a 3-fold increase in intestinal lipolytic activity and coincides with a 20-fold increase in total lipid stores during the last 2 days of larval life. Larval development is followed by metamorphosis, a stage that lasts several days and during which animals do not feed. This developmental switch in gut function could maximise nutrient intake and energy storage to support the progression through metamorphosis and the first hours of adult life. We are currently investigating this possibility and the signals controlling this switch. More broadly, our studies pave the way for a better understanding of how the function of specific organs is adjusted to support organismal fitness throughout the life cycle.

Keywords: Metabolism, Intestine, Physiology, Growth

*Speaker

Adenosine as a cellular energy and activity balance hub

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Due to its metabolic connection with AMP, ADP, and ATP, adenosine is determined to play an important role in energy metabolism. Our laboratory has already presented how *Drosophila melanogaster* activated immune cells release adenosine and in this way actively affect systemic metabolism to fulfill their energetic needs. To further understand adenosine role in energy metabolism, we decided to examine its metabolic origin and other fate inside the activated immune cells. Adenosylhomocysteinase (Ahcy) acts in S-Adenosylmethionine (SAM) cycle and converts S-Adenosylhomocysteine into homocysteine and adenosine. SAM cycle is part of one-carbon metabolism and facilitates biomolecule methylation, polyamines synthesis, cellular redox control, etc. There is growing evidence supporting the significance of this pathway for different functional aspects of the immune cells in the last few years. ¹³C-methionine tracing showed us that SAM cycle accelerates in activated immune cells and experiments with *Ahcy* RNAi confirmed its important role as an adenosine producer. The first step of the SAM cycle is a unique reaction in which the adenosyl moiety of ATP is consumed along with methionine to synthesize SAM. Since SAM cycle accelerates in activated immune cells, it consumes a huge amount of ATP. This ATP can be refilled by *de novo* purine synthesis or just quickly recycled back into ATP through the coordinated action of Adenosine kinase, Adenylate kinase, and glycolysis. ¹³C-adenosine tracing showed adenosine incorporation into AMP, ADP, and ATP. Moreover, adenosine incorporates into SAM and this incorporation is increasing upon infection reflecting the SAM cycle acceleration. Considering SAM cycle rate as a reflection of cellular activity and adenine nucleotides levels as a cellular energy state indicator, our data are implying that adenosine is a sensitive detector of cellular activity and energy state balance and can regulate systemic energy metabolism to further keep this balance.

Keywords: Adenosine, SAM cycle, energy metabolism, Adenosine kinase, Adenylate kinase, immunity.

*Speaker

Adipocyte-Derived Amino Acid Storage Proteins Regulate Distinct Steps of Oogenesis

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Nutrient availability, stress, age, and other physiological changes influence stem cell lineages. Adult stem cell populations mediate tissue homeostasis, which is intimately linked to the physiology of an organism. Multiple endocrine organs (such as adipose tissue, muscle, and the gut) relay physiological status to other organs (such as the ovary) through circulating factors to influence their function. However, disruption of signaling from endocrine organs often leads to pathologies such as obesity, increased cancer risk, or organ failure. My lab is interested in understanding how organs communicate their physiological status to one another to maintain optimal tissue function, including oogenesis. I previously found that the nuclear receptor, Seven Up (Svp) is required in adult female adipocytes to regulate distinct steps of oogenesis. RNA sequencing analysis from purified fat bodies and oenocytes showed that genes related to detoxification and amino acid storage are significantly decreased in the fat body when svp is reduced. Amino acid storage proteins are hexamerins that are primarily synthesized in the fat body during the larval feeding period and reabsorbed by the fat during metamorphosis to provide building blocks for adult tissues. My recently established lab has found that adipocyte-derived amino acid storage proteins are required in adult females to regulate distinct steps of oogenesis. We found that adipocyte-specific loss of the amino acid storage protein receptors Fat Body Protein 1 (Fbp1) and Fbp2 significantly decreases GSC number without any additional effects on oogenesis. Furthermore, the amino acid storage proteins Lsp1 alpha, Lsp1 beta, and Lsp1 gamma are required in adipocytes for survival of vitellogenic follicles. We are currently testing whether adipocyte-derived amino acid storage proteins are either: 1) secreted into the adult hemolymph and transported to the ovary to regulate distinct stages of the GSC lineage; or 2) required cell autonomously in the adult fat body to remotely control ovarian function by catabolizing amino acid storage proteins. Our work will provide insight to how adipose-derived secreted factors influence oogenesis and ovarian tissue homeostasis.

Keywords: adipocytes, amino acid storage proteins, oogenesis, inter, organ communication

*Speaker

Chronic ingestion of *Bacillus thuringiensis* spores promotes inflammation and metabolic disorders in the *Drosophila* intestine

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Bacteria of the genus *Bacillus* have a two-state life cycle: the vegetative cell capable of proliferation and the spore, a form resistant to adverse environmental conditions. The ingestion of spores, in contrast to vegetative bacteria, has been little studied and much remains to be discovered about their behavior and impact in the gut. Our laboratory focuses on the study of the Gram-positive *Bacillus cereus* group, and in particular on *Bacillus cereus* (*Bc*) and *Bacillus thuringiensis* (*Bt*) which are genetically very similar. Unlike *Bc*, *Bt* synthesizes during sporulation specific entomopathogenic toxins, named Cry, thanks to which several strains of *Bt* are widely used as insecticides in agriculture. *Bc* is known as an opportunistic pathogen responsible for gastrointestinal syndromes and is the second most common cause of foodborne outbreaks in France. We use *Drosophila melanogaster*, a non-target insect of *Bt* insecticides, to study their unintended effects. Here we show that chronic dietary ingestion of low environmental doses of *Bc/Bt* spores reduces the lifespan of *Drosophila*. We examined signs of inflammation and aging in the midgut, such as overall morphology, stem cell proliferation, progenitor mis-differentiation, enteroendocrine cell number, cell junction alteration, permeability and oxidative stress. We also analyzed the time course of spore germination in the gut. To further characterize the molecular processes involved in the deregulation of intestinal physiology, we performed a transcriptome analysis of *Drosophila* midguts. In addition to oxidoreduction and inflammation, the results have revealed changes in the expression of genes involved in sugar and lipid metabolism. We are currently performing measurements to assess metabolic changes in the midgut. Overall, our project strengthens the fundamental knowledge on the relationship between spore-forming allochthonous bacteria, which are widely present in the environment, and the intestinal response.

Keywords: *Bacillus thuringiensis*, spores, inflammation, metabolism, intestine

*Speaker

Coordination of the starvation response across cell types in a complex tissue

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¹, Marc Amoyel ¹

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The nutritional status of an organism has profound effects on its health and survival. During starvation, tissues undergo changes in response to systemic signals to better adapt to the limiting conditions. A common response of many tissues is to involute and undergo a reversible reduction in size, modulated by the regulation of stem cells that reside within the tissue. In the *Drosophila* testis, starvation leads to a drastic reduction in the number of both germline stem cells and somatic stem cells (called cyst stem cells) that reside in the tissue, as well as early differentiating cells. However, how the different cell types in the tissue coordinate the starvation response and which systemic signals drive these changes in the testis is still poorly understood. In one part of the study, we aim to understand whether different cell types respond to starvation autonomously or have non-autonomous influences from neighbouring cells. To this end, we upregulated starvation responsive Insulin signalling in cyst cells and observed that it rescued the starvation induced death of differentiating germ cells. This indicates that cyst cells have a non-autonomous effect on the survival of germ cells in starvation. We are currently performing experiments to understand the molecular basis of the rescue. In second part of the study, we want to understand if and which distant tissues have a role in driving the starvation response in testis. To this end, we are utilising a candidate-based genetic screen approach, whereby we knockdown metabolic and starvation response signalling pathways in the fat body and gut – critical nutrient sensing tissues – and determine if the starvation response in the testis is affected. Preliminary results indicate that downregulating sugar transporters in the fat body leads to a starvation-like phenotype in the testis under fed conditions, pointing towards a putative role of metabolites in starvation signalling. We are currently trying to dissect the molecular mechanism of how sugar transporters might be important in the process. Overall, our work will shed light on the systemic and tissue-level coordination of the starvation response in a complex tissue.

Keywords: Starvation, Stem Cells, Insulin Signalling, Testis

*Speaker

Deranged Larval Muscle Phenotype is Associated with Pupal Lethality in Alternative Oxidase-Expressing Flies Cultured on Low-Nutrient Diet

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The xenotopic expression of the alternative oxidase (AOX) from the tunicate *Ciona intestinalis* provides an alternative pathway for oxygen reduction and coenzyme Q reoxidation in mitochondria, with the possibility of rescuing deleterious phenotypes associated with respiratory chain complex III and/or IV diseases in humans. Despite this potential benefit, we have previously shown that the ubiquitous expression of AOX in *Drosophila* cultured under low-nutrient condition can lead to severely decreased pupal viability and body size, and an extensive rearrangement in the levels of all amino acids in L3 larvae. We hypothesized that the architecture of the larval musculature was compromised, so we quantified muscle area and general myofibril arrangement in different positions along the antero-posterior body axis of the larva, using confocal microscopy. We observed no changes in nuclei number per larval muscle fiber, indicating that the initial process of myoblast fusion during embryogenesis occurred normally. However, visceral larval (VL) 3 and VL4 muscles in AOX-expressing larvae cultured on a standard diet and in control larvae cultured on low-nutrient diet were 10-15% smaller than controls. Additionally, several muscle phenotypes were observed in AOX-expressing flies cultured on low-nutrient diet, including individuals with decreased muscle tone, others with F-actin bundles extending throughout the musculature, and animals without any detectable structural changes. These most severe structural phenotypes are correlated with significantly decreased larval mobility and with pupal lethality. Our results suggest that AOX expression leads to decreased muscle cell volume, which in combination with low nutrition is severely aggravated, resulting in poor pupal development. Further analysis is needed to establish the correlation between muscle mass, mitochondrial metabolism, and the dietary effects on muscle development.

Keywords: *Drosophila melanogaster*, AOX, Larval muscle

*Speaker

Dietary sugar and protein differentially regulate the insulin and IGF1 homologs Dilp2 and Dilp6 in *Drosophila*.

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The *Drosophila* genome encodes seven insulin-like peptides (Dilps) that are made by distinct cell types and predicted to bind to a single known insulin receptor to regulate growth and metabolism. A group of fourteen neurons in the brain secretes Dilps 2, 3 and 5 into circulation, and the fat body, a liver-like organ that also mediates the humoral response to infection, secretes Dilp6. A key question in an endocrine system with many ligands is whether they respond uniformly to different stimuli. In fact, our data show that the opposite seems to be the rule in the Dilp-insulin receptor system. We find opposing effects of infection/immune signaling and dietary nutrients on hemolymph levels of distinct Dilps. We used dual-epitope tagged *Dilp2* and *Dilp6* alleles to measure hemolymph levels of each hormone by ELISA in male and female larvae. Starvation of mid-third instar larvae for 24 hours led to a 90% reduction in circulating Dilp2 but only a 33% decrease in circulating Dilp6 levels. We used a nutritional geometry approach to assess responses of Dilp2 and Dilp6 to the major components of the fruit fly diet: sugar and protein, supplied as yeast extract. Hemolymph Dilp2 was dose dependently recovered from starved levels by feeding larvae yeast extract but not sucrose. Interestingly, elevated and imbalanced ratios of sucrose to yeast led to modest reductions in circulating Dilp2. In contrast, circulating levels of Dilp6 were increased by a sucrose-only diet, but surprisingly, hemolymph Dilp6 was strongly suppressed by yeast in the diet. Additionally, we find that whole-animal glycogen and triglyceride storage levels strongly and positively correlate with dietary sucrose and Dilp6 but not dietary yeast and Dilp2. These data show that Dilp2 and Dilp6 secretion are regulated differently by environmental conditions ranging from infection, as shown previously, to dietary composition. Our work raises the questions of how the single *Drosophila* insulin receptor makes sense of these divergent signals, what information is conveyed by each ligand, and whether target cells exhibit distinct responses to different Dilps.

Keywords: Dilp, growth, nutrient storage, fat body

*Speaker

Drosophila can utilize phosphatidylcholine supplied by commensal bacteria

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The gut microbiota plays a vital role in animal health. One major function of the host-microbe interaction is ameliorating malnutrition by supplying metabolites to its host. However, its molecular mechanism is yet to be fully elucidated. *Acetobacteraceae* and *Lactobacillaceae* are the two main families that make up *Drosophila* gut microbiota. Using metabolome analyses, we identified that *Acetobacter persici* but not *Lactiplantibacillus plantarum* can produce phosphatidylcholine (PC) from phosphatidylethanolamine. This pathway is also conserved in mammals, but not in *Drosophila*. We tested whether *A. persici* or lyso-PC, a PC metabolite, can rescue the phenotypes of choline deficiency such as repressed pupariation in larvae and decreased fecundity, increased starvation, and shortened lifespan in adult flies. We also investigated the involvement of *PmtA*, a gene required for PC biosynthesis in *A. persici*, on the phenotypes of flies under choline deficiency. In this presentation, I will discuss how a bacterial metabolite, or a bacterial gene is sufficient to alleviate the effects of a detrimental nutritional supply such as choline deficient diet.

Keywords: commensal, bacteria, phosphatidylcholine, holidic medium

*Speaker

Enzymes and interorgan communication controlling *Drosophila* female sterol and steryl ester homeostasis

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Sterols are essential lipids with multifaceted biological functions as diverse as signalling, the hormonal control of metabolism, and membrane homeostasis. Like all insects, *Drosophila* is sterol-auxotroph which makes this model particularly suitable to study the organismal management of dietary sterols in the context of reproduction and aging. Sterol metabolic flexibility of the fly is increased by the conditional storage and mobilization of steryl esters (SE) in intracellular lipid droplets mainly in the fat body. We functionally characterized the central fat body enzymes for sterol/SE interconversion: the anabolic Sterol *O*-acyltransferase (SOAT) and the catabolic Hormone-sensitive lipase (Hsl). Hsl is essential for the organ-specific SE mobilization from the fat body in the context of intergenerational sterol transfer from mother to embryo. Consistently, Hsl function safeguards female reproductive success under dietary sterol limitation. Conversely, SE stores are depleted in SOAT mutant flies, which are short-lived even under continuous dietary sterol supply. Importantly, our study reveals a metabolic crosstalk between ovaries and fat body in female sterol homeostasis, which prioritizes sterol supply to ovaries (and therefore reproduction) upon dietary sterol depletion. Ovary degeneration impairs the SE efflux from the fat body which causes SE accumulation in this organ and hypersterolemia in the hemolymph. Notably, ovary dysfunction not only compromises organismal sterol/SE homeostasis but causes profound changes in various classes of the female fly lipidome. Collectively, our study supports a model of ovary-induced SE mobilization from the fat body as part of the female post-mating response. Preliminary data involve ecdysone signalling in the mating-dependent control of sterol metabolism in *Drosophila* females.

Keywords: sterol, Hormone sensitive lipase, Sterol O acyltransferase, reproduction, fat body, ovary

*Speaker

FlyCyc: updating the metabolic network for *Drosophila melanogaster*

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BioCyc is a collection of metabolic networks for over 20,000 species. The BioCyc ‘Pathway Tools’ software can generate a metabolic network by matching reactions/pathways in its reference database (MetaCyc) with the set of enzymes encoded by given genome. The quality of the metabolic reconstruction therefore depends on the accuracy and completeness of enzymatic annotation of that genome. A BioCyc model for *Drosophila melanogaster* (FlyCyc) exists but is based on data from a FlyBase release 15 years ago and therefore does not include more recent changes to genomic and functional annotations.

We have conducted a systematic review of *Drosophila* enzymes, improving the coverage and accuracy of their functional annotations (Gene Ontology (GO) and Enzyme Commission (EC)) in FlyBase. Overall, we verified ~3,750 *Drosophila* enzymes and made ~4,000 changes to manual annotations. We have also improved access to enzymatic data within FlyBase by displaying EC information and chemical reaction graphics (from the RHEA database) in relevant gene reports, and by creating accessible ‘gene group’ pages representing each enzyme class/subclass.

The revisions to enzyme annotations have allowed us to compute a new FlyCyc that also incorporates the latest genomic and gene nomenclature data. Compared to the previous version, the updated FlyCyc includes > 50 additional metabolic pathways and identifies > 600 additional enzyme-encoding genes. However, a number of ambiguous enzyme mappings and ‘pathway holes’ remain - as far as possible, these are being resolved by correcting GO/EC annotations within FlyBase. Once finalized, the new FlyCyc will be made available on the BioCyc website and via FlyBase, thereby providing researchers with much-improved *Drosophila* metabolic pathway diagrams and enhanced capabilities to analyse metabolomic datasets.

Keywords: Metabolic pathway, metabolic network, enzymes, BioCyc, FlyCyc, FlyBase

*Speaker

Functional genomics of *Drosophila* larval gut response to *Lactiplantibacillus plantarum* reveals microbe-mediated and Ecdysone-dependent intestinal tissue growth

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The juvenile growth period is crucial, as both acute and chronic undernutrition can result in severe wasting, stunting, and, in extreme cases, childhood mortality. Using *Drosophila* and mouse gnotobiotic models, we have demonstrated the evolutionarily conserved impact of intestinal microbiota and selected lactobacilli strains on promoting linear growth in animals. In this context, the first aim of our study was to identify the molecular mechanisms promoting systemic growth in *Drosophila* larvae. Since the midgut acts as the interface between the entire organism and intestinal bacteria, we conducted a comprehensive larval midgut RNAseq analysis. Among the various biological processes, we identified a microbiota-mediated "Ecdysone signaling" emerged. Interestingly, it appears that intestinal Ecdysone signaling is not a limiting factor for systemic growth upon microbe association, but it is indeed required for microbe-mediated intestinal tissue growth. Finally, our study demonstrates that the midgut serves as a unique organ that benefits from an Ecdysone-mediated growth effect upon microbiota association. This discovery highlights a novel role for the pleiotropic hormone Ecdysone in microbe-mediated intestinal growth.

Keywords: Tissue Growth, Ecdysone, Microbiota

*Speaker

Functional-metabolic coupling in distinct renal cell types coordinates organ-wide physiology and supports healthy ageing in vivo

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The coordination of a cell's physiological role and its metabolic programming is emerging as a vital relationship underpinning tissue health and longevity. Despite its importance, this functional metabolic coupling within whole tissues remains poorly understood due to the complexity and plasticity of metabolic networks. Here, we establish the *Drosophila* renal system as a paradigm for linking mechanistic analysis of metabolism (at single cell resolution) to organ-wide physiology.

Kidneys are amongst the most energetically demanding organs, with the human kidney accounting for just ~0.4% of our body mass yet consuming ~7% of our total oxygen intake. This energy supports vital life-sustaining processes such as waste excretion and osmoregulation, roles that are facilitated by a heterogeneous population of renal cell sub-types. However, exactly how individual cells fine-tune their metabolism to meet their diverse and unique physiologies over the life course remains unclear.

Using the *Drosophila* renal (Malpighian) tubules and integrating *in vivo* live-imaging and spatio-temporal genetic perturbation, we have uncovered the distinct metabolic signatures of functionally diverse cell subtypes that are essential to support robust renal physiology. We find that diversion of key metabolic pools is vital to support cellular antioxidant regeneration and delay premature renal senescence, whilst intense energetic demands are met by partitioning of alternative metabolites. We suggest *Drosophila* renal tubules will offer a powerful platform to decipher complex intercellular metabolic relationships within intact heterogenous microenvironments *in vivo*.

Keywords: metabolism, kidney, renal, physiology, lipids, antioxidant, PPP, glutathione, malpighian, stress, in vivo, peroxisome

*Speaker

How diet-microbiome-host interactions remodel the fly metabolic landscape, appetite, and physiology

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Diet is a key determinant of life- and healthspan in all animals, including humans. While essential amino acids (eAAs) are nutrients that need to be acquired from the diet, AA overconsumption also negatively impacts lifespan and reproduction. Given the importance of a balanced dietary intake of AAs, organisms direct their feeding choices to homeostatically compensate both for the lack and over-ingestion of AAs. Furthermore, gut bacteria are now well-established modulators of host physiology and behavior. The impact of commensal bacteria on the host arises from complex microbial-diet-host interactions. Mapping metabolic interactions in gut microbial communities is therefore key to understanding how the microbiome influences the host. Our lab has shown that two of the main bacteria of the *Drosophila melanogaster* microbiome, *Acetobacter pomorum* (*Ap*) and *Lactobacillus plantarum* (*Lp*), reduce the protein appetite of flies deprived of eAAs. Using a combination of chemically defined diets, bacterial manipulations, and isotope-labeled metabolomics we showed that *Ap* and *Lp* exchange metabolites to become resilient to detrimental host diets and modulate food choice. We are now harnessing untargeted and Isotope-resolved metabolomics to understand the impact of both dietary perturbations and microbiome on the metabolism of different fly tissues. Both eAA deprivations and bacterial treatments led to significant tissue-specific changes in metabolism. Given that commensal bacteria suppress protein appetite in eAA-deprived flies, one would expect that the microbes would revert the metabolomics profile of flies back to a fully fed state. Surprisingly, eAA-deprived flies hosting gut bacteria showed a unique metabolic profile that differed both from the fully fed and eAA-deprived state of germ-free flies. This supports the concept that gut microbes do not simply alter behavior and physiology by providing flies with missing nutrients but induce a novel metabolic state that promotes changes in different host traits. We are currently complementing these data with isotope-resolved metabolomics to identify metabolites synthesized by *Ap* from dietary lactate. Furthermore, by combining these metabolomics approaches with nutritional interventions and bacterial and *Drosophila* genetics, we aim at causally identifying how these metabolic reorganizations contribute to changes in behavior and physiological traits.

^{*}Speaker

Keywords: microbiome, feeding behavior, Acetobacter, Metabolomics

Investigation of the neuroprotective effect of 1,3,4,10-tetrahydroacridin-9(2H)-one in *Drosophila melanogaster*

Andrielle Adelina Teodoro Jesus * ¹, Jessica Maria De Souza ¹, Lucas Campos Curcino Vieira ¹, Anderson Oliveira Souza ¹

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Chronic diseases make up the set of chronic conditions. In general, they are related to multiple causes, are characterized by a gradual onset, and the prognosis is usually uncertain, with a long or indefinite duration. They present a clinical course that changes over time, with possible periods of exacerbation that can lead to disabilities. Degenerative illnesses are characterized by an increase in oxidative and nitrosative stress, mitochondrial dysfunction, improper protein folding or aggregation, loss of synapses, reducing the neuronal survival. Animal models are widely used for understanding these diseases, such as the *Drosophila melanogaster*, which represents a non-traditional experimental model to obtain basic information about the genetics and pathophysiology of various human neurodegenerative diseases. This method has been widely employed to screen a variety of bioactive phytochemicals to prevent or enhance neurobiochemical processes induced in transgenics for Alzheimer's or Parkinson's disease. In this context, we have successfully employed the *D. melanogaster* model to assess the neuroprotective potential of various plant extracts and natural molecules. In this work, we aim to evaluate the effect of the compound 1,3,4,10-tetrahydroacridin-9(2H)-one in *D. melanogaster* constitutively expressing the amyloid precursor protein, involved in Alzheimer's disease. Our results demonstrated significant impairment in the climbing ability and development of *D. melanogaster*. Furthermore, the parameters showed a change in oxidative stress (levels of carbonyl protein and glutathiones in the reduced and oxidized states) and activity of crucial enzymes (superoxide dismutase, citrate synthase and acetylcholinesterase).

Keywords: synthetic compounds, neuroprotective, *Drosophila melanogaster*, biochemical parameters.

*Speaker

Macroglobulin Complement-Related protein (Mcr) acts as a chemoattractant signal for macrophages recruitment upon wound

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The ability to heal wounds is a prerogative of virtually all living organisms. One crucial and evolutionary conserved event taking place during tissue repair is the recruitment of immune cells, which fulfil the critical role of clearing cell debris and invading pathogens. Damaged tissues release damage-associated molecular patterns (DAMPs) that act as a chemoattractant for immune cells that are therefore recruited to the site of injury.

Exploiting the optical transparency and genetic manipulability of early pupal wing, we generate an extensive dataset to monitor haemocyte behaviour in response to laser-induced sterile tissue damage. To gain fresh insight on the nature of the chemoattractant signals, we employed mathematical modelling to identify novel candidate DAMPs, with biophysical characteristics and diffusion properties consistent with macrophage response to wound.

Our top candidate as a chemoattractant signal is the Macroglobulin complement-related protein (Mcr), a component of the septate junction known to play a pivotal role in developmental cell death and inflammation by modulating autophagy in neighbouring cells. Mcr's function involves the immune receptor Draper, implying a connection between autophagy, inflammation regulation, and migration to epithelial wounds.

Our preliminary data show that specific suppression of Mcr expression in the posterior compartment of the developing wing epithelium compromises the recruitment and directionality of circulating haemocytes.

Overall, our findings reveal that Mcr is required in epithelial cells for hemocyte migration to epithelial wounds, positioning it as an excellent candidate for chemoattraction.

Keywords: Macroglobulin Complement, Related protein (Mcr), macrophages, wound healing, chemoattraction

*Speaker

Mapping metabolic programs in a whole-animal at single-cell resolution

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Cellular metabolism converts nutrients into various metabolites that support tissue and organismal functions. Despite the critical role of specific metabolic pathways in regulating animal physiology, we still lack an understanding of the repertoire of metabolic programs in a whole animal and how these programs are organized in a cell-specific manner, at the whole organism and within organ level. This question is challenging due to the large cellular complexity and the lack of tools for comparing metabolic gene expression among individual cells across a whole animal. However, the availability of whole animal cell atlases such as the Fly Cell Atlas (FCA) makes such an approach feasible. Our goal was to use this powerful dataset to profile the transcriptional metabolic signature of all cells in the animal and identify the molecular mechanisms regulating their implementation.

To benchmark our approach, we quantified the transcriptional enrichment of enzymes involved in fatty acid metabolism across the cells in the fly body. We found this pathway to be highly enriched in the fat body and oenocyte cells compared to other cells in the body, consistent with the extensively studied role of these tissues in lipid metabolism. These findings demonstrate that cell-specific metabolic signatures can be identified using our approach.

Next, we expanded our analysis to comprehensively profile all cells in the head and body of the fly for a combination of 23 metabolic pathways. Our analysis revealed that cells could be clustered according to their metabolic signature, mostly recapitulating tissue-specific signatures. Our results show that most metabolic pathways analyzed are not ubiquitously expressed, which supports the idea that specific metabolic capacities could be a determinant of tissue-specific biological functions. To test this hypothesis, we characterized the functional relevance of a few cell-specific metabolic programs by manipulating them and analyzing their effects on tissue and whole-animal physiology. We demonstrate that the strategy we have developed allows for a comprehensive analysis of the metabolic organization of an animal across and within tissues and the discovery of new organ compartmentalizations and cell functions. This approach paves the way for a systematic functional dissection of metabolic programs in a whole animal.

Keywords: metabolism, single cell

*Speaker

Mating-induced digestive switch in *Drosophila* females

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The female's physiological and behavioral changes, such as increased food intake and altered digestive processes, are significant after mating. The female midgut is essential in modulating post-mating energy balance since it is responsible for digestion and nutrient absorption. Several studies have demonstrated that mated female fruit flies prefer a protein-rich diet; this preference was observed by offering mated flies a choice between yeast and sugar patches with varying nutrient contents. NPF (Neuropeptide F), a hormone derived from the gut, was reported to trigger a switch in dietary preference after mating in *Drosophila*. We provided standard fly food containing protein and sugar for the flies during the mating experiment. Compared to virgin flies, our RNA sequencing data sets of mated female flies revealed the upregulation of Jonah genes (serine-type endopeptidase) responsible for breaking down dietary proteins. We will also confirm this by directly testing more efficient protein digestion in mated flies compared to virgin ones as the control. Mated flies preferred digesting more protein than sugar from the provided food, possibly through the induction of endopeptidase enzymes. Jonah's family is expressed twice during the life cycle; it can be detected throughout all larval stages but disappears by the end of the third larval instar; however, it reappears in adults, particularly in the midgut. Ecdysone signaling regulates Jonah's gene expression during the early stages of development, but it is unknown if it does the same in mated adult female flies. We also detected a significant downregulation of genes associated with the oxidative-stress response, including Gsts and Ldh. We hypothesize that the upregulation of Jonah endopeptidase enzymes results in more efficient protein digestion, which provides mated flies with an increased supply of methionine. Methionine is a precursor for glutathione production, crucial in regulating the oxidative stress response. It is known that Juvenile hormone, ecdysone signaling, and male sex peptide regulate many post-mating effects, including mid-gut growth. We aim to determine whether ecdysone signaling plays a role in regulating the digestive switch towards protein consumption induced by mating and whether these changes can affect any aspect of the oxidative stress response in *Drosophila* females after mating.

Keywords: Mating, digestive switch, Jonah, Ecdysone

*Speaker

Neurotoxicological effects of agrochemicals and their probable degradation by-products in *Drosophila melanogaster*

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The increase in world population in the 20th century would not have been possible without a parallel growth in food production and was achieved due to agrochemicals, which are now essential components of the world agricultural systems, allowing for a remarkable increase in crop yields and the production of food during the last century. Among the compounds used in the formulation of agrochemicals, 1,1'-dimethyl-4,4'-bipyridinedichloride, Paraquat (PQ) is a non-selective herbicide belonging to the chemical class of bipyridines, polar and has been widely applied to control weeds in vegetable crops in several countries. However, its application is prohibited in several countries, mainly on the European continent, due to studies showing damage to public health. This herbicide shows similarity to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a potent dopaminergic neurotoxin. Given its properties, it is vital to find scientific solutions for water contaminated with pesticides that do not originate even more harmful compounds. Therefore, we used *Drosophila melanogaster* as a model, which presents relevant orthology to human genes related to neurobiochemical processes, such as dopaminergic and cholinergic transmission and clearance of proteins as amyloid proteins involved in Alzheimer's disease. In this study, we investigated the influence of the photocatalysis system (radiation and semiconductor) on the degradation of PQ. For this, the organic compounds were degraded only through irradiation (photolysis) and using the Ti/TiO₂ electrode in the presence of irradiation (photocatalysis). We evaluated the neurotoxicity of PQ by-products generated from photolysis and photocatalysis through behavioral and neurobiochemical parameters in *D. melanogaster*. Our results demonstrated significant impairment in the climbing ability and development of *D. melanogaster*. Furthermore, the parameters showed a change in oxidative stress (levels in the carbonyl protein and glutathiones in the reduced and oxidized state) and activity of crucial enzymes (superoxide dismutase, citrate synthase and acetylcholinesterase).

Keywords: Paraquat, Biochemical parameters, Photodegradation, Neurotoxicology.

*Speaker

Oenocytes orchestrate lipoprotein trafficking and systemic lipid metabolism during prolonged starvation in a Desat1-dependent manner

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Liver steatosis induced by starvation or malnutrition is characterized by the accumulation of lipids in hepatocytes. Moreover, the human liver plays important roles in regulating systemic lipid homeostasis under prolonged starvation or fasting and re-feeding cycles. Here, we use *Drosophila* as a model to investigate the role of hepatocyte-like cells, oenocytes, in lipid metabolism. By using a modified starvation holidic medium that allows adult *Drosophila* survival for up to 12 days in starvation, we show that oenocytes take up lipids from the surrounding fat body in the first days of fasting before releasing them back at later stages. We also show that the knockdown of Desat1 specifically in oenocytes leads to higher lipolysis in the fat body as well as higher saturation, shorter carbon length and lower amount of DAG in hemolymph lipids. This indicates a intimate crosstalk between fat body and oenocytes in starvation. Mechanistically, we identify a strong sequestering of Apolpp- and Apoltp-containing lipoproteins by the Desat1-deficient oenocytes. Lipoproteins were found in intracellular vesicles that are surrounded by a dense actin cytoskeleton, preventing lipid storage and release by the oenocytes. Additional cell-autonomous changes suggest that membrane fluidity is strongly perturbed in Desat1-deficient oenocytes. Together, our findings reveal a Desat1-mediated role of oenocytes in controlling lipoprotein trafficking, fat body lipolysis and systemic lipid metabolism in prolonged starvation.

Keywords: oenocytes, lipid metabolism, lipoproteins, desat1

Precancerous oncogenic signaling induces metabolic syndrome

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Host diabetes is a co-morbidity for cancer initiation and progression, implying that diabetic individuals have poor prognoses. However, diabetes is also detected post-cancer diagnosis. It is plausible that oncogenic hits may induce cell-autonomous precancer lesions on the one hand and systemically trigger host diabetes on the other. Here, we have tested this possibility and show that epithelial gain of oncogenic signaling in *Drosophila* systemically induces metabolic syndrome (MetS) in otherwise healthy host larvae. We found MetS-associated phenotypes i.e., obesity, diabetes and dyslipidemia in oncogene-expressing larval hosts culminating into larval mortality without neoplastic growth *per se*. Furthermore, oncogene-secreted Upd and ImpL2, the *Drosophila* homologs of human ILs and IGFBP7, respectively, underpin the systemic trigger for MetS. We further noticed that the severity of MetS induction is linked to the intensity of oncogenic signaling. For instance, an activated Yki oncogene, displaying loss of a single phosphorylation site, YkiS168A, induces MetS at a slow pace. By contrast, the loss of three phosphorylation sites, Yki3SA, displays rapid induction of MetS with accompanying muscle atrophy. Finally, we show that pharmacological suppression of diabetes in animals displaying oncogenic signaling rescues MetS. Overall, these findings suggest that mitigation of oncogene-induced host MetS is likely an adjuvant cancer therapeutic intervention.

Keywords: Cancer, metabolic syndrome, obesity, diabetes, dyslipidemia, muscle atrophy, Unpaired, ImpL2

*Speaker

Sexual Dimorphism in the *Drosophila* Fat Body, a Central Organ in Metabolic Homeostasis

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The last decades have seen a steep increase in the prevalence of obesity, a metabolic disorder characterized by abnormal accumulation of fat. Obesity is associated with diabetes type-2, cardiovascular diseases, and cancer. Importantly, the regulation of fat metabolism and the associated disorders differ between men and women. The mechanistic basis of the differences between sexes is still poorly understood. We investigated the sexual dimorphism of fat metabolism using *Drosophila melanogaster* as a model system and the combination of the *Drosophila* Genetic Reference Panel and Genome Wide Association Studies (GWAS). We analyzed the natural variation in the expression levels of the key genes *bmm*, *Lsd-1*, and *Lsd-2* and found that the expression level of all three is sexually dimorphic. GWAS for all three datasets provided evidence for extensive sexual dimorphism in the genetic and physiological mechanisms that regulate lipid homeostasis and phenotypes associated with fat metabolism. Furthermore, the regulation of *bmm*, *Lsd-1*, and *Lsd-2* expression levels appears to be largely non-overlapping. We identified a variety of candidate regulatory genes with possible roles in establishing and maintaining the sexual dimorphism in fat metabolism. We validated a subset of putative regulatory genes using RNAi-mediated knockdown and assessment of the effect on lipid metabolism using relative lipid area in fat body cells as a proxy. Taken together, our findings highlight the importance of including both sexes in research and provide a resource to identify novel regulatory genes involved in fat metabolism. We anticipate that our results will also facilitate studies of sexual dimorphism in metabolic regulation and associated disorders in other organisms including humans.

Keywords: lipid metabolism, fat body, sexual dimorphism

*Speaker

Spalt related (Salr), a novel metabolism regulator

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Nutrient intake is closely coupled to animal energy metabolism and growth. This is controlled through nutrient sensing pathways, which operate cell intrinsically in specific tissues, as well as through cell-to-cell communication by hormones. How these regulatory networks are wired and how they operate to maintain homeostasis in multicellular animals remains insufficiently understood. Here we uncover that transcription factor Spalt-related (Salr), a known target of the Dpp signaling pathway, is a key regulator of metabolism and growth upon nutrient starvation. We provide evidence that Salr inhibits organismal growth and lipid storage in the *Drosophila* fat body. Our results indicate that Salr inhibits growth by indirectly inhibiting mTOR complex 1 and directly regulating Insulin-like peptide 6 (dILP6). Moreover, we found that Salr promotes lipid catabolism and autophagy, while inhibiting mitochondrial biogenesis. In conclusion, our results reveal an unexpected metabolic role for a transcription factor earlier established as a developmental regulator.

Keywords: growth, lipid metabolism, mTOR, autophagy, starvation

*Speaker

Spontaneous Neurotransmitter Release is Regulated by Unc-5

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Spontaneous neurotransmitter release, also called miniature neurotransmission or ‘minis’, is the trans-synaptic process where single synaptic vesicles, released from presynaptic neurons, induce small postsynaptic electrical currents. Miniature events have been observed at every excitatory chemical synapse studied since their discovery over seventy years ago and are widely used as an electrophysiological proxy for synapse numbers, based upon the fundamental tenant that the frequency of these events is uniform, spontaneous and unregulated. Here, we show that miniature neurotransmission is not in fact a spontaneous process, but can be regulated by a novel synaptic function of the protein Unc-5, best known for its roles in axon guidance. We show that the frequency of miniature events can be independently and bidirectionally regulated by Unc-5 through interaction with the synaptic vesicle release machinery in a Netrin independent function. We further show that loss of synaptic Unc-5 activity, and consequently altered miniature neurotransmission, induces synaptic structural degeneration. Our data question the reliability of miniature event measurements to determine synapse numbers and further support that miniature neurotransmission is a simultaneous but independent parallel mode of synaptic communication with singular essential roles and also regulation.

Keywords: Miniature Neurotransmission, Netrin Signalling, NMJ, Synaptic Biology, Adult *Drosophila*

^{*}Speaker

THE OLFACTORY SYSTEM REGULATES DEVELOPMENTAL TIMING

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What triggers the juvenile-to-adult developmental transition (JDT) is a biological question that concerns all sexually reproductive organisms. Several observations highlighted the effect of non-nutritional environmental conditions in determining the right JDT timing. Emblematic examples include delayed maturation in isolated rats, accelerated development in chickens exposed to high temperatures, and premature pupation in infected female mosquito larvae. Despite these observations, the precise mechanisms by which non-nutritional signals integrate the neuroendocrine system to time the JDT remain elusive. An intriguing possibility is that the sensory organs, responsible for detecting and distinguishing various environmental cues, may provide input to the neuroendocrine axis. Accordingly, the recent complete reconstruction of the *Drosophila* first instar larva connectome revealed indirect inputs from sensory neurons to the insect neuroendocrine system. To investigate this further, we used *Drosophila* genetic tools to functionally screen for sensory neurons involved in timing JDT. This screening led us to pinpoint the *olfactory system as a key non-nutritional input upstream of the neuroendocrine system*. Specifically, individually silencing the activity of several Olfactory Sensory Neurons (OSN) resulted in a significant delay in the onset of JDT, implying their role in establishing the precise timing of this developmental process. Supporting this functional association, we used the trans-tango technique to label secondary neurons and identified PTTH neurons downstream of the olfactory projection neurons during the third instar larval stage, just before the transitional phase. We are currently investigating the molecular mechanisms underlying this connection and its physiological significance. Remarkably, a recent study demonstrated a strong correlation between an enlarged olfactory bulb volume and precocious puberty in humans, suggesting a *potential causal relationship between olfaction and JDT, even in higher organisms*.

Keywords: metamorphosis, PTTH, developmental timing, olfaction, neuroendocrine

*Speaker

The Drosophila Tumor Necrosis Factor Receptor, Wengen, couples energy expenditure with gut immunity

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It is well established that tumor necrosis factor (TNF) plays an instrumental role in orchestrating the metabolic disorders associated with late stages of cancers. However, it is not clear whether TNF/TNF receptor (TNFR) signaling controls energy homeostasis in healthy individuals. Here we show that the highly conserved Drosophila TNFR, Wengen (Wgn), is required in the enterocytes (ECs) of the adult gut to restrict lipid catabolism, suppress immune activity and maintain tissue homeostasis. Wgn limits autophagy-dependent lipolysis by restricting cytoplasmic levels of the TNFR effector, TNFR associated factor 3 (dTRAF3), while it suppresses immune processes through inhibition of the dTAK1/TAK1-Relish/NF- κ B pathway in a dTRAF2-dependent manner. Knocking down dTRAF3 or overexpressing dTRAF2 is sufficient to suppress infection-induced lipid depletion and immune activation, respectively, showing that Wgn/TNFR functions as an intersection between metabolism and immunity allowing pathogen-induced metabolic reprogramming to fuel the energetically costly task of combatting an infection.

Keywords: TNF gut homeostasis infection immunity metabolism

*Speaker

The effect of diet supplemented with *Caryocar villosum* (Aubl.) Pers extracts against cell aging in *Drosophila melanogaster*

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The current scenario shows a gradual aging of the Brazilian population, as a result of a reduction in mortality and fertility levels, improvement in the population's living conditions, as well as the evolution of medical technology. Due to one of the greatest biodiversity on the planet, located mainly in the Amazon region, Brazil has a high number of exotic fruits with substances capable of altering metabolic aspects related to the prevention or reduction of chronic and degenerative diseases. As the main mechanisms that cause brain damage are neuronal apoptosis, inflammatory reaction, disruption of the blood-brain barrier and oxidative stress, these antioxidant compounds act by neutralizing or inhibiting reactive oxygen species (ROS) or reactive nitrogen species (RNS). Studies with the hydroethanolic extract of *Caryocar brasiliense* showed a positive result when inhibiting cholinesterase in rats, as well as the hydroethanolic extracts of *Caryocar villosum* showed an increase in the ability to stabilize free radicals of both reactive oxygen species and reactive nitrogen species. In this context, the scientific study proposes to observe and analyze the results of the application of *C. villosum* extract in relation to the locomotion and climbing activity of *Drosophila melanogaster* and the antioxidant activities present in the fruit, as well as its possible contributions to neuroprotective mechanisms. Our results demonstrated significant impairment in the climbing ability and development of *D. melanogaster*. Furthermore, the parameters showed a change in oxidative stress (levels in the carbonyl protein and glutathiones in the reduced and oxidized state) and activity of crucial enzymes (superoxide dismutase, citrate synthase and acetylcholinesterase).

Keywords: Amazon compounds, neuroprotective, *Drosophila melanogaster*, biochemical parameters.

*Speaker

The influence of *Piranhæ trifoliata* and *Himatanthus sucuuba* extracts in *Drosophila melanogaster*

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The use of medicinal plants reflects the reality of a part of human history, and the Brazilian population with limited access to public health programs led to the development and conservation of ethnobotanical knowledge-rich information regarding medicinal plants. The Amazon rainforest is an essential source of scientific knowledge, so several research groups seek to understand the role of Amazonian compounds in diseases. Among the vast flora, *Piranhæ trifoliata* and *Himatanthus sucuuba* demonstrated significant biological activities. Studies suggest the use of the trunk bark of *P. trifoliata* in the treatment of inflammation in the uterus in sitz baths and for teas in the treatment of malaria. The use of latex and trunk bark from the *H. sucuuba* showed anti-inflammatory and analgesic action, therefore, indicated in the treatment of arthritis and edema. However, the neuroprotective action by *P. trifoliata* and *H. sucuuba* extracts remains unclear. Therefore, we used *Drosophila melanogaster* as a model, which presents relevant orthology to human genes related to neurobiochemical processes, such as dopaminergic and cholinergic transmission and clearance of proteins as amyloid proteins involved in Alzheimer's disease and aging. In this study, we investigated the development (mortality and axial ratio), behavioral (climbing ability), and biochemical parameters in *D. melanogaster* exposed to aging. Our results demonstrated significant impairment in the climbing ability and development of *D. melanogaster*. Furthermore, the parameters showed a change in oxidative stress (levels in the carbonyl protein and glutathiones in the reduced and oxidized state) and activity of crucial enzymes (superoxide dismutase, citrate synthase and acetylcholinesterase).

Keywords: Amazon plants, antioxidant assay, *Drosophila melanogaster*, ageing protection, natural product.

*Speaker

The role of ecdysteroid signaling in the regulation of adult metabolism

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Ecdysone is a well-known regulator of insect metamorphosis, but its post-developmental functions have received relatively little attention. Nevertheless, several studies suggest that ecdysteroid signaling also plays important roles in adult insects, although their ecdysone titer is relatively low. Our research aimed to investigate the potential link between the ecdysteroid pathway and fat metabolism in adult flies. We used two main approaches: we examined correlations between obesity and ecdysone levels, and performed direct genetic manipulations targeting the ecdysteroid pathway. First, we induced obesity through dietary manipulations and assessed the concomitant changes in the expression of ecdysone biosynthesis genes and levels of ecdysone. In females, we also analyzed the impact of obesity on the organs secreting ecdysone - ovaries. Subsequently, we down-regulated ecdysteroid signaling using GeneSwitch-driven RNAi and studied its consequences on metabolism. Our results showed that obesity alters the expression of the examined ecdysteroidogenic genes and ecdysone titer. Obesogenic diets also induced notable changes in the morphology of ovaries. In addition to the whole-body manipulations, we have conducted fat body-specific RNAi, which revealed tissue-autonomous roles of this pathway in energy metabolism. Altogether, our experiments indicate that ecdysteroid signaling is essential for energy homeostasis in adult fruit flies. The study thus also expands our understanding of the multifaceted roles of ecdysone beyond its well-established role in insect metamorphosis.

Keywords: ecdysone, ecdysteroid signaling, fat metabolism, obesity

*Speaker

The structural basis of sugar metabolite recognition by the conserved sugar tolerance transcription factor Mondo

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Organisms sense and respond to nutrients by tuning gene activity, typically through signal transduction. In prokaryotes, carbohydrate metabolites also directly bind transcriptional switches, yet no direct interactions have been demonstrated for eukaryotic transcription. Here, we show that the conserved transcription factor Mondo directly binds to key metabolites, including specific sugar metabolites. We identified these metabolites through a candidate ligand screen and complementary biophysical assays that validated their binding to Mondo's glucose sensing module. We determined the molecular basis for the selective recognition of a specific sugar metabolite using crystallography, mutagenesis and ligand analogs. Point mutations that selectively impaired metabolite interaction disrupted the glucose-induced transcriptional activation of physiological targets in cultured primary mouse hepatocytes and sugar tolerance during *Drosophila* development *in vivo*. Our findings reveal a direct interface between glucose metabolism and transcription in eukaryotes, establish a conserved and vital sugar-tolerance function of the metabolite-sensing sugar receptor Mondo, and enable transcription factor-targeted small molecule therapies for metabolic diseases.

Keywords: transcription, metabolism

^{*}Speaker

Transcriptional mechanisms regulate secretory capacity in response to nutrient uptake

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Secretory capacity is vital for an organism to utilize nutrients appropriately. Secretion is important for digestion and distribution of metabolites. Particularly, lipids require secretion into circulation either to be stored or utilize throughout the body. In addition, in response to nutrients secretion is needed for organ-organ communications via hormones, such as insulin. We have found that endoplasmic reticulum (ER) protein sorting machinery is transiently upregulated upon feeding. Our previous studies have identified the transcription factor CrebA in *D. melanogaster*, as a key regulator of these genes in response to nutrients. We have been investigating the mechanisms regulating CrebA in response to nutrient uptake. The unfolded protein response (UPR) pathway has been shown to regulate CrebA transcription family members (Khan et al 2019). However, in response to feeding our data indicates that the UPR pathway is not involved, unlike the mechanistic target of rapamycin (mTOR) pathway. Here we show the mTOR pathway regulates CrebA protein and transcript level. The kinetics of mTOR activation was extremely rapid, within 5 minutes, whereas CrebA protein level's peak in 2 hours after refeeding. The CrebA transcript is upregulated within 1 hour which suggests that mTOR regulates CrebA on the transcriptional level. We are investigating the nutrient-dependent transcriptional regulation of CrebA by mTOR pathway.

Keywords: Secretion, CrebA, mTOR, nutrients

*Speaker

Transcriptome and proteome analysis to investigate sex differences and the reproductive plasticity of the intestine

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Male and female flies differ in many aspects of their development, physiology, and behaviour. Previous work has revealed extensive sex differences at the transcriptional level in non-reproductive organs such as the adult intestine. These sex differences are physiologically important: they affect metabolism, the gut's susceptibility to tumours and infection, and its ability to resize postmating to sustain reproduction. Whether and how these transcriptional sex differences impact the proteome has not been comprehensively tested, and we currently lack information about how the proteome of non-reproductive organs differs between the sexes.

Here we use bulk RNA sequencing and mass spectrometry to examine the transcriptome and proteome of virgin male, virgin female, and mated female flies, as well as their dissected adult midguts. By analysing these datasets, we obtain differentially expressed genes/proteins between conditions, which describe sex differences (comparison between virgin male and virgin female flies) and reproductive plasticity (comparison between mated female and virgin female flies). By conducting a comparative analysis between the transcriptional and protein datasets, we confirm previously described sex and reproductive differences in carbohydrate and lipid metabolism, but also reveal sex and reproductive differences that are only apparent at the protein, but not transcriptional level.

Our dataset will provide a valuable resource for future studies aimed at investigating sex differences and reproductive plasticity in non-reproductive organs.

Keywords: Sexual dimorphism, Post-mating responses, Metabolic pathways, RNAseq, Label-free quantification

*Speaker

Transgenerational changes in glucose metabolism due to maternal zinc deficiency in *Drosophila melanogaster*: effects on *dilp2* and *Pepck* mRNA expression

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Maternal nutrition is crucial for optimal pregnancy outcome and offspring's later life health. In this study, we investigated the effects of maternal zinc deficiency on glucose metabolism in three generations of fruit flies. Virgin female flies reared on a zinc-deficient diet (diet supplemented with 100 μ M of TPEN (N,N,N',N'-tetrakis (2-pyridylmethyl)-ethylenediamine)) were mated with normal diet-fed male flies to generate F1, F2, and F3 offspring. Offspring were maintained on a normal diet for seven days and analyzed for biochemicals (glucose, trehalose, glycogen, triglycerides) and gene expression (*dilp2* and *Pepck* mRNA). The parent (F0) showed a significant increase in glucose levels, while there was no difference observed in male and female offspring at F1 and F2. However, a significant reduction in glucose levels was observed in F3. Similarly, trehalose levels significantly increased in F0, reduced in F1, but significantly increased again in F2 and F3. Glycogen levels were reduced in F0, increased in F1 (male), but significantly decreased in F2 and F3. Triglyceride levels increased in F0 and F1 but reduced in F2 and F3. The fold change in *dilp2* mRNA from F0 to F3 showed a significant increase, while *Pepck* mRNA significantly increased in F0 but decreased in F1. For F2 offspring, male had an increase in *Pepck* mRNA while the female showed a decrease. Furthermore, F3 male and female flies had a significant increase in *Pepck* mRNA. Although various homeostatic mechanisms exist in flies to regulate biochemical indices, epigenetic programming could determine biochemical outcomes through gene expression regulation. Our study suggests that maternal zinc deficiency could programme for various biochemical perturbations with transgenerational implications. The findings highlight the importance of maternal zinc status as an important variable in metabolic disease prevention.

Keywords: zinc deficiency, glucose metabolism, maternal, transgenerational, *dilp2*, *pepck*

*Speaker

Unbalanced Cellular Redox Caused by the Expression of the Alternative Oxidase During *Drosophila* Development

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AOX, a mitochondrial alternative oxidase found in tunicates like *Ciona intestinalis*, but not in vertebrates and insects, shows potential therapeutic applications for treating human mitochondrial diseases. The enzyme bypasses respiratory chain complexes III and IV, providing an alternate pathway for oxygen reduction and coenzyme Q reoxidation, ensuring continued metabolic flux even when the electron transfer system is compromised. We have previously shown that AOX expression in *Drosophila* causes a dramatic rate of pupal lethality when the larvae are cultured under nutritional limitation. AOX-expressing flies appear to downregulate the glycerophosphate shuttle and upregulate Lactate dehydrogenase (Ldh), two complementing systems for cytosolic NAD⁺ reoxidation, necessary for larval growth. Here, we aimed at testing the genetic interactions between *AOX*, *Ldh* and *Gpo1*, the main coding gene for the mitochondrial component of the glycerophosphate shuttle in flies. Overexpressing *Gpo1* did not result in any visible developmental alterations under a standard rich diet, but remarkably caused nearly complete rescue of the AOX-induced lethality in low-nutrient diet. Overexpression of *Ldh*, on the other hand, severely enhanced the lethality phenotype and affected the development of AOX-expressing flies even when cultured on standard rich diet. It is possible that *Gpo1* overexpression caused a metabolic compensation towards an increased use of the glycerophosphate shuttle, and a decreased use of Ldh, restabilizing normal development. It is yet to be shown why AOX drives this unbalanced developmental redox state. Our findings demonstrate a genetic interaction between *AOX*, *Ldh* and *Gpo1*, and may contribute to our understanding of the effectiveness of possible future treatments using AOX as a therapeutic enzyme.

Keywords: respiratory chain alternative enzymes, oxidative phosphorylation, lactate dehydrogenase, glycerophosphate shuttle

*Speaker

Immunity & symbiosis

A method for long-term maintenance of germ-free flies using aseptic isolator

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The generation and maintenance of germ-free (GF) and gnotobiotic (GB) animals serves as an important strategy for investigating the causal effect of microbiota on hosts. Commonly, germ-free *Drosophila* is generated by sterilizing embryos by hypochlorite treatment, followed by transfer to a vial containing autoclaved food sometimes supplemented with antibiotics. In this study, to avoid any possible side effects of early-life hypochlorite exposure on fly biology, the usage of antibiotics, and the risk of microbe contamination certainly, we established a method that can provide GF and GB flies of several generations later upon sterilization by maintaining those flies for multiple generations. Our method consists of an optimized procedure for embryo sterilization, a vinyl isolator system, and the use of GF and GB flies from the second generation onward. In this presentation, we would like to show the effect of micronutrients provided by microbiota by examining traits of GF flies using our technique and discussing the usability of our germ-free fly method.

Keywords: microbiota

*Speaker

Approaching the mechanistic basis of Disease tolerance in *Drosophila* *melanogaster*

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Immune response against infections can be divided into mechanisms of Resistance that ensure active pathogen elimination, and of Disease Tolerance, which include host-tissue protection and repair processes that will return the host to physiological homeostasis. Studies trying to understand host responses to infection have mostly targeted mechanisms of Resistance, and consequently, these are now well-described in both vertebrates and invertebrates. However, the mechanistic basis of Disease Tolerance is poorly understood, in part because both mechanisms interact and are difficult separate. To tease apart these components, we have developed a protocol of oral exposure of *Drosophila melanogaster* to inactivated *Pseudomonas entomophila*, hence, minimizing the Resistance component of the host response. To get to the mechanisms underlying the variation in host Disease Tolerance, we have applied this protocol to ~200 *Drosophila melanogaster* isogenic lines (DGRP) and measured fitness traits (survival and reproduction). Using this protocol, we observed considerable host genetic variation for these traits that show weak correlation with one another. The observed host variation is highly sexually dimorphic and differs according to the level of pathogenicity of the bacterium species to which it was exposed. In addition, we will be presenting a preliminary account of a genome-wide association study on these lines, for these traits, that includes functional validation of candidate genes and the first glimpse at the genetic bases for Disease Tolerance mechanisms. Furthermore, we will compare these data with two more datasets, one using exposure to non-virulent strains and, the other, utilizing host mutants incapable of mounting immune responses. With this comparison, we partition the effects and mechanisms of Disease Tolerance into its immunopathology and infection-induced damage components.

Keywords: *Drosophila* immunity, Disease tolerance, Immunopathology, GWAS

*Speaker

Edin, another AMP in the whole?

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Invertebrates, including insects, rely exclusively on the physico-chemical barrier and the innate immune system to cope with pathogens and maintain tissue homeostasis. To date, two main components of the innate immune system have been characterized: the cellular response and the humoral response. While the cellular response is mediated by specific types of immune cells called hemocytes, the humoral response is mainly characterized by the induction and secretion of specific proteins such as antimicrobial peptides (AMPs). Although they have been studied over the last few decades, it is only recently that it has emerged that AMPs are specific to certain pathogens. They are controlled by two signaling pathways, the Toll and Imd pathways. We have recently started to look at a gene called edin (elevated during infection). Previous studies have shown that edin is required for resistance to *Listeria monocytogenes*, that it is involved at the larval stage in the fight against wasp parasitism, but that it is also required to induce neural stem cells division in the event of brain injury. These different phenotypes therefore require a more in-depth understanding of this gene. We discovered that edin is required to resist infections by fungi and yeasts, while being dispensable against Gram-positive and Gram-negative bacteria. In addition, the Edin protein localizes to the midgut during oral and systemic infections. Moreover, loss of edin is associated with a shorter lifespan and increased proliferation of midgut stem cells. This phenotype is linked with a dysbiosis characterized by an increased number of *Acetobacter* bacteria. Edin therefore appears to be a key regulator of the *Drosophila* microbiota, suggesting a potential role as an AMP.

Keywords: antimicrobial peptides

*Speaker

Gut Microbiome Exacerbates Infections by Altering Host Immune Response: Insights from *Drosophila* Model

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Gut microbiome promotes host resistance to colonization by pathogenic microbes. However, recent studies have shown that gut microbiome can also exacerbate certain infections. The mechanisms by which gut microbiome facilitates infections are little studied in part due to the complexity and genetic intractability of gut microbiome in most animals. The fruit fly *Drosophila melanogaster* has been a model of choice to study host-microbiome interactions due to its simple, genetically amenable, and cultivable microbiome, simplicity of generating axenic and gnotobiotic animals, and extensive genetic toolkit. We found that the presence of gut microbiome can exacerbate infections by the pathogen *Pseudomonas entomophila* in wild type fruit flies. Specifically, we found that the presence of a bacterial species, *Lactiplantibacillus plantarum*, in the gut microbiome was associated with increased mortality and higher pathogen loads in fruit flies. Furthermore, flies colonized with *L. plantarum* showed enhanced intestinal and systemic immune responses compared to germ-free flies after *P. entomophila* infection. We then found that the increased mortality in flies colonized with *L. plantarum* was eliminated in immune-deficient fly mutants (*PGRP-SDsk1* and Δ AMPs). Moreover, the deletion of a specific antimicrobial peptide (AMP) *Metchnikowin* (*MtkR1*) protects flies from increased mortality caused by *L. plantarum*, while the deletions of other AMP genes did not have this effect. This suggests that the production of *Metchnikowin* is responsible for the exacerbation of the *P. entomophila* infection in the presence of *L. plantarum*. Overall, this study highlights the complex role of gut microbiome in host-microbe interactions and how specific bacterial species can worsen infections by altering the immune response of the host. The findings may have implications for the development of treatments for infectious diseases that target the gut microbiome.

Keywords: Gut microbiome, Pathogen infection, Antimicrobial peptide, Metchnikowin

*Speaker

Identification and functional analysis of endogenous ligands for STING in *Drosophila* upon virus infection

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Innate immunity, operating in all living organisms, is the first line of defense against viral infections. It relies on pattern recognition receptors (PRRs) that recognize viral nucleic acids, leading to the activation of downstream adaptor molecules and transcription factors, which further regulate the expression of antiviral effectors to combat virus infection. In mammals, the enzyme cGAS acts as a PRR sensing the presence of DNA in the cytosol and synthesizes the cyclic dinucleotide (CDN) 23-cGAMP. This CDN binds to and activates the protein STING to trigger antiviral immunity.

We previously reported that the STING pathway is conserved in *Drosophila melanogaster* and that two cGAS-like receptors (cGLR1 and cGLR2), act upstream of STING in flies. cGLR1 recognizes double stranded (ds)RNA, leading to the synthesis of 32-cGAMP and small amounts of 23-cdiAMP, while cGLR2, for which the activating ligand has not been identified yet, can synthesize 32-cGAMP and 23-cGAMP. Both 32-cGAMP and 23-cGAMP act as agonists for STING, resulting the activation of the kinase IKKb, which regulates the NF-κB like transcription factor Relish to control the expression of antiviral genes. Of note, the three reported CDNs produced by *Drosophila* cGLRs were identified with *in vitro* assays using either purified recombinant cGLR1 or extracts from HEK293T human cells transfected with cGLR2. Thus, how STING gets activated *in vivo* in the context of viral infection remains unknown.

Here, we report a method to detect the production of CDNs in fly extracts and hemolymph. Using this assay, we show that 23-cGAMP, 23-cdiAMP, 32-cGAMP, but also a novel CDN, 23-cdiGMP, are produced in *Drosophila* in response to infection by *Drosophila* C virus or Nora virus. Production of these CDNs is significantly reduced in flies double mutant for cGLR1 and cGLR2, but not in single mutant flies. Furthermore, CDNs can still be detected in double mutant flies, suggesting that a third cGLR may participate in antiviral immunity in *D. melanogaster*. Overall, our data provide insight on the *in vivo* function of a previously unrecognized important family of PRRs, the cGLRs.

*Speaker

Keywords: cyclic dinucleotides, secondary messenger, cGAS, like receptors, STING pathway, virus, innate immunity

Immune role of *Drosophila melanogaster* putative Kazal-type serine protease inhibitor CG14933

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Serine protease inhibitors (serpins) exhibit major regulatory functions in the proteolytic cascades of both arthropods and mammals, but despite their important function in *Drosophila melanogaster* immunity, few have been fully characterized and knowledge of their precise mechanism of action is still sparse. In this study, we investigate the immunological role of the previously uncharacterized gene *CG14933*. *CG14933* contains a Kazal domain, a protein domain that is often indicative of serpins. By using *CG14933SK1* flies, a mutant devoid of any *CG14933* expression, as well as RNAi knockdown and deficiency models, we show that flies lacking *CG14933* exhibit increased susceptibility to infection with *Pseudomonas entomophila* and *Providencia alcalifaciens*, respectively. This suggests involvement of *CG14933* in *Drosophila* immunity, although *CG14933* itself is not induced by infection. We show that *CG14933SK1* larvae harbor increased numbers of crystal cells, specialized immune cells that store the key enzymes of the melanization cascade, the prophenoloxidasases, and that adult *CG14933SK1* flies also have significantly increased levels of active phenoloxidasases compared with wild type flies. We were able to rescue the sensitivity phenotype to *P. entomophila* infection by employing a double mutant devoid of both *CG14933* and *PPO1*, the prophenoloxidasase activated in early stages of infection. We also observed higher levels of iron in the hemolymph of *CG14933SK1* flies, which may indicate a lower efficiency of the iron sequestration response, explaining the observed susceptibility to pathogens known to require iron for their virulence. Overall, we propose that *CG14933* plays a role in negatively regulating the melanization response in *Drosophila*. Furthermore, we provide evidence of a possible link between excessive melanization and reduced iron sequestration that leads to increased susceptibility to *P. entomophila* and *P. alcalifaciens* infection.

Keywords: *P. entomophila*, *P. alcalifaciens*, systemic infection, melanization, iron sequestration

*Speaker

Investigating the interaction of the immune system with metabolism and the central nervous system in the *Drosophila* model

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Interaction between the immune system and other body systems, including metabolism and the central nervous system (CNS), remains poorly understood. Increasingly, the role of the immune system in pathologies such as neurodegeneration and metabolic disorders is being investigated. Factors such as stress and diet also have influence on the immune system. In this project we are using the *Drosophila melanogaster* (fruit fly) model to investigate these relationships. We have identified two peptides, IBIN and IBIN-like, that are highly expressed in the fly during systemic infection with pathogens including gram-positive and gram-negative bacteria and parasitic wasps. The role of these peptides in the immune response remains unclear, however our unpublished results show increased susceptibility to infection in flies with both genes absent. The peptides have also been shown to be expressed in the CNS when flies are socially isolated or visually exposed to parasitic wasps. This apparent role of immune-relevant peptides in the CNS allows us to investigate the relationship between these two systems. In addition to describing the role of these peptides in the immune response, we are working to answer questions including: are immune molecules in the CNS expressed under the control of the same pathways as in the case of systemic infection; does expression of immune molecules in the CNS in response to stress cause pathology over the lifetime of the animal, or a shorter lifespan; and does the stress-related immune response in the CNS convey any protection from infection to the animals, or to their offspring.

Keywords: CNS, metabolism, infection, immunity

^{*}Speaker

Investigating the role of Tolls in the *Drosophila* intestine

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The intestine represents one of the largest interfaces between the internal and external environment. It plays a key role in sensing and coupling environmental inputs, such as nutrients and microbial derivatives, with intestinal stem cell (ISC)-driven adaptive responses. Despite the physiological divergence between insects and mammals, studies have shown that flies are a model system well suited for studying ISC physiology during aging, stress, and infection. In the adult fly gut, ISCs are regulated by multiple signals released from the surrounding niche, which includes enterocytes (ECs), enteroendocrine cells (EECs), enteroblasts (EBs) and the visceral muscles (VMs). During the past year, we carried out a functional screen to identify signals integrated by the ISC niche to control ISC activity and/or cell fate. We used RNAi-mediated knockdown of 600 receptors in the ECs, EECs, VMs and progenitor cells to screen for genes required to maintain gut homeostasis in physiological conditions or during infection-triggered regenerative growth. Out of approximately 70 candidate hits, several were members of the Toll receptor family. With nine Toll receptor members, the Toll family is involved in a variety of processes, including cell competition, pattern formation, neuronal structural plasticity and immunity. However, little is known regarding their role and function in the gut. Here, we aim to explore the potential roles certain members of the Toll family in immunity and gut homeostasis.

Keywords: Toll, intestine, 18 wheeler, Tehao, immunity

*Speaker

Investigations into the metabolic interplay of *Drosophila melanogaster* and its microbiota

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The *Drosophila* microbiota has far-reaching, profound effects on host physiology, ranging from training the immune response, altering host development time, body-size, triglyceride levels, and gut morphology. Further, the microbiota plays a nutritional role by provisioning essential nutrients to its host. Despite being low-complexity; the microbiota is comprised of a few species predominantly from the Acetobacteraceae and Lactobacillaceae families, the microbiota is functionally conserved and behaves like that of more complex mammalian microbiotas. Previous research has demonstrated that *Acetobacter* species are the functional members of the microbiota affecting host physiology, however, the exact nature of how they do this is not well understood. One potential candidate is through the generation of the SCFA acetic acid. We set out to confirm if acetic acid production is important with our laboratory *Acetobacter pomorum* strain. We found that in liquid culture, it is a net acetate user rather than producer. Further, despite influencing host physiology on standard SYA fly diet, *A. pomorum* did not produce acetate, rather (iso)butyrate was the most predominant SCFA being produced. To better understand what important metabolites the microbiota may be providing, we compared the metabolomes of germ-free, *Levilactobacillus brevis*, and *A. pomorum* colonized whole flies as well as of specific tissues: the gut, head, fat-body, thorax, and abdomen. We consistently found that in flies colonized with *A. pomorum*, specific B-vitamins (B2 and B5) and their derivatives were more abundant in their tissues. Further, metabolites involved in nucleic acid and nitrogen metabolism were also more prevalent in *A. pomorum* associated flies, suggesting a potential host-microbe feedback loop.

Keywords: host, microbe, Acetobacter, metabolomics, SCFAs, Lactobacillus

*Speaker

Metabolic profiling of *Drosophila* larval hemocytes

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Energy metabolism is a fundamental biological process in cellular physiology that involves the conversion of nutrients into ATP. While the significance of metabolic alterations during immunity has been widely indicated, metabolic profiles of *Drosophila* hemocytes been comprehensively elucidated. In this study, we utilized a Seahorse XFe96 Analyzer to investigate the metabolic activities of *Drosophila* hemocytes. We found that larval hemocytes in the third instar primarily rely on mitochondrial oxidative phosphorylation for ATP production. Hemocytes undergo dynamic changes in metabolic rates during development, with hemocytes in earlier developmental stages exhibiting higher metabolic rates compared to their late-stage counterparts. In addition, we observed a significant increase in metabolic activity in response to active innate immune responses, which is reduced by an inhibition of sugar metabolism. Overall, our results provide novel insights into the metabolic profiles of larval hemocytes and highlight the crucial role of metabolic activity in hemocyte development and immunity.

Keywords: Hemocyte, Energy metabolism, OXPHOS, Glycolysis

*Speaker

Microbiota cell surface charges mediate the resistance to host antimicrobial effectors during the infection and the onset of *Drosophila* aging

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Resilience to short-term perturbations, like inflammation, is a fundamental feature of microbiota, yet the underlying mechanisms of microbiota resilience are incompletely understood. Here we show that *Lactiplantibacillus plantarum*, a major *Drosophila* commensal, stably colonizes the fruit fly gut during infection and is resistant to *Drosophila* antimicrobial peptides (AMPs). By transposon screening, we identified *L. plantarum* mutants sensitive to AMPs. These mutants were impaired in peptidoglycan O-acetylation or teichoic acid D-alanylation, resulting in increased negative cell surface charge and higher affinity to cationic AMPs. AMP-sensitive mutants were cleared from the gut after infection and aging-induced gut inflammation in wild-type, but not in AMP-deficient flies, suggesting that resistance to host AMPs is essential for commensal resilience in an inflamed gut environment. Thus, our work reveals that in addition to the host immune tolerance to the microbiota, commensal-encoded resilience mechanisms are necessary to maintain the stable association between host and microbiota during inflammation.

Keywords: Microbiota, Infection, AMPs, *Drosophila*

*Speaker

Microbiota reconstruction analysis reveals core yeast species and microbe-microbe interactions impacting larval growth of *Drosophila* in the wild

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The microbial community associated with animals significantly influences host development. In nature, *Drosophila* lives in association with yeast and bacteria. Axenic larvae that lack these microbial partners fail to develop on fresh fruits in the laboratory. Nevertheless, the precise impact of host-microbe and microbe-microbe interactions on larval development remains largely unexplored. To address this, we sampled bananas that were fed on by wild *Drosophila* species and fermented by fly-associated microbes to different degrees, yielding "early-stage" and "late-stage" foods, and we demonstrated significant changes in the composition of fungi and bacteria during fermentation. Regarding fungi, yeasts predominated in both stages, but the dominant species changed between the stages. As for bacteria, *Enterobacteriales* were prevalent in the early stage, whereas lactic acid bacteria (LAB) and acetic acid bacteria (AAB) dominated in the late stage. We then isolated yeast and bacterial strains from the food samples and tested their ability to support larval development on a banana-agar medium. *Hanseniaspora uvarum* (*H. uvarum*), a yeast species dominant in the early stage, was alone sufficient to support larval growth. In contrast, most of the late-stage microbes tested failed to efficiently promote larval growth when inoculated individually. However, the acetic acid bacteria *Acetobacter orientalis* (*A. orientalis*) effectively promoted larval growth when coexisting with LAB or late-stage yeast species. Our analyses on larvae under different microbial environments, including transcriptomic analyses of first-instars, strongly suggest that *A. orientalis* has the potential to stimulate a growth-promoting response in larvae, and the ability requires microbe-microbe interactions in the late-stage microbiota. Finally, we investigated the molecular basis underlying the distinct effects of the yeast species, including the supportive *H. uvarum* from the early-stage food and non-supportive *Pichia kluyveri* and *Starmerella bacillaris* from the late-stage food, on larval growth. Surprisingly, all yeast species exhibited strong growth-promoting effects upon heat killing, indicating that all species produce sufficient nutrients for larval development, but larvae

*Speaker

are unable to utilize those produced by the live non-supportive species. Our metabolomic analysis and metabolite supplementation assay suggest that only supportive yeast cells can release critical metabolites for larval growth, including branched-chain amino acids such as leucine and isoleucine. Collectively, our findings highlight the core microbial species, their interactions, and the yeast species-dependent supply of nutrients that contribute to *Drosophila* larval development in the wild during the transition of the microbiota.

Keywords: microbes, yeast, lactic acid bacteria, acetic acid bacteria, larval growth, multiomics

Role of hemocytes in regulating intestinal homeostasis upon ingestion of pathogens or xenobiotics in *Drosophila melanogaster*

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The ingestion of pathogens or xenobiotics perturbs intestinal homeostasis. Host organisms employ resistance and resilience mechanisms to protect against such attacks. Resistance targets microorganisms for neutralization or elimination whereas resilience allows the host to tolerate the pathogen and repair damage. Previous studies with *Drosophila melanogaster* show that exposure to the pore-forming toxin hemolysin from the pathogenic bacterium *Serratia marcescens* or xenobiotics such as ethanol or caffeine induces the rapid extrusion of a large part of the apical cytoplasm of enterocytes in the gut lumen, leading to a fast thinning of the intestinal epithelium, followed by a recovery phase. This process of epithelial thinning, the enterocyte cytoplasmic purge, is a resilience mechanism that may help in the elimination of damaged organelles and pathogens/toxins. This novel host defense mechanism is conserved across species. Interestingly, a prior exposure to *S. marcescens* or xenobiotics primes *Drosophila* enterocytes against a recurring cytoplasmic purge.

Unexpectedly, we show that elimination of hemocytes by genetic ablation or blocking phagocytosis inhibits the cytoplasmic purge. Hemocyte-specific expression of the TNF ligand, Eiger and enterocyte-specific expression of the TNF receptor, Wengen are essential for the process to occur. A candidate-based RNAi screen showed that the Death regulator Nedd2-like caspase, Dronc is required for thinning of the gut epithelium. Clonal analysis revealed that Dronc is required cell-autonomously in enterocytes to regulate the cytoplasmic purge.

In conclusion, our initial findings suggest that in response to ingestion of pathogens or xenobiotics, enterocytes signal to hemocytes and likely recruit them to the gut. In return, hemocytes signal back to the enterocytes via TNF signaling to regulate the cytoplasmic purge. In this process, the initiator caspase Dronc acts downstream of hemocyte signaling. Identifying conserved signaling mechanisms that regulate the crosstalk between hemocytes and enterocytes may be relevant in understanding inflammatory gut disorders in vertebrates.

Keywords: *Serratia marcescens*, xenobiotics, *Drosophila melanogaster*, hemocytes, gut, TNF, enterocyte, cytoplasmic purge

*Speaker

Sexual dimorphism in *Drosophila melanogaster* susceptibility to gut infection

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Response to gut infection consists of complex, highly regulated interactions between distant immune compartments determining the disease outcome. However, although sex differences in gut physiology and morphology have been previously described, whether and how these differences contribute to intestinal immunocompetence has not yet been fully understood. We use *Drosophila melanogaster* to investigate the mechanisms underlying sexual dimorphism in the outcome of gut infections. Here, we used enteric infection with *Drosophila* natural pathogen *Pseudomonas entomophila* to decipher the mechanisms underpinning the generally higher susceptibility of female flies to gut infection. Firstly, we used transcriptomics and proteomics to describe the sexually dimorphic gut response to infection. Then, genome-wide association analyses (GWAS) using the *Drosophila melanogaster* Genetic Reference Panel (DGRP) lines exposed to *P. entomophila* in environment-controlled conditions identified candidate genes mediating sexual dimorphism in *Drosophila* susceptibility to this infection. This study suggests that sex differences in cell-to-cell signaling, development, and metabolism might influence the sexually dimorphic outcome of gut infection. Using *Drosophila* to understand how one sex suffers from infectious disease symptoms while the other does not will broaden our global understanding of variation in susceptibility to infectious diseases.

Keywords: *D. melanogaster*, sexual dimorphism, infectious diseases, gut immunity, DGRP, GWAS

*Speaker

Single-cell sequencing of tumor-associated macrophages in a *Drosophila* model

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Tumor-associated macrophages may act to either limit or promote tumor growth, yet the molecular basis for either path is poorly characterized. Using a larval *Drosophila* model that expresses a dominant-active version of the Ras-oncogene (RasV12) to study dysplastic growth during early tumor progression, we performed single-cell RNA-sequencing of macrophage-like hemocytes to characterize these cells in tumor- compared to wild type larvae. Hemocytes included manually extracted tumor-associated- as well as circulating cells. We identified 5 distinct hemocyte clusters. In addition to RasV12 larvae we included a tumor model where the activation of effector caspases was inhibited, mimicking an apoptosis-resistant setting. Circulating hemocytes from both tumor models differ qualitatively from control wild-type cells – they display an enrichment for genes involved in cell division, which was confirmed using proliferation assays. Split analysis of the tumor models further reveals that proliferation is strongest in the caspase-deficient setting. Similarly, depending on the tumor model, hemocytes that attach to tumors activate different sets of immune effectors – antimicrobial peptides dominate the response against the tumor alone, while caspase inhibition induces a shift toward members of proteolytic cascades. Finally, we provide evidence for transcript transfer between hemocytes and possibly other tissues. Taken together, our data support the usefulness of *Drosophila* to study the response against tumors at the organismic level.

Keywords: *Drosophila melanogaster*, macrophages, tumor model, hemocyte, single, cell transcriptomics, scRNA, seq

*Speaker

Treatment with tetracycline antibiotics mitigates survival outcomes of Flock House virus infection in both young and aged *Drosophila melanogaster*

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Older organisms experience increased susceptibility and mortality to viral infections. This presents a major challenge for humans, as the elderly population in many regions is projected to double by 2050. Organisms possess two strategies to fight disease: resistance and tolerance. Resistance mechanisms directly reduce pathogen loads, and disease tolerance mechanisms limit tissue damage caused by a pathogen or immune response. Resistance mechanisms are well characterized, while disease tolerance mechanisms have not been studied as extensively. Our lab utilizes *Drosophila melanogaster* and Flock House virus (FHV) as a model system to investigate mechanisms that improve an aged host's survival to virus infection. FHV is a single-stranded, positive sense, RNA virus pathogenic to flies, and older flies display accelerated mortality to FHV compared to young flies. The accelerated mortality of aged hosts is not characterized by increased FHV loads, suggesting that aged flies die more quickly from impaired disease tolerance. Aged flies mount a unique transcriptional response to FHV, characterized by larger significant changes for genes functioning in metabolic pathways. Additionally, we have shown that FHV significantly reduces metabolic rate relative to controls and the inability to modulate metabolic rate in aged flies is associated with survival outcomes of virus infection. Therefore, we hypothesized that modulating host metabolism could affect survival after infection. To accomplish this, after FHV infection we treated young and aged flies with tetracycline antibiotics, which disrupt mitochondrial protein synthesis because of mitochondria's bacterial ancestry. Many electron transport chain proteins are encoded by the mitochondrial genome, so tetracycline treatment affects host metabolism. Our results show that tetracycline treatment allows young and aged *Drosophila melanogaster* to survive significantly longer following FHV infection than control treatment. No significant differences in virus loads were observed between tetracycline treated flies in comparison to controls. Bacterial loads are not significantly different in FHV-injected and control-injected flies. These results indicate that tetracycline's protective effects are likely a result of improving disease tolerance mechanisms and independent from its antibiotic properties that could potentially mitigate secondary bacterial infections.

Keywords: aging, RNA virus, disease tolerance, metabolism, tetracycline, antibiotics

*Speaker

Tumor markers in body fluids – tools for cancer diagnosis, prognosis and functional analysis of tumor progression

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Cancer is a severe disease described as the second leading cause of death in humans. While healthy cells are in balance within the organism, cancer cells show uncontrolled cellular division and growth. Tumour formation and progression can use different metabolic systems to produce extra energy and substances required for their survival. The characterization of tumour formation, progression and development can be studied with *Drosophila melanogaster*. The fruit fly has been used to induce tumour growth in a tissue specific manner and for mimicking invasion behaviour. There are several hallmarks of cancers, in which chronic inflammation is included. Tumour cells interact with pro-inflammatory cells such as hemocytes (the insect blood cells), and other cellular components forming the inflammatory tumour microenvironment (TME). In response to inflammation, the fat body releases into hemolymph (the insect blood equivalent) different antimicrobial peptides to fight the wound region of the tumour cell. One of these peptides is Drosomycin (Drs). The immune response and the metabolic interaction of the transformed, healthy cells and TME is still poorly understood. However, it has been shown that fragments of tissues can signal other tissues using hemolymph as a vehicle. During several years, we have induced, and characterized, tumours in salivary glands (SG) of *Drosophila melanogaster* larvae by the expression of a dominant-active form of the oncogene RAS (RasV12) and the effects of rescue promoted with Drs when expressed in RasV12 background. As a consequence, from transcriptome profiling in SG, fat body, and tumor-associated macrophage-like cells we demonstrated the transfer of information among these tissues through nano-vesicles, also known as extracellular vesicles (EV). EVs are membrane-vesicles that are released to the hemolymph and taken to the target tissues by unknown mechanisms. EVs are thought to be mediators of physiological reactions, immune and metabolic response throughout tumor development. Here, we mapped the metabolites present in EVs and hemolymph of controls, RASv12 and the rescue fly line, Drs expressed in RasV12 background using the LC-MS-based target metabolomic. Our results showed a differential regulation in amino acid metabolism and in fatty acid biosynthesis in tumorigenic SG when compared with control ones. Furthermore, we characterized two different metabolic profiles, one specific for Ras and other distinct for Drs. Finally, upon the EV purification, we mapped the EV metabolome and compared it with the hemolymph metabolome, to uncover the specific signals to soluble metabolites and EVs metabolites interconnection.

*Speaker

Keywords: Immunology, Metabolomics, Immunomodulators, EVs, Soluble metabolites, Hemolymph Metabolome, Evs Metabolome

Using *Drosophila* to dissect the molecular players required for immune cell extravasation

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The initiation and timely resolution of inflammation is critical for triggering an optimal immune response to any tissue insult. Leukocyte extravasation from the circulation is an early and rate-limiting step of inflammation and is a highly complex, polygenic and regulated trait, involving the interaction of multiple molecules expressed on both leukocytes and endothelial cells. We have developed a *Drosophila* pupal wing extravasation model to dissect potentially conserved mechanisms of extravasation between fly and man, and reveal novel molecular players in the process. Our model has already shown that *Tre1*, a GPCR necessary for germ cell migration in *Drosophila* embryos, is also essential for hemocyte extravasation from wing veins in response to a laser induced wounds. Deletion of the murine orthologue of *Tre1*, GPR84, results in a similar inhibition of leukocyte extravasation from the vasculature under induced inflammation. Extrapolating from the germ cell literature, we are now investigating known Tre1 interactors for potential roles in extravasation. Tre1 and GPR84 share a highly conserved Rho1 binding domain which is pivotal for germ cell migration, and here we explore the effects on Rho1 when Tre1 is depleted. Further, we have investigated the role of *wunens* in hemocyte recruitment and extravasation. During germ cell migration, *wunens* compete with and deplete an unknown Tre1-activating ligand and their dynamic expression helps direct migration of germ cells via local depletion of Tre1-activating ligand. Hemocyte-specific knockdown of *wunens* had little effect on hemocyte recruitment, whereas global knockdown of *wunens* significantly reduced recruitment – suggesting *wunens* act on hemocytes in a non-autonomous manner. Our *Drosophila* extravasation model has demonstrated that at least some of the mechanisms that drive immune cell extravasation are conserved between fly and man, and that developmental mechanisms appear to be recycled during the wound inflammatory response.

Keywords: immune cell, extravasation

*Speaker

mTOR signaling in hemocytes is needed for full hemocyte activation

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Immune cell activation and differentiation are closely linked with metabolic changes at cellular and systemic levels. The main signaling cascade connecting energy and nutrient levels to cellular functions such as growth, proliferation and survival, is the evolutionarily conserved mechanistic Target of rapamycin mTOR signaling pathway. The mTor protein forms two complexes, mTORC1 and mTORC2, which mediate different processes in the cell. mTORC1 is well-known for its regulation of protein synthesis, metabolism and autophagy, while mTORC2 connects insulin signaling to cell proliferation and controls cytoskeletal polarization and reorganization. We studied the role of mTOR signaling in the hemocytes of *Drosophila* larvae. We found that activating mTOR signaling in hemocytes by overexpressing mTor led to differentiation of hemocyte types normally induced by the activation of the cellular immune system upon wasp parasitisation. Furthermore, blocking mTOR activity reduced both the efficiency of the immune response and the numbers of immune-induced hemocytes after parasitisation. This reduction required the silencing of both mTOR complexes, either by expressing a dominant negative form of mTor or by simultaneously silencing the TORC1 binding partner raptor and the TORC2 binding partner rictor. An RNA sequencing experiment showed that while mTor overexpression and wasp infection induced largely similar changes in hemocyte gene expression, mTor overexpression led to expressional changes in mitosis-linked genes not observed in hemocytes activated by wasp infection. Moreover, our data indicated that immune-induced hemocytes upregulated the oxidative branch of the pentose phosphate pathway to produce NADPH, likely to generate reactive oxygen species to aid in killing the parasitoid. Our data highlight the key role of mTOR signaling in controlling hemocyte fate and uncover novel features of hemocyte function.

Keywords: parasitisation, wasp, hemocyte, mTOR

*Speaker

Other

Characterization of the brain proteome in *Drosophila melanogaster* as a function of sex and age

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The brain accumulation of aberrant proteins or proteins that lose their functionality is characteristic of many neurodegenerative diseases and aging (DOI:10.1016/j.cell.2013.05.039). To understand the pathogenesis of neurodegenerative diseases and the alterations that accompany aging, it is therefore necessary to start from a thorough knowledge of protein expressions in wild-type organisms. Although there are currently documents that, using a proteomic approach in *Drosophila melanogaster*, describe changes in the expression of head proteins during aging (DOI:10.1021/pr070224h; doi:10.1016/j.ijms.2018.01.003), there are currently no studies that consider sex as a factor in itself that can differently influence the expression of these proteins during aging.

The aim of this work was to carry out a comparative proteomic analysis of proteins expressed in the head of wild-type *Drosophila melanogaster* not only in function of age, but also of sex (males *vs* females, M or F respectively).

An initial population of ~450 animals/sex was used to obtain the three subgroups of adult wild-type flies (Canton S strain) that were bred until the specified age of harvest: at 15, 30, 45 days. Flies were supplied with Formula 4–24 ® media (Carolina Biological, Burlington, NC, USA) and maintained into culture vials of 35 flies/each, at constant temperature (25°C) and humidity (60%) with a 12/12h light–dark cycle. Yeast pellets (*Saccharomyces cerevisiae*) were added to each tube after diet hydration. The vials were changed every three days. Eighty heads were pooled for each experimental time point, for both M and F flies. A combination of specific proteomic techniques such as bidimensional electrophoresis (2-DE) and mass spectrometry (MALDI-TOF/TOF) were adopted.

The SameSpots software (Cleaver Scientific, UK) was utilized to process raw data and gave as result 1200±138 spots. The mass lists were searched against the Uni-ProtKB reference proteome (*Drosophila melanogaster* data-base, ID: UP000000803, release: 2023_02, 22,066 proteins) database.

*Speaker

The comparative analysis between groups revealed a significant different expression of proteins in F with respect to M, and such differences increased with aging.

These results confirm that sex is a key parameter to consider to fully understand cellular mechanisms at the base of aging and the development of neurodegenerative diseases such as Parkinson and Alzheimer's Disease. Moreover, taking into consideration that female flies live longer compared to male flies, the possibility to identify specific proteins responsible for this difference could be an important molecular target to increase both male and female lifespan.

Keywords: brain proteins, proteome, aging, sex.

Convergent evolution in glutamate dehydrogenase activity in *Drosophila* and human

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Gene duplication opens the way of neofunctionalization of proteins which can drive evolution. Both in human and *Drosophila melanogaster* there are a duplicate of glutamate dehydrogenase coding genes. The human duplicate is evolutionary young, while *Drosophila* duplicates are well established in many Dipterian species. Human GLUD1 and *Drosophila* Gdh are housekeeping genes with general expression, meanwhile human GLUD2 and *Drosophila* Bb8 show tissue-specific expression. Glutamate dehydrogenases presenting a crucial branchpoint between amino acid and energy metabolism. Their role is widely known in a variety of biological processes from neuronal function to cancer development. *Drosophila* Bb8 is required for male fertility and the normal development of post-meiotic mitochondrial derivatives of spermatids. Testis-specific genes are less conserved and often gain new functions, which question whether Bb8 has enzymatic activity. Our results indicate that Bb8 indeed has glutamate dehydrogenase enzymatic activity, however this activity differs from the housekeeping Gdh activity. We found it interesting, to test the human glutamate dehydrogenases' (GLUD1 and GLUD2) functionality in *bb8* mutant spermatids, and our results showed that they are capable of rescuing the male sterile mutant phenotype. However, the tissue-specific GLUD2 outperforms the housekeeping GLUD1, which suggest functional similarity of Bb8 to GLUD2. We also tested the role of three conserved amino acids between Bb8 and GLUD2 in Gdh mutants, which mutations increased the Gdh rescue capacity, further proving the similarity between Bb8 and GLUD2. Our results suggest that *Drosophila* Bb8 and the human GLUD2 is a novel example of convergent molecular evolution.

Keywords: glutamate dehydrogenase, *bb8*, GLUD2, mitochondria, spermatogenesis

*Speaker

Identification of a new player preventing apoptosis during meiotic recombination in *Drosophila*

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During the formation of tissues and organs, cells divide multiple times, distributing their genome with high precision. Importantly, during cell proliferation the DNA is exposed to lesions that need to be repaired to generate functional daughter cells, a process that requires mitotic arrest. When DNA damage is not repaired, cells may malfunction and ultimately undergo cell death or uncontrolled proliferation. Bruno is a translational repressor that acts over critical targets during *Drosophila* female gametogenesis such as oocyte specific factors and mitotic cyclins. Proper gamete formation in the *Drosophila* ovary involves germline mitosis and meiosis, processes that take place in the germarium. Interestingly, meiosis implicates the generation of serious DNA damage in the form of double strand breaks (DSBs) during meiotic recombination. However, in normal conditions the germline does not undergo cell death, indicating the existence of surveillance and repair mechanisms acting during early oogenesis. We will show that Bruno is responsible for the prevention of apoptosis during meiotic recombination in *Drosophila*. Our observations indicate that loss of *bruno* i) increases DNA damage and apoptosis in germline cells during early oogenesis, ii) affects oocyte positioning and development and iii) induces excess proliferation in the follicular epithelium. Altogether, our results point to an essential role for Bruno during oocyte specification, follicular epithelium homeostasis and in the prevention of apoptosis during meiotic recombination in the *Drosophila* ovary. We are currently trying to understand the connection between DSB repair, p53 activity and Bruno function(s) in early oogenesis.

Keywords: Oogenesis, apoptosis, meiotic recombination, bruno

*Speaker

Nuclear genetic background influences the phenotype of the *Drosophila tko25t* mitochondrial protein-synthesis mutant

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The *Drosophila tko25t* point mutation in the gene encoding mitoribosomal protein S12 produces a complex phenotype of multiple respiratory chain deficiency, developmental delay, bang-sensitivity, impaired hearing, sugar and antibiotic sensitivity and impaired male courtship. Its phenotypic severity was previously shown to be alleviated by inbreeding, and to vary with mitochondrial genetic background. Here we show similarly profound effects conferred by nuclear genetic background. We backcrossed *tko25t* into each of two standard nuclear backgrounds, Oregon R and w1118, the latter used as recipient line in many transgenic applications requiring selection for the white minigene marker. In the w1118 background, *tko25t* flies showed a moderate developmental delay and modest bang-sensitivity. In the Oregon R background, males showed longer developmental delay and more severe bang-sensitivity, and we were initially unable to produce homozygous *tko25t* females in sufficient numbers to conduct a meaningful analysis. When maintained as a balanced stock over 2 years, *tko25t* flies in the Oregon R background showed clear phenotypic improvement though were still more severely affected than in the w1118 background. Phenotypic severity did not correlate with the expression level of the *tko* gene. Analysis of *tko25t* hybrids between the two backgrounds indicated that phenotypic severity was conferred by autosomal, X-chromosomal and parent-of-origin dependent determinants. Although some of these effects may be *tko25t*-specific, we recommend that, in order to minimize genetic drift and confounding background effects, the genetic background of non-lethal mutants should be controlled by regular backcrossing, even if stocks are usually maintained over a balancer chromosome.

Keywords: mitochondria, nuclear background, ribosome, semi, lethality

*Speaker

The Role of Hox Proteins in Cellular Plasticity

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The development of multicellular organisms is a sophisticated process that necessitates the precise spatial and temporal positioning of each cell type. To achieve this, distinct cell groups initially establish their identity and subsequently adopt the morphological and functional characteristics that delineate each cell type. Throughout this process, the transcriptional program of each lineage must be meticulously maintained, ensuring that cells do not alter their identity. This fidelity is accomplished by suppressing genes that would drive cells towards alternative developmental fates. A prime example is the inhibitory function of the Hox transcription factor Ultrabithorax (Ubx) in the mesodermal lineage during *Drosophila melanogaster* embryogenesis. Our prior research underscores the proclivity of this protein to bind to genes associated with the evolution of alternative cell lineages. In this context, Ubx collaborates with a Polycomb Group Complex member, Pleiohomeotic (Pho), to promote the accumulation of repressive chromatin marks (H3K27me). While the molecular mechanisms underlying this repression are well-defined, the degree to which they modulate the mesoderm's propensity to alter its identity remains elusive. To probe this, we will test the cellular plasticity of this tissue by aberrantly expressing transcription factors pivotal for other lineage developments, such as Ventral nervous system defective (Vnd). This factor has previously demonstrated the ability to partially transdifferentiate mesodermal cells into neurons. By juxtaposing ectopic *Vnd* expression against various *Ubx* expression contexts (both knockout and overexpression), our goal is to delineate the relationship between Ubx and mesodermal cellular plasticity. To rigorously assess the identity of these cells, we will undertake transcriptomic analysis via RNA-seq and evaluate chromatin accessibility using ATAC-seq under the stated genetic conditions. Given reports suggesting functional overlaps among Hox genes, our study will also incorporate tissue-specific knockouts of Hox genes *Antennapedia* (*Antp*) and *abdominal-A* (*abd-A*) in tandem with *Ubx*.

Keywords: Hox genes, Cellular Plasticity, Development, embryogenesis

*Speaker

The Structure and Function of Orphan Peptides of the *Drosophila* Male Accessory Gland and Ejaculatory Duct

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The seminal fluid (SF) of *Drosophila melanogaster* is rich in proteins and small peptides that are made by, and secreted from, the paired male accessory glands (MAGs) or paragonia, the ejaculatory duct (ED) and the ejaculatory bulb (EB). They are transferred with sperm to the female during copulation, where they help to secure paternity by forming a mating plug, aiding sperm storage and by manipulating female reproductive physiology (e.g. elevated oviposition) and behaviour (e.g. rejection of courting males). Much attention has focused on the role of the SF sex peptide (SP) in changing the physiology and behaviour of the female by activation of the neuronal MIP/SP receptor. SP is a 36 amino acid peptide that undergoes extensive post-translational modifications (PTM) that are functionally important. SP is not the only SF peptide made by the secretory cells of the MAG and ED, but there is little structural or functional information available for the mature forms of these other peptides. We have used a peptidomics strategy to determine the structures of over 20 additional mature peptides that are secreted from the MAG and ED. Our results show that they undergo various PTMs (for example, oxidation of pairs of cysteines to form cyclic peptides, deamidation of the N-terminal glutamine to form cyclic pyroglutamate, proline hydroxylation, glycosylation and C-terminal amidation). These modifications could offer stability and protection from peptidases present in both the SF and the female reproductive tract. BLAST searches indicate that orthologues of the 20 peptide-encoding genes are restricted to *Drosophila* within the subgenus *Sophophora* and are therefore likely to be evolutionarily ‘young’ genes. Peptide modifications for protection in hostile proteolytic environments could be critical for these ‘young’ SF peptides to acquire physiological roles in the post-mated female. In an effort to improve our understanding of the role of SF peptides in reproduction, we are also seeking to identify physiological or behavioural effects of these orphan MAG/ED peptides using the GAL4/UAS system for the ectopic expression of peptide cDNA in virgin female *D. melanogaster*.

Keywords: Peptidomics, Seminal Fluid, *Drosophila melanogaster*, Behaviour, Physiology

*Speaker

The ecdysone receptor promotes or suppresses proliferation according to ligand level

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Steroid hormones control various cellular activities in a context-dependent manner. For example, ecdysone, which acts through a type II nuclear receptor, has seemingly opposite effects in *Drosophila* wing precursors, promoting proliferation during larval stages, and triggering proliferation arrest at pupariation. We find that, whether ecdysone is present or not, complete removal of the ecdysone receptor (EcR) enables normal proliferation during larval wing growth, suggesting that ecdysone overrides a default anti-proliferative activity of the receptor. By contrast, termination of proliferation at the end of larval life requires both ligand and receptor. The switch from one mode of regulation to the other is determined by ligand level, as measured with a calibrated EcR transcriptional reporter and *ex vivo* proliferation assays. Accordingly, RNA Seq analysis uncovers distinct transcriptional responses to different doses of ecdysone. Some genes are only activated at high doses (high threshold targets) and likely to comprise genes that stop proliferation at pupariation, when ecdysone titers are high. We find that other target genes respond to the whole range of physiologically relevant ecdysone concentrations. Some are known to promote proliferation and could therefore account for the pro-proliferation activity of low-level ecdysone, but only in part since they are still expressed in the absence of ecdysone, albeit at reduced levels. Finally, we show mathematically and with synthetic reporters that relatively simple combinations of regulatory elements can recapitulate the behaviour of both types of target genes.

Keywords: Hormone signalling, growth control, proliferation control, ecdysone, *Drosophila*, wing imaginal discs, nuclear hormone

^{*}Speaker

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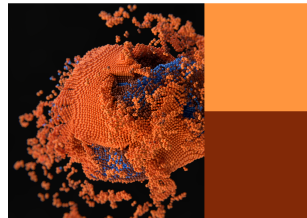
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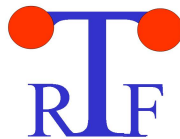


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