XXI ITALIAN DROSOPHILA RESEARCH CONFERENCE

L' AQUILA
JULY 7-9

IDRC 2026

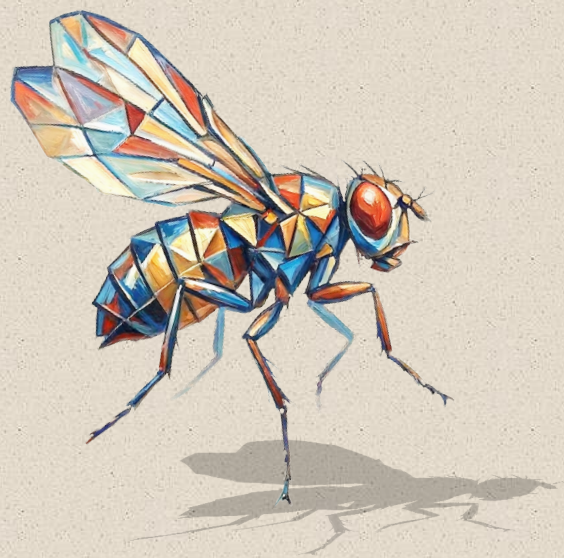
«Alan Turing» building, University of L'Aquila, Coppito

Hosted by Patrizia Morciano, Daniela Grifoni & CancerEvolutionLab

University of L'Aquila – Dept. «Life, Health and Environmental Sciences»

UNIVERSITÀ DEGLI STUDI DELL'AQUILA

MESVA
Dipartimento di Medicina Clinica, Sanità Pubblica, Scienze della Vita e dell'Ambiente



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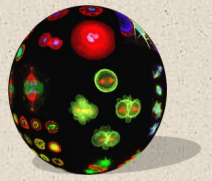
University of L'Aquila – Dept. «LIfe, Health and Environmental Sciences»

Tuesday, July 7

Opening Talk
h. 15.00-16.00



Maurizio Gatti
Dipartimento di Biologia e Biotecnologie "C. Darwin", Sapienza Università di Roma

«Everything you always wanted to know about *Drosophila* male meiosis but have never been told»

Drosophila male meiosis has unique properties that make it a fascinating subject for study. I will summarize some of these properties, focusing on (i) spindle size, (ii) spindle formation and (iii) meiotic exit.
Drosophila male meiotic spindle largely exceeds the size of mitotic spindles. To address this fact, we examined *Drosophila* species with sperm flagella ranging in length from 0.3 mm in *D. persimilis* to 58.3 mm in *D. bifurca*. Males of these species showed striking variations in meiotic spindle size, which positively correlated with sperm length. This links the spindle size to a precise developmental requirement, suggesting that the spermatocytes of these species manufacture and store amounts of tubulin that are proportional to the axoneme length, and use these tubulin pools for spindle assembly. *Drosophila* somatic cells can assemble functional bipolar spindles in the absence of centrosomes, exploiting microtubules (MTs) generated by the kinetochores/chromosomes. In contrast, in male meiotic cells, centrosomes are necessary for spindle assembly. Remarkably, in these cells, centrosomes are also sufficient for spindle formation. Indeed, secondary spermatocytes devoid of chromosomes have the unique property of forming robust bipolar spindles that undergo the same ana-telophase morphological transformations that characterize normal spindles. In animal systems, the only case of third meiotic division not preceded by DNA replication has been described in males of *Drosophila roughex* mutants. We have recently identified another *Drosophila* gene, *terno* (*teo*), that regulates meiotic exit in males. In *teo* mutants, many cysts with 64 haploid spermatids undergo an extra division. Teo interacts with cyclin A and B, and components of the APC/C complex. In *teo* mutant testes, the levels of these cyclins are higher than in *wild type*, and downregulation of either cyclin rescues the *teo* mutant phenotypes. These results suggest that Teo facilitates targeted degradation of the cyclins, so that in *teo* mutants there is an increase in Cdk1-CycA and Cdk1-CycB activities, resulting in an extra meiotic division.

Sponsor Time
h. 16.00-16.45

TEMA RICERCA

Dr. Simone Di Giacomo, Product manager

Biosigma

Dr. Maria Giovanna Rossetto

Novogene
Advancing Genomics, Improving Life

Dr. Caterina Baldi, Senior account manager
Dr. Asia Zonca, Technical support

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Biosigma is a company specialized in the production and distribution of laboratory instruments and consumables, part of the international Dutscher Group, a European leader in life sciences. It offers high-quality, certified products, both under its own brand and selected from qualified suppliers. It focuses particularly on solutions for *Drosophila*, including consumables, growth media, and anesthesia systems. The company guarantees comprehensive support through qualified technical service and a dedicated sales network, ensuring reliability, ongoing assistance, and compliance with international standards.

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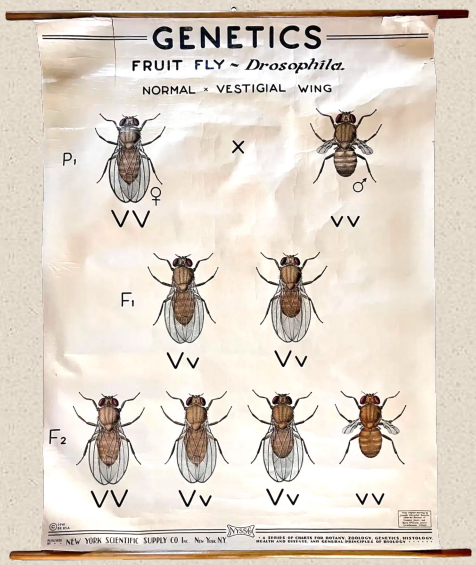
Novogene is a leading provider of sequencing services, operating in more than 90 countries through a global network of laboratories. We support research, clinical, and industrial applications, collaborating with universities, research institutes, hospitals, and companies worldwide. Leveraging state-of-the-art sequencing platforms, Novogene offers both short- and long-read technologies, enabling comprehensive multi-omics approaches spanning genomics, epigenomics, transcriptomics, proteomics, and metabolomics. Our workflows are further enhanced by the proprietary Falcon automation system, designed to streamline processes, reduce turnaround times, and improve reproducibility.
Novogene Europe provides dedicated local support, assisting clients throughout the entire project workflow, from sample shipment to bioinformatic analysis.

Poster Highlights
h. 16.45-17.00



Daniela Beghelli
University of Camerino

Giulio D'Agostini
University of L'Aquila

Rossella Gratton
SISSA, Trieste

Lucia Graziadio
University of Rome Sapienza

Ignazia Mocci
National Research Council Cagliari

Roberta Schirru
University of Milan

Sonia Sonda
University of Padua

Luca Zanfini
IRCCS Fondazione Santa Lucia Rome

P1. Dietary bioactives shape healthspan through metabolic, immune and sex-specific responses in *Drosophila melanogaster*
Daniela Beghelli
Scuola di Bioscienze e Medicina Veterinaria, Università di Camerino, Camerino /MC), Italia

P2. Cell Polarity Disruption in Indolent *RAS*-mutated Follicle Cells Primes Aggressive Behaviours in *Drosophila* and Human Models of Ovarian Cancer
Giulio D'Agostini
Department of "Life, Health and Environmental Sciences", University of L'Aquila, L'Aquila, Italy
INFN-Laboratori Nazionali del Gran Sasso, 67100 Assergi, Italy

P3. Investigating the role of m6A RNA methylation in glia cells during brain development
Rossella Gratton
International School for Advanced Studies (SISSA), Trieste, Italy

P4. RNase H1 counteracts DNA damage and ameliorates SMN-dependent phenotypes in a *Drosophila* model of Spinal Muscular Atrophy
Lucia Graziadio
Department of Biology and Biotechnology Sapienza Università di Roma, 00185 Rome, IT

P5. The novel IMiD 3-Monothiopomalidomide Confers Neuroprotection in *Drosophila* Models of Parkinson's Disease
Ignazia Mocci
National Research Council of Italy, Institute of Translational Pharmacology (IFT), Cagliari, Italy

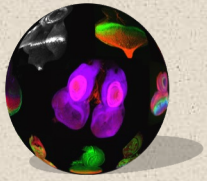
P6. A novel *Drosophila* model of Type B Kufs disease (CLN13) shows neurodegeneration and defects in lysosomal homeostasis
Roberta Schirru
Dipartimento di Bioscienze, Università degli Studi di Milano, Milano, Italy

P7. Towards a unifying cellular mechanism for the pathophysiology of Hereditary Spastic Paraplegia
Sonia Sonda
Neuroscience Institute, National Research Council, Padua, Italy

P8. The protective role of clomipramine in modulating SOD1-induced DNA damage
Luca Zanfini
Experimental Neuroscience and Neurological Disease Models, IRCCS Fondazione Santa Lucia, Rome
Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome, Rome

Talk Session 1
h. 17.15-18.45

Developmental signalling and patterning
Chair: Laura Ciapponi



Thomas Vaccari
«TUSC3 serves as a rate-limiting gatekeeper of a glycan-mediated ER Triage Checkpoint for BMP4/Dpp»

Gabriella Mazzotta
«Circadian regulation of mitochondrial dynamics in the retina of Drosophila melanogaster»

Erika Donà
«From timing to wiring: a global temporal code for neuronal identity»

TUSC3 serves as a rate-limiting gatekeeper of a glycan-mediated ER Triage Checkpoint for BMP4/Dpp

Antonio Galeone [1,8,§], Emilio Solazzo [1,4,§], Francesco Lavezzari [2], Seung Yeop Han [3], Gaia Consonni [2], Bruna My [4], Riccardo Rizzo [1], Giuseppe Gigli [1,5], Hamed Jafar-Nejad [3,6,7,*] and **Thomas Vaccari** [2,*]

1 Institute of Nanotechnology, National Research Council (CNR-NANOTEC) and Tecnomed Puglia - Tecnopolo per la medicina di precisione (Biotech Lecce Hub), Lecce, Italy.
2 Department of Biosciences, University of Milan, Milan, Italy.
3 Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, USA.
4 Department of Mathematics and Physics, University of Salento, Lecce, Italy.
5 Experimental Medicine Department, University of Salento, Lecce, Italy.
6 Genetics & Genomic Graduate Program, Baylor College of Medicine, Houston, USA.
7 Development, Disease Models & Therapeutics Graduate Program, Baylor College of Medicine, Houston, USA
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How are cell-cell signaling outputs controlled in developmental contexts? Is N-glycosylation involved in setting signaling thresholds? Are co-and post-translational modifications during secretion a general determinant of protein quality control? Recent and upcoming work in the Vaccari lab uncovers the consequences of the impairment of two key step modification of the bone morphogenetic protein 4 (BMP4) and its *Drosophila* homolog Dpp in the endoplasmic reticulum (ER). In particular, soon-to-be-published work reveals that trimming of the three glucose residues decorating nascent N-glycoproteins is a critical step for their entry into the ER quality control (ERQC) and recognition by ER chaperones. In fact, we find that TUSC3, a component of the oligosaccharyltransferase (OST) complex, regulates G2 to G1 trimming on N-glycosylated in BMP4/Dpp to promote their ERQC entry. Loss- and gain-of-function genetic experiments and biochemical assays in mammalian cells and flies indicate that TUSC3 serves as a dosage-sensitive gatekeeper that influences the decision between proper folding and secretion *versus* elimination by ER-associated degradation for BMP4 molecules, thereby tuning BMP signaling. Together, these data reveal an unrecognized role for an OST component in early glycoprotein maturation, relevant to a major developmental signaling pathway.

Circadian regulation of mitochondrial dynamics in the retina of *Drosophila melanogaster*

Nadia Ceccato [1], Justyna Kadluczka [2], Davide Colaianni [1], Milena Damulewicz [2], Gabriella **M. Mazzotta** [1]

1 Department of Biology, University of Padua, Padua, Italy
2 Department of Cell Biology and Imaging, Jagiellonian University, Kraków, Poland

Nearly all metabolic processes follow 24-hour rhythmic patterns closely tied to the circadian clock, with mitochondria playing a crucial role in meeting the body's changing energy demands. However, the mechanisms by which mitochondria adapt to these daily cycles remain poorly understood.
We used retinal photoreceptor cells as a model system, as they are among the most metabolically active neurons and experience pronounced daily changes in energy demand required for vision. In *Drosophila*, these cells also contain an autonomous circadian clock that regulates visual sensitivity. Using this system, we investigated whether mitochondrial dynamics in the eye follow rhythmic patterns.
Our analyses reveal that mitochondrial abundance oscillates across the day in distinct patterns among photoreceptor subtypes. These rhythms are shaped by each subtype's light sensitivity and synaptic connectivity and depend on both circadian signalling and direct light input. In addition, mitochondrial size and morphology in the retina are under circadian control: a functional, entrainable clock is required to maintain proper mitochondrial organisation.
Together, our findings indicate that intrinsic circadian mechanisms coordinate mitochondrial dynamics with daily environmental cycles. This work provides new insight into how circadian regulation intersects with cellular metabolism and may help illuminate links between circadian disruption, mitochondrial dysfunction, and neurodegenerative diseases.

From timing to wiring: a global temporal code for neuronal identity

Sebastian Cachero [1], Myrto Mitletton [1], Isabella R. Beckett [1], Elizabeth C. Marin [2], Laia Serratosa Capdevila [3], Marina Gkantia [2], Jelly H.M. Soffers [4], Haluk Lacin [4], Gregory S.X.E. Jefferis [1,2], **Erika Donà** [5]

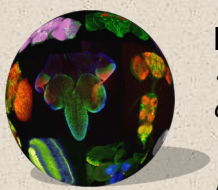
(1) Neurobiology Division, MRC Laboratory of Molecular Biology, Cambridge, UK
(2) Department of Zoology, University of Cambridge, Cambridge, UK
(3) Aelysia Ltd., Bristol, UK
(4) School of Science and Engineering, Division Biological and Biomedical Systems, University of Missouri-Kansas City, MO, USA
(5) Institute of Neuroscience, Consiglio Nazionale delle Ricerche (CNR), Vedano al Lambro (MB), Italy

The assembly of functional neural circuits relies on the generation of diverse neural types with precise molecular identity and connectivity. Unlocking general principles of neuronal specification and wiring across the nervous system requires a systematic and high-resolution characterisation of its diversity, recently enabled by advances in single-cell transcriptomics and connectomics. However, linking the molecular identity of neurons to circuit architecture remains a key challenge.
Here, we present a high-resolution developmental transcriptional atlas for the *Drosophila melanogaster* nerve cord, the central hub for sensory-motor circuits. With an unprecedented 38× aggregate coverage relative to its reference connectome, our atlas captures extensive molecular diversity and enables robust mapping to the adult connectome.
We identified three developmental principles underlying neuronal diversity in the nerve cord. First, timing of neurogenesis shapes diversification of molecular identity: embryonic-born neurons diverge faster than larval-born neurons, as also observed in the adult connectome. Second, 17 transcription factors common to neurons from all lineages provide a global molecular identity code for birth order. Consistently, ectopic expression of one of these transcription factors outside its normal temporal window drives changes in neuronal identity. Lastly, by mapping sex-specific transcriptional profiles to the connectome, we uncovered female-specific apoptosis and transcriptional divergence as key global drivers of sex specification.
By revealing key organisational axes of molecular identity, this atlas opens new avenues to dissect the molecular mechanisms underpinning the development and evolution of neural circuits.

Wednesday, July 8

Talk Session 2
h. 10.00-11.30

Brain development and disease
Chair: Cristiano De Pittà



Roberto Feuda
«Evolutionary conserved transcriptional regulators control monoaminergic neuron development»

Alessia Soldano
«m6A RNA methylation: how shaping the glia epitranscriptome affects brain development»

Diana Pendin
«From ER shaping to ER function: imaging neurodegeneration in Drosophila»

Evolutionary conserved transcriptional regulators control monoaminergic neuron development

Clifton Lewis [1,2], Matthew Goulty [1,3], Aniela Wroblewska [1], Nicola Croxall [4], David Onion [4], Sue Robinson [5], Robert P. Zinzen [6], Jordi Solana [7,8], Charlambos P. Kyriacou [1], Ezio Rosato [1] and **Roberto Feuda** [1,9]

1 Neurogenetics Group, Division of Genetics and Genome Biology, School of Biology and Biomedical Sciences, College of Life Sciences, University of Leicester, Leicester, LE1 7RH, UK
2 Centre for Chromosome Biology, Biomedical Sciences Building, University of Galway, Galway, H91 W2TY, Ireland
3 The Francis Crick Institute, London, UK
4 Faculty of Medicine & Health Sciences, Queen's Medical Centre, University of Nottingham, Nottingham, NG7 2UH, UK
5 Division of Molecular and Cell Biology, College of Life Sciences, University of Leicester, Leicester, LE1 7RH, UK
6 Systems Biology Imaging Technology Platform, Max Delbrück Centre for Molecular Medicine (MDC-BIMSB), Berlin, Germany
7 Living Systems Institute, University of Exeter, Exeter, UK
8 Department of Biosciences, University of Exeter, Exeter, UK
9 Department of Biology, Geology and Environmental Sciences, University of Bologna, Bologna, Italy

To what extent conserved developmental programs specify homologous cell types is a central biological question. To address this, we reconstructed the developmental program underlying monoaminergic neuron specification in *Drosophila melanogaster* embryos using time-resolved single-cell genomics, spatial transcript mapping, and targeted metabolomics. We identified a defined set of transcription factors activated prior to biosynthetic enzyme expression, revealing a temporal hierarchy underlying fate commitment. We then tested the evolutionary conservation of this regulatory program by comparing monoaminergic developmental trajectories across *Drosophila*, zebrafish, and sea urchin single-cell atlases. These analyses reveal conservation in the temporal deployment of key orthologous transcription factors across ~550 million years of bilaterian evolution. Simultaneously, our results indicate that lineage-specific regulatory innovations contributed to the diversification of monoaminergic subtypes across species,

m6A RNA methylation: how shaping the glia epitranscriptome affects brain development

Rossella Gratton, Anja Kobal, Chiara Ercolano, Elena D'Alessandris, Silvia Bizzarri, **Alessia Soldano**

Laboratory of Neurogenetics, International School for Advanced Studies (SISSA), Neuroscience Area

During neurodevelopment, precise spatiotemporal coordination of gene expression profiles and signaling pathways is fundamental to properly regulate cell fate, differentiation, and proliferation. Beyond the classical mechanisms regulating gene expression, the epitranscriptome, including various RNA modifications, has emerged as a critical and dynamic layer of post-transcriptional control, affecting the rapid cellular responses required during tissue morphogenesis. Among these modifications, N6-methyladenosine (m6A) is the most abundant and extensively characterized. Highly enriched within the brain, m6A is involved in modulation of various neurobiological functions across both at early and late stages of neural development. However, the precise tissue and time-specific distribution and consequences of m6A in the brain are still elusive. In our lab, we study the role of m6A in glia cells development and function, combining the powerful genetic toolkit offered by *Drosophila*, together with transcriptomic techniques (scSEQ, DART-seq) and 3D cellular models, such as hIPSCs derived brain organoids.
In my presentation, I will describe some of our latest findings describing the involvement of m6A in glia cells development.

From ER shaping to ER function: imaging neurodegeneration in *Drosophila*

Manuela Santalla [1], Sonia Sonda [1], Nicola Vajente [1], Ambra Bertocco [2], Paola Pizzo [2], Elisa Greotti [1,3], **Diana Pendin** [1]

1 Neuroscience Institute, Italian National Research Council, Padua, Italy; 2 Department of Biomedical Sciences, University of Padua, Padua, Italy; 3 Padova Neuroscience Centre (PNC), University of Padua, Padua, Italy

Hereditary Spastic Paraplegias (HSPs) are a genetically heterogeneous group of neurodegenerative disorders characterized by progressive degeneration of corticospinal neurons. Despite the identification of more than 80 disease-associated genes, the cellular mechanisms underlying HSP pathogenesis remain only partially understood. *Drosophila melanogaster* has proven to be an exceptional model for investigating HSP-associated proteins whose biological functions were previously unknown, owing to its experimental tractability and limited genetic redundancy. Our previous studies identified Atlastin as the ER-shaping GTPase mediating homotypic fusion of endoplasmic reticulum (ER) membranes, and the *Drosophila* Reticulon orthologue Rtnl1 as the functional antagonist of Atlastin, promoting ER membrane fission. These findings revealed the requirement for a dynamic balance between fusion and fission processes to maintain ER organization. Subsequent studies on additional HSP proteins, including regulators of microtubule dynamics and ER-phagy, further established ER network organization and positioning as key cellular determinants in neurodegeneration, identifying ER membrane dynamics as a central pathogenic theme in HSP. Understanding how alterations in ER morphology impact ER function in neurons is now a critical challenge. Since the ER is the major intracellular Ca^{2+} store and Ca^{2+} handling is essential for neuronal physiology and survival, we investigated the relationship between ER organization and Ca^{2+} homeostasis. Using Drosophila HSP models displaying distinct ER morphological defects, together with newly developed in vivo Ca^{2+} imaging tools, we demonstrated that disruption of ER architecture profoundly affects store-operated Ca^{2+} entry (SOCE) and intracellular Ca^{2+} handling, independently of the primary cause of ER deformation. These findings support the idea that ER Ca^{2+} dyshomeostasis represents a convergent pathogenic mechanism in HSP. Together, our work highlights how *Drosophila* genetics and advanced imaging approaches can uncover fundamental mechanisms linking ER organization to neuronal function and neurodegeneration.

Students' session
h. 11.45-12.15

AI tools for *Drosophila* research
Chair: Roberto Piergentili

Federica Rosati: *MultiCell*
federica.rosati1@student.univaq.it

Eleonora Paradisi: *DeepMEL*
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Alice Bove: *FlyWire*
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Iara Sfarra: *FlydAI*
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Martina Iafrate: *FlyVISTA*
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Davide Di Giulio: *JAABA*
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Marta Santone: *LabGym*
marta.santone@student.univaq.it

AI Tools for *Drosophila* Research

Federica Rosati, Eleonora Paradisi, Alice Bove, Iara Sfarra, Martina Iafrate, Davide Di Giulio, Marta Santone

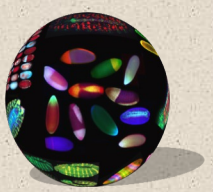
University of L'Aquila

Drosophila melanogaster is one of the primary model organisms for studying fundamental biological processes. The integration of artificial intelligence into *Drosophila* research is revolutionizing the analysis of genomic, morphological, and behavioral data. This session presents some of the most recent tools based on machine learning and deep learning applied to this organism.
MultiCell is a model developed by MIT that uses graph-based neural networks to predict embryonic development processes, achieving high accuracy in predicting the loss of cell-cell junctions during morphogenesis. DeepMEL also employs neural networks, leveraging them to understand the regulatory logic of enhancers and design synthetic enhancers specific to certain cell types. FlyWire is a platform for reconstructing the complete *Drosophila* brain connectome, combining collaborative annotation and artificial intelligence tools for mapping neuronal connections.
In the field of automated phenotyping, FlydAI uses image recognition algorithms for counting and phenotypic classification of flies, achieving high levels of accuracy. FlyVISTA combines high-resolution video acquisition, tracking via DeepLabCut, and automatic classification of sleep behaviors. JAABA enables supervised learning of behavioral patterns through user-provided examples, automating the analysis of large amounts of video data. Finally, LabGym leverages deep learning techniques to identify and classify complex animal behaviors directly from videos, reducing the need for manual annotations and increasing the reproducibility of analyses.
Together, these tools demonstrate how artificial intelligence is transforming *Drosophila* research, enabling faster, more accurate, and more reproducible analyses across fields ranging from developmental biology to regulatory genomics, from neurobiology to computational ethology.

MultiCell: Yang H et al**.** MultiCell: geometric learning in multicellular development. Nature Methods 23, 617-625 (2026).
DeepMEL: Taskiran, I.I., Spanier, K.I., Dickmänken, H. et al. Cell-type-directed design of synthetic enhancers. Nature 626, 212–220 (2023).
FlyWire: Nature (2024) - "The FlyWire connectome"; NeuroTrailblazers; FlyWire; Neuroscience News (2026).
FlydAI: Gálvez Salido, A., de la Herrán, R., Robles, F., Ruiz Rejón, C., and Navajas-Pérez, R. 2024. Automatic counting and identification of two *Drosophila melanogaster* (Diprera: Drosophilidae) morphs with image-regognition artificial intelligence. The Canadian Entomologist.
FlyVISTA: Keleş et al., 2025 FlyVISTA, an integrated machine learning platform for deep phenotyping of sleep in *Drosophila*
JAABA: Interactive Machine Learning for Automatic Animal Behavior Annotation", Kabra, Robie, et al., Nature Methods, 2012.
LabGym: Hu et al. (2023), Cell Reports Methods

MiniTalk Session 1
h. 12.15-13.15

Chair: Cinzia Volontè



Gianni Cenci
«Loquacious (Loqs) and its human ortholog PACT affect the response to IR in a dosage-dependent manner»

Chiara Stefanelli
«Functional characterization of the hnRNP Hrb87F through combined HyperTRIBE and CRISPR/Cas9 approaches»

Giulia Vitale
«Drosophila models of intractable Visceral Myopathy caused by ACTG2 mutations»

Marta Marzullo
«TDP-43-mediated polyamine dysregulation drives neuromuscular defects in ALS models»

Loquacious (Loqs) and its human ortholog PACT affect the response to IR in a dosage-dependent manner

Andrea D'Alessandro, Maria Assunta Graziano, **Giovanni Cenci**

Department of Biology and Biotechnologies "C. Darwin"; Fondazione Cenci Bolognetti/Istituto Pasteur, SAPIENZA, Università di Roma

Radiotherapy is a key cancer treatment, but its long-term success is often limited by tumor recurrence driven by intrinsic or acquired radioresistance. This resistance arises from cellular mechanisms that enable tumor cells to withstand genotoxic stress better than healthy tissue. Understanding these mechanisms is essential for improving clinical outcomes. *Drosophila melanogaster* offers a valuable platform to study radiation responses. We found that three copies of loqs, which encodes a double-stranded RNA-binding protein involved in small non-coding RNA biogenesis, confer radiosensitivity in Drosophila mitotic cells. This contrasts with the radioresistance previously observed in heterozygous *loqs* mutants, indicating that *loqs* function in DNA damage response is dosage-sensitive. These effects are consistent across tissues and cell types. We also demonstrated that *PACT*, the human ortholog of *loqs*, plays a similar role in human cells, including cancer cells. Depletion of PACT -known for its roles in small RNA biogenesis, antiviral response, and PKR activation- conferred radioresistance in both tumor and non-tumor cell lines. Conversely, *PACT* overexpression in radioresistant embryonal rhabdomyosarcoma cells increased X-ray sensitivity. Additionally, we observed that modulating *PACT* or *loqs* affects chromatin state in human and *Drosophila* cells, respectively, potentially underpinning the observed resistance or sensitivity.

Functional characterization of the hnRNP Hrb87F through combined HyperTRIBE and CRISPR/Cas9 approaches

Chiara Stefanelli [1], Sara Provesi [1], Eugenio Caldart [1], Pietro Antolini [1], Davide Colaianni [1], Gabriele Sales [1], Francesco Chemello [2], Cristiano De Pittà [1], Gabriella M. Mazzotta [1]

1 Department of Biology, University of Padova
2 Department of Pharmacy and Biotechnology (FaBiT), University of Bologna

Heterogeneous nuclear ribonucleoproteins (hnRNPs) are a large family of RNA-binding proteins (RBPs) playing essential roles in various aspects of nucleic acid metabolism. In *Drosophila melanogaster*, Hrb87F participates in omega speckle formation, alternative splicing, and telomere maintenance, and its dysregulation results in developmental abnormalities, reduced female fertility, and impaired stress tolerance.
Notably, Hrb87F is a homolog of the human hnRNP A/B subgroup, which has been linked to several neurodegenerative diseases, and increasing evidence from *Drosophila* models of neurodegeneration points to a potential role for Hrb87F in neuronal function in flies as well.
To gain deeper insight into Hrb87F activity, we identified its cell-specific RNA targets *in vitro* using the HyperTRIBE technique (Targets of RNA-binding proteins Identified By Editing). The identified targets support the established functions of Hrb87F while also highlighting potential roles in neuronal homeostasis. To validate these findings, we are generating *Hrb87F* knockout cells via CRISPR/Cas9 and extending our approach *in vivo* by applying HyperTRIBE in *Drosophila* brains.
This work establishes a strong foundation for a comprehensive analysis of Hrb87F function in *D. melanogaster*. Given the widespread roles of hnRNPs in RNA processing, identifying cell-type specific RNA targets is essential for understanding their functional significance.

Drosophila models of intractable Visceral Myopathy caused by ACTG2 mutations

Giulia C. Vitale 1, Kaan Kozluka [1], Paolo Prina [1], Emma Terzi [1], Antonio Galeone [2], Federica Viti [3], Margherita Marchi [4], Thomas Vaccari 1

1 Department of Bioscienze, University of Milan, Milano, Italy
2 Instituto di Nanotecnologia, National Research Council, Lecce, Italy
3 Institute of Biophysics, National Research Council, Genova, Italy
4 Istituto Neurologico Carlo Besta, Milano, Italy

Primary visceral myopathies (VSCM) are a group of rare genetic diseases characterized by severe smooth muscle disfunction reducing efficient movement of air and nutrients through the bowel and impairing bladder emptying and uterine contraction (Hashmi et al 2021). VSCM often causes chronic pseudo-obstruction (CIPO), a life-threatening condition requiring immediate medical attention. VSCM diagnosis is challenging due to its low-incidence among population and non-specific clinical symptoms. Therapies are non resolutive and usually consist of partial or total parenteral nutrition, ostomy and in extreme situations, even intestinal transplantation (Di Nardo et al 2017; Chen et al 2025). Among types of VSCM, the most debilitating are associated with dominant variants in the *actin gamma 2* gene (*ACTG2*), with disease severity that depends on the specific amino acid change (Wangler et al 2014). Preliminary studies suggest that disease-associated variants may affect protein stability, actin polymerization and/or depolymerization, and interaction with other proteins (Ceron et al 2024). The study of ACTG2 VSCM has been very limited so far, and no efficient genetic models have been established for functional analysis. To develop a *Drosophila melanogaster* model, we have generated transgenic lines expressing either human *ACTG2* or pathogenic variant, *R40C*, *Y70C*, *R148C*, *R178C*, *R178H*, or *R257C*, which are known to represent most phenotypic variability in patients. So far, we have demonstrated that *ACTG2* or *ACTG2R257C*, the most common pathogenic variant, can be expressed at high levels in fly muscles and can be recovered as full-length proteins. We also find that expression of *ACTG2R257C* but not of *ACTG2* increases the insoluble actin fraction, alters gut peristalsis and prevents food transit. We propose to use our models to compare variants by analyzing tissues, organs and animal behavior in vivo. Eventually, we hope to identify new disease targets and pharmacological intervention to develop effective and possibly variant-specific treatments.

TDP-43-mediated polyamine dysregulation drives neuromuscular defects in ALS models

Marta Marzullo [1,2], Marta Terribili [3], Nadia Zappaterreni [3], Alberto Macone [4], Sonia Coni [5], Gianluca Canettieri [5] and Laura Ciapponi [3]

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2 Section of Human Anatomy, Department of Neuroscience, Università Cattolica del Sacro Cuore, Rome, Italy
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4 Department of Biochemical Sciences, Sapienza University of Rome, Rome, Italy;
5 Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy;

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive motor neuron degeneration. A central pathological hallmark of ALS is the dysfunction of the RNA-binding protein TDP-43, which undergoes cytoplasmic mislocalization and aggregation in the majority of both familial and sporadic cases. TDP-43 regulates multiple aspects of RNA metabolism, and its dysfunction broadly impacts downstream cellular pathways. Despite its central role in ALS pathology, the mechanisms by which TDP-43 alterations drives neurodegeneration remain unclear. In particular, the possible role of polyamine in ALS is still under investigation and whether TDP-43 contributes to disease by altering polyamine homeostasis has not yet been established.

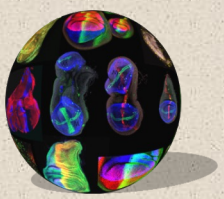
Here, we demonstrate that TDP-43 directly binds the mRNA of Ornithine Decarboxylase 1 (ODC1), the rate-limiting enzyme in polyamine biosynthesis, thereby regulating its stability, and that this interaction is conserved in both *Drosophila* and humans. In *Drosophila melanogaster*, loss of the TDP-43 orthologue TBPH leads to reduced ODC1 levels and a consequent decrease in polyamine content. Functionally, disruption of TDP-43 function, results in locomotor impairment and neuromuscular junction defects. Importantly, both dietary polyamine supplementation and genetic restoration of ODC levels rescue these phenotypes.

Together, our findings identify the ODC-polyamine axis as a key downstream effector of TDP-43-mediated neurotoxicity and uncover polyamine dysregulation as a mechanistic link between TDP-43 and neurodegeneration in ALS.

Talk Session 3
h. 15.30-17.00

Stress signalling, genome integrity & cancer

Chair: Grazia Daniela Raffa



Paola Bellosta
«Nucleolar NOC1/CEBPZ protein controls rRNA Maturation and Genome Integrity: A Novel Nucleolar Surveillance Link with MYC and p53 controlling Apoptosis»

Laura Fanti
«Heat shock affects genome stability and centromere identity in Drosophila"»

Chiara Angioli
«Vitamin B6 deficiency shapes tumor behavior in Drosophila models»

Nucleolar NOC1/CEBPZ protein controls rRNA Maturation and Genome Integrity: A Novel Nucleolar Surveillance Link with MYC and p53 controlling Apoptosis

Valeria Manara [1], Shivani Bajaj [1], Andrea Vutera-Cuda [1], Marianna Penzo [2,3], Guglielmo Rambaldelli [2,3], **Paola Bellosta** [1,4]

1 Dept-CIBIO, University of Trento, IT
2 Dept-DIMEC University of Bologna, IT
3 IRCCS-Policlinico Sant'Orsola Bologna, IT
4 Dept-Medicine, New York University Grossman School of Medicine, NY, USA

Ribosome biogenesis is essential for cellular homeostasis, and its disruption is increasingly associated with cancer development. Among the nucleolar factors involved in this process, NOC1, a C/EBP family transcription factor, regulates pre-rRNA processing and 60S ribosomal subunit maturation through its interaction with NOC2 and NOC3 [1, 2].
Using Drosophila as a model system, we demonstrate that NOC1 downregulation disrupts prerRNA processing, inducing nucleolar stress, altered nucleolar morphology, and DNA damage. These defects activate a nucleolar surveillance response characterized by MYC and p53 induction [3] and mediated by Xrp1, a stress-induced C/EBP transcription factor involved in cell competition and tissue homeostasis. In this context, p53 exerts a protective role by limiting the propagation of damaged cells and promoting apoptosis when nucleolar stress becomes excessive. Importantly, NOC1 depletion is also associated with increased R-loop accumulation, supporting a link between impaired ribosome biogenesis and transcription-associated genomic stress within the nucleolus. We further show partial conservation of this mechanism in the human homolog CEBPZ. In addition, CEBPZ, NOC2L, and NOC3L display altered expression across multiple tumor types [4], supporting a potential link between nucleolar stress regulation and genome stability pathways in cancer.

[1] P. Milkereit, O. Gadal, A. Podtelejnikov, S. Trumtel, N. Gas, E. Petfalski, D. Tollervey, M. Mann, E. Hurt, H. Tschochner, Maturation and intranuclear transport of pre-ribosomes requires Noc proteins, Cell, 105 (2001) 499–509.
[2] F. Destefanis, V. Manara, S. Santarelli, S. Zola, M. Brambilla, G. Viola, P. Maragno, I. Signoria, G. Viero, M.E. Pasini, M. Penzo, P. Bellosta, Reduction of nucleolar NOC1 leads to the accumulation of pre-rRNAs and induces Xrp1, affecting growth and resulting in cell competition, J Cell Sci, 135 (2022).
[3] A. Vutera Cuda, S. Bajaj, V. Manara, P. Bellosta, Noc1 downregulation induces nucleolar stress and upregulates p53 isoforms, with a robust increase of the truncated p53E isoform in Drosophila wing discs, G3 (Bethesda), (2026).
[4] G. Rambaldelli, V. Manara, A. Vutera Cuda, G. Bertalot, M. Penzo, P. Bellosta, Drosophila and human cell studies reveal a conserved role for CEBPZ, NOC2L and NOC3L in rRNA processing and tumorigenesis, J Cell Sci, 138 (2025).

Heat shock affects genome stability and centromere identity in *Drosophila*

Laura Leo [1,2], Erika Andreuccioli [1], Nunzia Colonna Romano [1], Domiziana Trasmondi [1], and **Laura Fanti** [1]

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2 Ospedale Pediatrico Bambino Gesù

Organisms are continually subjected to a variety of both biotic and abiotic stress conditions that alter the physiological state of cells. Previous studies have shown that heat shock can exert deleterious effects on cells, including protein unfolding, cytoskeletal defects, loss of proper organelle localization, disruption of intracellular transport processes, and cell cycle arrest, all of which may ultimately lead to cell death. Furthermore, several lines of evidence indicate that heat shock can perturb mitotic processes and compromise genome stability. However, although many studies have provided insights into the cellular response to environmental stress, the genomic instability and chromosomal alterations induced by stress conditions remain poorly understood.
We show how heat shock affects centromere organization and function by inducing chromosomal instability (CIN) in *Drosophila melanogaster*, a particularly suitable and useful model for directly observing potential chromosomal damage.

Vitamin B6 deficiency shapes tumor behavior in *Drosophila* models

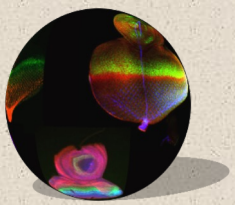
Chiara Angioli, Angelo Ferriero, Beatrice Agostini, Fiammetta Vernì

Department of Biology and Biotechnologies "Charles Darwin", Sapienza University of Rome, 00185, Rome, Italy

Vitamin B6 is an antioxidant molecule whose active form, pyridoxal 5′-phosphate (PLP), acts as a crucial cofactor in DNA and amino acid metabolism. Several human studies have reported an inverse correlation between vitamin B6 levels and cancer risk; however, the underlying mechanisms remain unclear. Previous work showed that vitamin B6 deficiency induces chromosome aberrations (CABs) in both *Drosophila* and human cells, suggesting that genome instability may serve as a driver of cancer. Using the Ras^{V12} *Drosophila* cancer model, we demonstrate that PLP depletion promotes the conversion of benign tumors in eye imaginal discs into malignant forms by inducing DNA and chromosomal damage mediated by reactive oxygen species (ROS). ROS accumulation results from both the loss of the antioxidant activity of vitamin B6 and impaired one-carbon metabolism, reflecting the role of PLP as a cofactor of serine hydroxymethyltransferase (SHMT), whose dysfunction increases oxidative stress. Furthermore, by investigating the relatively underexplored tumor suppressor role of SHMT in the Ras^{V12}/Dlg-deficient tumor model, we show that the combined depletion of PLP and SHMT induces massive DNA damage that triggers apoptosis, thereby limiting tumor growth**.** This finding demonstrates that simultaneous targeting of PLP availability and SHMT activity can effectively restrain tumor progression when SHMT function is critical. Building on this, we propose that a similar strategy could be exploited in cancers characterized by SHMT overexpression, in which SHMT inhibitors alone have shown limited efficacy.

Talk Session 4
h. 17.15-18.45

Pathogenic mechanisms 1
Chair: René Massimiliano Marsano



Damiano Guerrini
«The role of transcriptional stress in Spinal Muscolar Atrophy»

Gaia Consonni
«Mesodermal-specific MECP2 expression in Drosophila induces visceral and skeletal muscle defects rescued by butyrate supplementation»

Francesco Liguori
«Modeling SOD1-ALS in Drosophila: from pathogenic mechanisms to drug repurposing»

The role of transcriptional stress in Spinal Muscolar Atrophy

Damiano Guerrini [1], Paolo Maccallini [1], Livia Scatolini [1], Lucia Graziadio [1], Roberto Piergentili [2], Maurizio Gatti [1,2], Alessio Colantoni [3], Grazia D. Raffa [1]

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2 Institute of Molecular Biology and Pathology, CNR IBPM, CNR, 00185 Rome, IT
3 UniCamillus-Saint Camillus International University of Health Sciences, 00131 Rome

Italy Spinal Muscular Atrophy (SMA) is a severe neurodegenerative disease caused by loss of the Survival Motor Neuron (SMN) protein, a key regulator of snRNP biogenesis and splicing fidelity. Trimethylguanosine synthase 1 (TGS1), a direct SMN interactor involved in snRNA cap hypermethylation, has emerged as a conserved genetic modifier of SMN in both *Drosophila* and *C. elegans*. TGS1 depletion causes neurological defects in flies and neurodegeneration in nematodes and zebrafish, suggesting a protective role in SMA-related pathways. Here, we show that TGS1 depletion in *Drosophila* larval brains phenocopies SMN loss, inducing accumulation of DNA:RNA hybrids (R-loops) and DNA damage, detected by S9.6 immunostaining and γH2Av *foci*. These defects are associated with widespread alternative splicing alterations identified by Illumina and Nanopore sequencing, together with defective snRNA 3′ end processing, particularly of U2 snRNA, likely contributing to spliceosome dysfunction and further R-loop formation. Strikingly, TGS1 depletion also causes strong downregulation of replication-dependent histones at both mRNA and protein levels, accompanied by increased RNA Polymerase II pausing at histone clusters, defective histone pre-mRNA 3′ end processing, and altered chromatin accessibility at histone loci. In early embryos, loss of TGS1 broadly impairs Pol II elongation and increases pausing at snRNA loci and telomeric regions, supporting a global role in transcription regulation. Transcriptomic analyses reveal partially overlapping expression signatures between Tgs1- and Smn-depleted brains, with shared dysregulated genes enriched for innate immune pathways, including antimicrobial peptides, cytokine homologs (Upd2/Upd3), and replication-dependent histones, suggesting that R-loop-driven genome instability activates inflammatory responses in both conditions. Importantly, increased dosage of U2 and U5 snRNAs partially rescues R-loop accumulation, DNA damage, neurodevelopmental defects, and cytokine dysregulation in both Tgs1- and Smn-depleted flies. RNase H1 overexpression similarly suppresses DNA damage in Smn mutants and is further enhanced by TGS1 co-expression. Together, these findings establish TGS1 as a critical SMN co-factor whose loss recapitulates core molecular hallmarks of SMA and provide new insight into mechanisms underlying motor neuron vulnerability.

Mesodermal-specific MECP2 expression in Drosophila induces visceral and skeletal muscle defects rescued by butyrate supplementation

Gaia Consonni [1], Mustafa Kaan Kozluca [1], Maria Cristina Gagliani [4], Alessandro Mineo [3], Katia Cortese [4], Irene Miguel-Aliaga [3], Aglaia Vignoli [2], Elisa Borghi [2], Antonio Galeone [5], Thomas Vaccari [1]

1 Department of Biosciences, University of Milan, Milan, Italy;
2 Department of Health Sciences, University of Milan, Milan, Italy
3 The Francis Crick Institute, London, United Kingdom
4 University of Genoa, Genoa, Italy
5 National Research Council (CNR), Lecce, Italy

Patients affected by Rett syndrome (RTT) and MECP2 duplication syndrome (MDS) experience disabling muscle weakness and gastrointestinal dysmotility of unclear origin. Whether these defects arise cell-autonomously, rather than secondarily to neural dysfunction, and which developmental windows are most vulnerable to MeCP2 disfunction remains unresolved. MeCP2 is a dosage-sensitive transcriptional regulator, whose functions are tightly linked to chromatin states. Because short-chain fatty acids (SCFAs) are known to inhibit histone deacetylases (HDACs), a tractable *in vivo* model is needed to test the effect of HDAC modulation on muscle defects.

We misexpressed human MECP2 in the *Drosophila melanogaster* mesoderm that gives rise to skeletal and visceral muscles. We analyzed quantitatively their morphology and function. To assess the effects of SCFA supplementation, we also supplemented diets with sodium butyrate (NaB), Lalbaay®, a NaB-containing supplement, acetate (AcOH), and valproate (VPA).

MECP2 misexpression caused pre-eclosion lethality, thinning of larval skeletal fibers with nuclear mispositioning and altered mitochondria. Functionally, it reduced locomotion, decreased food transit and gut peristalsis. Phenotypes were strongest when expression began during development. NaB and VPA supplementation rescue most of these phenotypes, consistent with their histone-deacetylase (HDAC) activity. Defects were not observed upon comparable misexpression of an RTT-associated MeCP2 loss-of-function variant, indicating that they might be relevant to pathogenesis of MECP2-related disorders.

Our genetic *in vivo* analysis models peripheral effects of MeCP2 dysregulation and their amelioration, supporting the possibility of HDAC-targeted strategies for MECP2-related muscle and gastrointestinal dysfunction.

Modeling SOD1-ALS in Drosophila: from pathogenic mechanisms to drug repurposing

Francesco Liguori [1,2], Luca Zanfini [1,3], Susanna Amadio [1,2] and Cinzia Volonté [2,1]

1 Experimental Neuroscience and Neurological Disease Models, IRCCS Fondazione Santa Lucia, Via del Fosso di Fiorano 65, 00143 Rome; 2 Institute of System Analysis and Computer Science (IASI), National Research Council (CNR), Via dei Taurini 19, 00185 Rome; 3 Dept. of Biology and Biotechnology “Charles Darwin”, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome.

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive loss of motor neurons and muscle weakness, generally leading to death within 2-5 years of symptom onset. The Superoxide Dismutase 1 (SOD1) gene is the second most common ALS-associated gene, with more than 200 identified point mutations. Despite extensive research, currently approved drugs offer limited clinical benefit. To bypass this therapeutic letdown, we employed a network medicine-based drug repurposing strategy to identify potential therapeutic candidates for ALS treatment. Using the SAveRUNNER algorithm (Fiscon et al., 2021), we selected clomipramine, mianserin, and modafinil, and validated their efficacy in vivo using two *Drosophila melanogaster* models pan-neuronally expressing human mutant *SOD1* (*hSOD1A4V* and *hSOD1G85R*).

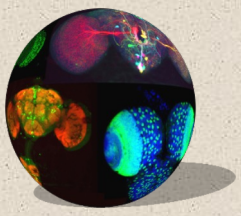
The expression of these pathogenic constructs significantly impairs fly survival and motor performance. At the cellular level, mutant *hSOD1* induction determines gliosis and up-regulates the IMD/Toll immune pathways through the expression of antimicrobial peptides. Furthermore, we identified significant chromosome aberrations in larval brains and interference with oxidative metabolism, suggesting that genomic instability and inflammatory responses are key components of *SOD1*-induced pathogenesis (Liguori et al., 2024). Among the pharmacological compounds emerged from our drug-repurposing analysis, clomipramine resulted as the most promising, significantly ameliorating lifespan, improving climbing abilities, and mitigating both genomic instability and neuroinflammation (Liguori et al., 2026).

Building on these results, we are currently characterizing the molecular mechanism by which clomipramine exerts its protective role, with a specific focus on the ability to resolve DNA damage and restore genomic integrity. Our preliminary findings establish an action of clomipramine on the DNA damage response pathway. Taken together, our data highlight the efficacy of *Drosophila* as a powerful ALS model and platform where to identify promising targets for therapeutic intervention and provide significant support for clomipramine as a potential add-on treatment for SOD1-ALS.

Thursday, July 9

Talk Session 5
h. 10.00-11.30

Pathogenic mechanisms 2
Chair: Daniela Passarella



Marta Terribili
«A Novel Role for Autophagy Deregulation in Myotonic Dystrophy Type 2 Pathogenesis»

Maria Antonietta Casu
«An overview of the effects of micro-nanoplastic exposure in Drosophila melanogaster, with a focus on mechanisms relevant to Parkinson's disease»

Bibiana Sgalletta
«From mitochondrial dysfunction to neurodegeneration: investigating the metabolic role of C19orf12»

A Novel Role for Autophagy Deregulation in Myotonic Dystrophy Type 2 Pathogenesis

Terribili M. [1], D'Amico R. [2], Marzullo M. [1,4,5], Matkovic T. [6], Sigrist S. [6], Bordone R. [2], Coni S. [2], Canettieri G. [2,3] and Ciapponi L. [1]

1 Department of Biology and Biotechnologies "C. Darwin", Sapienza University of Rome, 00185 Rome, Italy
2 Department of Molecular Medicine, Sapienza University of Rome, 00161 Rome, Italy
3 Istituto Pasteur Italia, Fondazione Cenci Bolognetti, 00161 Rome, Italy
4 Istituto Di Biologia e Patologia Molecolari, CNR, Sapienza Università Di Roma, Rome, Italy
5 Section of Human Anatomy, Department of Neuroscience, Università Cattolica del Sacro Cuore, Rome, Italy
6 Freie Universität Berlin, Institute for Biology and Genetics, Berlin, Germany.

Myotonic dystrophy type 2 (DM2) is a progressive degenerative disease that primarily affects skeletal muscle and is caused by the expansion of unstable CCTG repeats in intron 1 of the CNBP/ZNF9 gene. Several mechanisms have been associated with disease pathogenesis, including CNBP loss of function, repeat RNA toxicity, and protein toxicity. The contribution of CNBP loss of function has been successfully modelled in *Drosophila melanogaster* to dissect DM2 pathogenic mechanisms, where CNBP silencing is associated with locomotor defects and pupal lethality. Autophagy is a fundamental process required for the degradation and recycling of damaged cellular components to maintain tissue homeostasis. Dysfunctions of the autophagy pathway are involved in a range of human diseases, including cancer, neurodegenerative disorders, and myotonic dystrophies.
Here, we show that CNBP depletion in *Drosophila* causes autophagy activation in muscle cells, together with significant alterations in key autophagy markers. We also observed that CNBP-deficient muscles exhibit abnormal mitochondrial morphology, accumulation of enlarged vacuolar structures, and prominent multilamellar membrane bodies, consistent with defective autophagic processing and suggestive of altered mitophagy. Remarkably, we found that the locomotor defects and reduced viability of CNBP-depleted flies can be rescued by Atg7 silencing.
Taken together, these data reveal a previously unrecognized mechanism of autophagy deregulation in DM2 that may significantly contribute to muscle dysfunction, one of the most debilitating symptoms in DM2 patients.

An overview of the effects of micro-nanoplastic exposure in *Drosophila melanogaster*, with a focus on mechanisms relevant to Parkinson's disease

Maria Antonietta Casu [1], Ankit Siwach [2], Maria Dolores Setzu [2], Patrizia Muroni [2], Liana Fattore [3], Ignazia Mocci [1]

1 CNR-Institute of Translational Pharmacology, Cagliari, Italy
2 Department of Biomedical Sciences, University of Cagliari, Italy
3 CNR-Institute of Neuroscience, Cagliari, Italy

Micro- and nanoplastics (MNPs) are pervasive environmental pollutants that can enter the body through ingestion, inhalation, or dermal contact. The public health risks associated with such exposure are only beginning to be characterized, and the underlying pathophysiological mechanisms remain poorly understood. Recent evidence from animal studies indicates that MNPs may contribute to neuroinflammation, oxidative stress, neurotoxicity, and even transgenerational effects. Commonly used *Drosophila melanogaster* (Dm) *wild-type* strains (Canton-S, Oregon-R, and w^{1118}), assessed in embryonic, larval, pupal, and adult developmental stages, have revealed critical information about the pathways, internalization, and distribution of MNPs. These particles are typically administered via ingestion, absorbed through the digestive tract, and subsequently translocated into the hemolymph of both larval and adult flies. As a result, MNPs can accumulate in the excretory Malpighian tubules, in reproductive organs, and in the brain, determining a strong increase in oxidative stress, reduction in longevity, motor problems, and cytotoxicity (1). Many of these effects are also implicated in the progression of neurodegenerative disorders such as Parkinson's disease (PD), which is characterized by impaired motor function, elevated oxidative stress and neuroinflammation, and progressive loss of dopaminergic neurons. Studies in invertebrate, vertebrate, and rodent models support an association between MNP exposure and an increased risk of PD (2–4). The pathogenesis of PD involves both genetic susceptibility and environmental factors. In particular, mutations in the Leucine-rich repeat kinase 2 (LRRK2) gene are a major contributor to familial forms of PD (5).

To investigate the impact of MNP exposure on PD-related phenotypes, we employed a Dm model carrying a loss-of-function mutation in the WD40 domain of LRRK2 (LRRK2WD40), which exhibits PD-like features including motor impairment, dopaminergic neuron loss, and mitochondrial dysfunction (6). We evaluated the effects of three different concentrations of polystyrene MNPs (P-MNPs) on lifespan and motor performance. Our findings indicate that chronic exposure to P-MNPs alters PD-related phenotypes in LRRK2WD40 flies, suggesting a potential interaction between environmental MNP exposure and genetic risk factors for PD.

Given the continued global rise in plastic production and use, there is an urgent need for further research into MNP-related neurological disorders.

From mitochondrial dysfunction to neurodegeneration: investigating the metabolic role of C19orf12

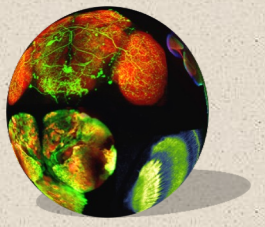
Bibiana Sgalletta*, Francesco Agostini*, Ana TerrienteFelix**, Tamás Maruzs***, Gabor Juhasz***, Alex Whitworth**, Marco Bisaglia*

** University of Padua, ** University of Cambridge, *** University of Szeged*

The high energetic demand of the brain makes neurons critically dependent on mitochondria, the central hub of ATP production. Consequently, mitochondrial dysfunction is a major driver of neurodegeneration. In this context, C19orf12 is a mitochondrial orphan protein associated with mitochondrial membrane protein-associated neurodegeneration (MPAN), a rare infantile-onset neurological disorder characterized by cognitive and locomotor impairments. Even though C19orf12 function remains poorly characterized, it is known that *C19orf12* is evolutionarily conserved and has two *Drosophila* orthologs, *CG3740* and *nazo*. Using a CG3740/nazo double knock-out (DKO) *Drosophila* model, I investigated the function of C19orf12 and its role in maintaining neuronal homeostasis. CG3740/nazo DKO flies exhibit locomotor deficits, reduced lifespan, and dopaminergic neuron loss, thereby recapitulating key features of the human pathology. In addition, MPAN flies display an impaired adaptive response to nutrient deprivation, showing reduced mitochondrial respiration and an increased reliance on glucose metabolism during starvation. Moreover, MPAN flies present a marked reduction in basal lipid droplet content. Beyond serving as energy reservoirs, lipid droplets also protect cells from the accumulation of peroxidized lipids. Consistently, MPAN flies exhibit elevated oxidative stress levels in the brain. The metabolic instability is closely associated with hyperactivation of the AMPK signaling pathway, likely reflecting a compensatory cellular response aimed at restoring energetic homeostasis. Altogether, these findings demonstrate that metabolic dysfunction plays a central role in MPAN pathogenesis, where impaired mitochondrial activity, depletion of lipid droplets, and increased oxidative stress collectively contribute to neuronal vulnerability and degeneration.

MiniTalk Session 2
h. 11.45-12.45

Chair: Patrizia Morciano



Alessandra Caragiuli
«eIF4G1 silencing impairs neuroblast proliferation and promotes apoptosis in the Drosophila melanogaster larval brain»

Davide Colaianni
«Functional characterization of a novel miR-210 target (dAcbp1) in Drosophila lipid metabolism»

Claudia Di Dio
«A reversible torpor-like state in Drosophila melanogaster enhances resistance to ionizing radiation and simulated microgravity»

Maira Certini
«Reversing time: OSKM-mediated rejuvenation enhances lifespan and healthspan in Drosophila melanogaster»

eIF4G1 silencing impairs neuroblast proliferation and promotes apoptosis in the *Drosophila melanogaster* larval brain

Alessandra Caragiuli, Federico Ghirelli, Sara Miglioranza, and Paola Bellosta

Dept-CIBIO, University of Trento, IT

Proper brain development relies on the precise orchestration of cell proliferation, neural stem cell maintenance, apoptosis, axonal growth and guidance, synaptic connectivity, epigenetic programming, and translational control. Yet the role of core translation initiation machinery in neural tissue growth remains poorly understood.
eIF4G1, the scaffold subunit of the eIF4F translation initiation complex, is essential for cap-dependent mRNA translation and has been implicated in human neurodegenerative disorders. Here, we investigated the role of eIF4G1 in the developing *Drosophila melanogaster* central nervous system (CNS) using RNAi-mediated silencing in third-instar larval brains. eIF4G1 knockdown driven by Elav-Gal4, Repo-Gal4, and Insc-Gal4 resulted in a pronounced reduction in optic lobe size across all conditions, producing a brain morphology resembling the second-instar larval stage and suggesting impaired overall brain growth. Immunofluorescence analysis revealed a significant reduction in Deadpan-positive neuroblasts and phospho-histone H3-positive mitotic cells, indicating defective neuroblast maintenance and proliferation. In addition, cleaved Caspase-3 staining under pan-neuronal knockdown showed increased apoptotic activity.
Together, these findings demonstrate that eIF4G1-dependent translational regulation is essential for neuroblast proliferation and survival during larval brain development. Loss of eIF4G1 causes arrested neurodevelopment and apoptosis, establishing eIF4G1 as a critical regulator of CNS growth in *Drosophila* and a potential model for translation-linked neurodevelopmental defects.

Functional characterization of a novel miR-210 target (*dAcbp1*) in *Drosophila* lipid metabolism

Davide Colaianni, Chiara Stefanelli, Cristiano De Pittà

Department of Biology, University of Padova, Padova, Italy

Previous studies from our group and others have shown that depletion of the highly conserved miR-210 in *D. melanogaster* leads to progressive retinal degeneration characterized by retinal lipid droplet accumulation and widespread alterations in lipid metabolism. Starting from a set of putative miR-210 target genes involved in lipid homeostasis, we performed gene expression analyses in miR-210 knock-out (KO) and overexpression (OE) *Drosophila* models to identify high-confidence candidate targets exhibiting expression patterns inversely correlated with miR-210. This approach led to the identification of the *Acyl-CoA binding protein 1* (*Acbp1*) gene, which was subsequently validated as a direct miR-210 target using a luciferase reporter assay. Although predicted to bind fatty acyl-CoA and participate in fatty acid metabolism, its physiological role and involvement in lipid metabolic pathways remain to be fully elucidated. Similarly, the limited evidence available for its mammalian ortholog, *Acyl-CoA Binding Domain Containing 7* (*ACBD7*), suggests an important role in lipid metabolic pathways, energy homeostasis, and feeding regulation; however, its physiological functions remain poorly understood. For these reasons, to gather information on the role of *Acbp1* and potentially of its ortholog *ACBD7*, we undertook the characterization of *Acbp1* KO and OE fly models. By combining organismal survival and behavioural assays with gene expression analysis, we demonstrated the physiological relevance of Acbp1 and its role in energy homeostasis. We also identified dysregulation of genes involved in specific lipid metabolic pathways following Acbp1 loss, highlighting a subset most closely associated with its function. Overall, our preliminary characterization provides a foundation for a more comprehensive understanding of this novel putative regulator of lipid metabolism and energy homeostasis in fruit flies and mammals.

A reversible torpor-like state in *Drosophila melanogaster* enhances resistance to ionizing radiation and simulated microgravity

Claudia Di Dio [1,2], Giovanni Cenci [2,3], Francesca Cipressa [2], Sergio Galanti [4], Giuseppe Esposito [1]

1 Istituto Superiore di Sanità (ISS), V.le Regina Elena 299, 00161 Rome, Italy
2 Dipartimento di Biologia e Biotecnologie "C. Darwin", Sapienza Università di Roma, Rome, Italy
3 Istituto Pasteur Italia, Fondazione Cenci Bolognetti, Rome, Italy
4 ADM, laboratorio centrale, Rome, Italy

Long-duration space exploration exposes organisms to ionizing radiation and microgravity, with potential consequences for genome stability and muscular and reproductive function. In this context, the development of biological countermeasures represents a major challenge in space biology. Among emerging approaches, particular attention has focused on hypometabolic states such as torpor, naturally observed in hibernating organisms and associated with decreased oxidative stress
and enhanced DNA repair capacity.
We present an experimental model of reversible torpor in *Drosophila melanogaster*, induced by temporary immersion in water. This condition triggers a transient inactive state characterized by reduced metabolic activity, followed by full physiological recovery after stimulus removal. Preliminary results indicate a significant radioprotective effect associated with torpor state. In larvae exposed to 10 Gy of gamma radiation, the frequency of chromosomal breaks decreased from 0.8 breaks per cell in irradiated normometabolic controls to 0.3 breaks per cell in irradiated individuals maintained in torpor. At 50 Gy, a dose associated with 50% survival in irradiated controls under standard physiological conditions, mortality is decreased to about 20% in torpor-treated samples. In adult males, a better preservation of residual fertility is also observed compared with irradiated controls. Integrated analysis under simulated microgravity using an RCCS system (RCCMax, Synthecon) shows that six hours of exposure do not affect viability, locomotion, or lifespan in torpor-induced adults. Microgravity appears to partially reduce the protective effect on fertility after irradiation; however, values remain higher than in untreated controls.
Overall, the data suggest that experimentally induced torpor may represent an effective biological strategy to mitigate ionizing radiation damage. It is plausible that metabolic downregulation contributes to limiting reactive oxygen species production and modulating DNA repair pathways, making this approach particularly promising for space medicine applications and future long-duration missions.

Reversing time: OSKM-mediated rejuvenation enhances lifespan and healthspan in *Drosophila melanogaster*

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Aging is a physiological process leading to systemic decline, often resulting in cardiac dysfunction and chronic inflammation. A promising strategy to slow aging is partial reprogramming, involving intermittent expression of Yamanaka transcription factors Oct4, Sox2, Klf4, and c-Myc (OSKM) to rejuvenate cells. Although applied in rodent models, this approach faces high costs and long generation times, limiting large-scale research. To address these issues, we created a transgenic *Drosophila melanogaster* model expressing OSKM in intestinal stem cells to investigate partial reprogramming. We assessed lifespan, healthspan, and intestinal integrity in OSKM-expressing and control flies. We also monitored response to an intestinal inflammatory stimulus by assessing intestinal integrity, a health indicator known to deteriorate with age. Pulsatile OSKM expression increased lifespan by 20% compared to controls. Healthspan, evaluated through a negative geotaxis assay, showed that OSKM flies over 100 days performed better than controls. The effect was more pronounced in males, suggesting sex-related impact. No significant difference in intestinal permeability was observed, although OSKM flies showed a tendency to resist chemically induced death more effectively. Furthermore, we sought to determine whether observed lifespan and healthspan improvements were driven exclusively by intestinal rejuvenation or whether intestinal OSKM expression could exert systemic effects on distal organs not directly targeted, such as the heart. Therefore, we evaluated age-related cardiac function in OSKM-expressing flies. Preliminary cardiac data showed no improvement in age-related bradycardia compared to controls. In conclusion, we successfully exploited partial reprogramming through intestinal OSKM expression in a transgenic *Drosophila melanogaster* model, demonstrating feasibility in extending lifespan and improving healthspan. Preliminary data suggest that beneficial effects of intestinal partial reprogramming may not translate into cardiac rejuvenation, supporting tissue-specific effects of this approach.

Posters
in alphabetical order

P1. Dietary bioactives shape healthspan through metabolic, immune and sex-specific responses in *Drosophila melanogaster*

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Nutrition plays a key role in shaping aging and metabolic health. *Drosophila melanogaster* represents a powerful *in vivo* model to investigate how dietary interventions modulate lifespan, healthspan, and underlying molecular mechanisms.
Our research focuses on the effects of dietary bioactive compounds and functional food components on organismal physiology, integrating phenotypic, molecular, and systems-level approaches. Notably, several of the investigated compounds are derived from food by-products, promoting a circular economy perspective aimed at valorizing waste materials and enhancing sustainability. Through a series of integrated studies, we evaluated lifespan, locomotor activity, metabolic parameters, and genomic stability, together with gene expression and proteomic analyses. Our methodology also accounts for biological complexity by utilizing models of neurodegeneration and investigating sex-specific responses.
Our findings demonstrate that dietary interventions modulate conserved metabolic and stress-response pathways, including AMPK signaling, mitochondrial function, and oxidative stress regulation. In addition, we provide evidence that dietary compounds influence host physiology through immunomodulation and gut microbiota remodeling. Specifically, we observed that dietary fiber supplementation alters gut microbial composition, reduces gut inflammation, which correlates with changes in brain protein expression, supporting a functional link along the gut–brain axis. These systemic effects highlight the complex interplay between metabolic, immune and neural responses in maintaining organismal homeostasis. A key result of our work is the dissociation between lifespan and healthspan: while some interventions extend longevity, several compounds primarily enhance functional parameters, such as vitality, locomotor performance and metabolic resilience, without significantly affecting overall lifespan. Importantly, the observed sex-dependent differences in response to dietary interventions underline the importance of including biological variability in aging studies.
Overall, our research work underscores how dietary modulation influences aging through interconnected pathways, involving metabolism, immunity, gut–brain communication and sex-specific regulation, positioning *Drosophila* as a valuable model to dissect the systemic impact of nutrition on healthy aging and sustainable resource use.

P2. Cell Polarity Disruption in Indolent RAS-mutated Follicle Cells Primes Aggressive Behaviours in Drosophila and Human Models of Ovarian Cancer

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Serous ovarian cancers (SOC) are the most lethal gynaecological tumours. They are classified into low-grade (LGSOC-10%) and high-grade (HGSOC-90%) subtypes, with HGSOC emerging as *de novo* malignant masses associated with TP53 aberrations, and LGSOC developing from pre-existing borderline lesions displaying RAS/MAPK mutations. No dedicated therapies are currently available for LGSOC, which undergo the same treatments as HGSOC but, given their indolent nature, barely respond to chemotherapy and about 70% of women die from this disease. Despite most low-grade and high-grade ovarian tumours are clinically distinct, some hybrid serous cancer samples contain both low- and high-grade areas bearing the same RAS/MAPK founder mutation, suggesting a clonal relationship between the two histotypes. Therefore, it appears clinically relevant to identify the molecular switch underlying the progression from low- to high-grade lesions.
In *Drosophila*, single cells carrying RAS/MAPK activation and mutant forms of the "*lethal giant larvae*" (*lgl*) polarity gene are able to grow into overt tumours in the larval epithelia, but evidence is missing about similar mechanisms driving malignant growth in the ovaries. We previously demonstrated that Lgl function in cell polarity maintenance is evolutionarily conserved, and we found the human orthologue HUGL-1 mislocalised in human ovarian tumours. We thus explored the phenotypic consequences of combined aberrant RAS signalling and cell polarity disruption in the *Drosophila* ovary.
We show that, while RAS activation in single cells of the follicular epithelium promoted mild hyperplasia with no immediate consequences on organ function, cells bearing simultaneous RAS activation and *lgl* KO bloom into overt ovarian cancers. The follicular epithelium indeed displays loss of proper architecture, aberrant growth, high mitotic activity, nuclear atypia, upregulation of oncogenic and anti-apoptotic proteins, genetic instability, diffuse pyknosis and invasive abilities, typical features of high-grade ovarian cancers, associated with abdominal swelling, organ failure, brain metastases and untimely death.
We are currently using this model to characterise relevant chemo-associated parameters, with the aim to find the best drug combination for this neglected class of female cancers. In parallel, we are trying to move out findings from bench to bedside by studying the phenotypic consequences of polarity disruption in human RAS-mutated LGSOC cells.

P3. Investigating the role of m6A RNA methylation in glia cells during brain development

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A properly developing brain depends on precise spatial and temporal coordination of specific gene expression profiles and signaling pathways that concertedly regulate cell fate determination, differentiation and proliferation. Amongst the mechanisms that synergistically fine-tune gene expression during neurodevelopment, RNA modifications – collectively known as the epitranscriptome- have recently emerged as a dynamic layer of post-transcriptional regulation that enables rapid cellular responses that are required in a fast-developing tissue. N6-methyladenosine (m6A) is the most abundant and well-characterized internal mRNA modification. It is highly enriched in the brain and plays pivotal roles in fundamental neurobiological functions required for both early and late stages of brain development. The functional effects of m6A arise from the strictly coordinated activity of three classes of effector proteins: RNA-modifying or "writer" enzymes, that catalyze the deposition of m6A; RNA-binding proteins or "readers", involved in the recognition and binding of m6A-marked transcripts; demethylases or "erasers", mediating the removal of m6A. While most research has focused on neurons, how m6A regulates mRNA in a spatially and temporally controlled manner in glia cells remains still poorly understood. This represents a relevant gap of knowledge in m6A functions, considering that glia cells are key players in the maintenance of homeostasis in the central nervous system and in neuron-glia communication. Our research seeks to decipher the contribution of m6A to various aspects of brain development, leveraging distinct developmental phases of Drosophila melanogaster. Previous studies have established an important role of m6A in neurons, particularly in controlling axonal growth across multiple circuits. However, its function in other nervous system cell types, including glia, remains insufficiently investigated. We are therefore currently dissecting the role of m6A during neurodevelopment in glia cells. In the poster session, I will present our previous findings and share novel insights from our ongoing work on m6A-mediated regulation during brain development.

P4. RNase H1 counteracts DNA damage and ameliorates SMN-dependent phenotypes in a *Drosophila* model of Spinal Muscular Atrophy

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Spinal Muscular Atrophy (SMA) is caused by a deficiency of the Survival Motor Neuron (SMN) protein. Mutations in *SMN* disrupt mRNA splicing and translation, leading to maladaptive changes in transcriptomes, proteomes, neuroinflammation, and metabolism, which drive motor neuron degeneration in SMA patients. Using a *Drosophila* SMA model, we found that systemic depletion of *Smn* leads to accumulation of RNA:DNA hybrids (Rloops), increased DNA damage, dysregulation of amino acids and sugar metabolism and activation of the innate immune response, recapitulating key pathological features reported in mammalian models and severe SMA patients. Persistent DNA damage in *Smn*-deficient flies alters cell proliferation rates in larval brains and induces extensive cell death in the developing eye. Importantly here, we show that stimulating the resolution of RNA:DNA hybrids with transgenic human RNAse H1 prevents the accumulation of DNA damage and attenuates the transcriptome and amino acid alterations induced by Smn depletion, mitigating the Smn-dependent cellular and developmental abnormalities, in Smn-deficient flies. Our data suggest that depletion of Smn causes an accumulation of aberrant transcripts and chronic DNA damage, which - along with the altered metabolomic profiles associated with Smn deficiency - trigger systemic inflammatory responses, ultimately affecting neuronal function and survival.

P5. The novel IMiD 3-Monothiopomalidomide Confers Neuroprotection in *Drosophila* Models of Parkinson's Disease

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by dopaminergic neuron loss in the substantia nigra, accumulation of misfolded α-synuclein (α-Syn), mitochondrial dysfunction, and synaptic impairment, with neuroinflammation playing a central role in disease progression (1). Immunomodulatory imide drugs (IMiDs), which suppress pro-inflammatory cytokines, have recently emerged as promising therapeutic candidates (2). In this study, we evaluated the neuroprotective efficacy of the novel IMiD derivative 3-monothiopomalidomide (3MP) in two *Drosophila melanogaster* models of PD: a genetic LRRK2WD40 mutant model and a transgenic model overexpressing human α-Syn (3-4). Male and female flies were chronically treated with 3MP, and behavioral, survival, neuroanatomical, and ultrastructural analyses were performed, with particular attention to sex-dependent effects. In the LRRK2WD40 model, vehicle-treated females exhibited a higher baseline number of TH-positive neurons than males, particularly in PPL1 and PPM3 clusters. Moreover, chronic treatment with 3MT significantly extended lifespan in both sexes, restoring survival beyond 80 days, while having no effect on *wild-type* flies. 3MT also significantly improved motor performance and preserved dopaminergic neurons in both sexes without affecting wild-type controls. The ultrastructural analyses revealed that 3MT reduced mitochondrial damage (swollen cristae) and increased T-bar density in both sexes. Importantly, females displayed milder mitochondrial alterations and higher synaptic density compared to males. In the human α-syn overexpressing model, as in the LRRK2WD40, females showed a higher number of TH-positive neurons than males. Notably, 3MT effects were partially sex-dependent. 3MT extended lifespan in both sexes and robustly prevented dopaminergic neuron loss across all clusters. However, motor performance was consistently improved by 3MT in males, while no significant changes were observed in females. At the ultrastructural level, 3MT mitigated mitochondrial abnormalities and restored T-bar density in both sexes, without altering the total number of
mitochondria. Female α-syn flies again exhibited less severe mitochondrial damage than males. Overall, 3MT exerts strong neuroprotective effects across two PD models, improving motor function, neuronal survival, lifespan, mitochondrial and synaptic integrity. While therapeutic benefits are evident in both sexes, baseline sex differences-favouring females in neuronal preservation and mitochondrial resilience-highlight the importance of incorporating sex as a biological variable in preclinical PD research.

1- Tansey et al., 2022; Joers et al., 2017
2- Chen et al., 2025; Hsueh et al., 2025
3- Feany and Bender, 2000
4- Casu et al., 2020

P6. A novel *Drosophila* model of Type B Kufs disease (CLN13) shows neurodegeneration and defects in lysosomal homeostasis

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Type B Kufs disease (CLN13) is a rare, adult-onset neuronal ceroid lipofuscinosis, caused by mutations in the CTSF gene. CTSF encodes Cathepsin F, a highly conserved lysosomal cysteine protease. The clinical presentation of CLN13 is characterized by neurological impairments, progressive dementia, and motor dysfunction, all associated with the accumulation of autofluorescent lipopigments within the nervous system. Despite the severity of these symptoms, the absence of versatile genetic models has significantly limited the functional characterization of specific patient-derived mutations.
To address this gap, we established a *Drosophila melanogaster* model by generating and studying mutants null for *CtsF*, the fly ortholog of human *CTSF*. Our findings demonstrate that *CtsF* mutants successfully recapitulate the cardinal pathological hallmarks of the human disease, including neuronal loss and a marked decline in climbing ability indicating neuromotor deficits. Detailed molecular analysis reveals that this phenotype is associated with disruption in autophagic flux,
which leads to the deleterious accumulation of toxic proteinaceous cargoes and lysosomal defects. Mutant flies appear to be protected from oxidative stress, a process that increases cellular damage, suggesting incorrect activation of lysosomal homeostasis.
Our data will contribute to clarify how the lack of Cathepsin F leads to neuronal death and will contribute to identify new pathways that could be targeted for future treatments.

P7. Towards a unifying cellular mechanism for the pathophysiology of Hereditary Spastic Paraplegia

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Hereditary Spastic Paraplegias (HSPs) are a group of inherited neurologic disorders in which lower extremity weakness and spasticity are the predominant symptoms. HSPs are characterized by high genetic heterogeneity. Nevertheless, alterations in morphology or distribution of the Endoplasmic Reticulum (ER) appear to be a critical pathogenic factor, as over 60% of patients carry mutations in genes encoding proteins influencing ER morphology (e.g., Atlastin-1, Reticulon-2, responsible for SPG3A and SPG12, respectively). More recently, mutations in two genes encoding crucial enzymes of the plasmalogens-ether phospholipids abundant in ER membranes-biosynthetic pathway, i.e., SPG81 and SPG82, have been identified in HSP patients.

Neuronal-selective downregulation of the fly orthologue of the aforementioned proteins recapitulates locomotion defects observed in patients, consistent with the hypothesis that disease-associated mutations primarily act through loss-of-function mechanisms.

Interestingly, fly models of SPG3A and SPG12 exhibit altered neuronal ER morphology. Importantly, downregulation of the SPG81 orthologue also causes significant alterations in ER shape, suggesting that ER dysfunction may represent a common pathogenic feature even in HSP forms linked to plasmalogen metabolism. Moreover, SPG3A and SPG12 models display defects in intracellular Ca^{2+} handling, indicating that altered ER morphology is associated with impaired ER functionality. Transcriptomic analysis performed on brain tissue from aging HSP models suggests that alteration in Ca^{2+} handling might be a common hallmark for SPG81 and SPG82 as well.

By employing in vivo Ca^{2+} imaging tools that we recently developed, we aim at investigating ER Ca^{2+} dyshomeostasis as a convergent pathogenic mechanism in HSP.

P8. The protective role of clomipramine in modulating SOD1-induced DNA damage

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder primarily affecting motor neurons and leading to death by progressive paralysis culminating into respiratory failure. Our established *Drosophila melanogaster* SOD1-ALS models (pan-neuronally expressing the human mutations A4V or G85R of the SOD1 gene, causative for 15-20% of ALS cases) exhibit extensive chromosome aberrations (CABs) and genome instability, among other pathological features (Liguori et al., 2024). We recently demonstrated that clomipramine, a tricyclic antidepressant potentially repurposable for ALS treatment, is able to reduce the frequency of CABs and the levels of pH2AV, a marker of DNA double-strand breaks (Liguori et al., 2026). To investigate the underlying mechanisms, we explored the clomipramine capability to modulate the expression of key genes involved in DNA repair and DNA Damage Response (DDR) pathways, through qPCR. Moreover, we investigated the DNA binding capacity of mutant SOD1 and the potential modulation by clomipramine, by polytene chromosomes immunofluorescence analysis. We cross-validated these results by assessing the extent of clomipramine-sensitive DNA damage, by western blot and immunofluorescence for pH2AX, ATM and Check2 as markers of DDR signaling, in the human neuronal cell line SH-SY5Y transiently transfected with plasmids expressing either human SOD1WT, SOD1A4V, or SOD1G85R. By bridging our findings from *in vivo Drosophila* model to in vitro human neuronal cell line, our preliminary results hypothesize that clomipramine exerts protective action by modulating the DDR signaling pathway.

1) Liguori F, Alberti F, Amadio S, et al. Pan-neuronal expression of human mutant SOD1 in Drosophila impairs survival and motor performance, induces early neuroinflammation and chromosome aberrations. Biochim Biophys Acta Mol Basis Dis. 2024; 1870(5):167192.
2) Liguori F, Amadio S, Angioli C, et al. Validation in Drosophila of the in silico predicted clomipramine as repurposable for SOD1-ALS.*Neurotherapeutics.* 2026; 23(1):e00793.

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