Paris Brain Institute

April 2 - 5, 2025

Abstract book

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DEVELOPMENT 12:30 PM -3:00 PM

CRISTINA PUJADES : Cell lineage reconstruction reveals the differential temporal contribution of neurog1-expressing progenitors to the hindbrain glutamatergic and GABAergic functional circuits

Matthias Blanc and Cristina Pujades

In the hindbrain the neurogenic capacity is differentially allocated along the anteroposterior (AP) axis, with the asynchronous onset of proneural genes' expression. Although proneural genes are expressed in similar domains along the hindbrain AP axis, their expression differs along the dorsoventral (DV) axis. Since many neurons can produce, store, and release multiple neurotransmitters, one of the long-lasting questions in neurobiology is whether specific progenitor pools drive neuronal function specificity.
Thus, in this work we address the contribution of the neurog1-expressing progenitors to glutamatergic and GABAergic neuronal circuits by reconstructing neurogenesis and the emergence of coordinated neuronal activity. We tracked neurog1 cell lineages and activity in the entire developing circuit upon hindbrain morphogenesis. Thereby, we characterized circuit development and activity with single-cell resolution and continuous temporal coverage throughout development, while discerning cell types by using progenitor-restricted intersectional fate mapping, which facilitates the exclusive genetic labelling of the progeny of neurog1 progenitors. We developped computational methods to extract and quantify the contribution of neurog1 progenitors and the changes in the neuronal network upon functional perturbation. Finally, we integrated these analyses with the optogenetic sensor recordings to model the emergence of patterned activity.
Within this work, we demonstrate that neurog1-progenitors contribute both to glutamatergic and GABAergic lineages with a different temporal input. Early-born GABAergic neurons are generated from neurog1 progenitors, whereas late-born ones derived from other progenitor pools, mainly ptf1a-expressing progenitors. CRISPR-based loss-of-function experiments demonstrate that indeed neurog1 cells give rise to GABAergic and glutamatergic neurons and unveiled the contribution of other proneural gene-expressing progenitors to the distinct differentiated neuronal populations. Moreover, we study the importance of progenitor origin and birthdate in the emergence of neuronal activity in the hindbrain.

MIGUEL MORENO MATEOS : RNA-targeting CRISPR-Cas optimizations and screenings to understand early development of vertebrates

Miguel A. Moreno-Mateos

The Maternal-to-Zygotic transition (MZT) orchestrates the reprogramming of early vertebrate development, encompassing zygotic genome activation (ZGA) and the clearance of maternally-provided RNAs. While some regulators of MZT have been identified, the vast majority of maternal RNAs remain functionally uncharacterized. Using our optimized CRISPR-RfxCas13d/CasRx technology, we performed a screening targeting maternally provided mRNAs encoding protein kinases and phosphatases in zebrafish, uncovering Bckdk as a novel post-translational regulator of MZT. Depletion of bckdk mRNA led to epiboly defects, global ZGA deregulation, reduced H3K27ac levels, and partial impairment of miR-430-dependent maternal RNA degradation. Beyond, phospho-proteomic analysis upon Bckdk depletion revealed a reduced phosphorylation of Phf10, a chromatin remodeling factor also involved in ZGA. Indeed, this lower Phf10 phosphorylation ultimately triggered the developmental phenotype observed in the absence of Bckdk.
Despite our CRISPR-RfxCas13d optimizations in vivo, the activity of CRISPR-RfxCas13d can be further enhanced in vivo, and its application has generated controversy due to the recently described collateral activity in mammalian cells and mouse models. In our lab, we have continued to improve the CRISPR-RfxCas13d system for an enhanced RNA targeting in vivo using zebrafish embryos as animal model and by different and compatible approaches. Indeed, we demonstrated that i) chemically modified gRNAs increase and maintain mRNA knockdown during early development, ii) specific nuclear RNA targeting can be more efficient using optimized nuclear localization signals, and iii) we show that in vitro-based computational models can predict gRNA efficiency in vivo but with a relatively modest accuracy. Furthermore, we showed that transient CRISPR-RfxCas13d approaches such as ribonucleoprotein complexes or mRNA-gRNA effectively deplete mRNAs in zebrafish embryos without inducing collateral activity, except when targeting extremely abundant and ectopic RNAs. To circumvent this potential issue, we have implemented alternative RNA-targeting CRISPR-Cas systems such as CRISPR-DjCas13d or CRISPR-Cas7-11 with reduced or absent collateral activity upon highly expressed RNA knockdown in zebrafish embryos.
Altogether, our findings i) demonstrate the potential of CRISPR-RfxCas13d to uncover novel early zebrafish developmental factors shedding light on the role of Bckdk as post-translational regulator of MZT and ii) contribute to optimize CRISPR-Cas technology for RNA targeting in zebrafish through transient approaches, promoting the development of in vivo CRISPR-Cas knockdown therapies.

ELKE OBER : Organ size: Mechanosensing controls liver growth

Ronja L. S. Heyne*, Iris A. Unterweger*, Pia R. Lundegaard, Julien Vermot, Elke A. Ober

During development, organs like the liver grow to a specific organ-to-body size ratio. Organ size is reestablished precisely during regeneration. Yet, relatively little is known about how organs sense their size and how compensatory growth is controlled.
Here, we investigate mechanisms controlling organ size using the prt/wnt2bb zebrafish mutant, exhibiting a severe liver specification phenotype in early development, yet developing into healthy and fertile adults. We show that prt/wnt2bb livers recover to wild-type size. Importantly, compensatory growth only starts when prt/wnt2bb livers are differentiated and functional, making it a model for studying postembryonic organ growth control independent of injury.
Combining morphological analyses and live imaging revealed faster blood flow in the liver, showing that mutant livers experience significantly higher shear stress compared to wild type embryos shortly before the start of compensatory liver growth. Modulating flow velocity, we demonstrate that elevated blood flow serves as a trigger initiating compensatory growth. Candidate sensor molecules were identified by cell type-specific transcriptomics.
Concomitantly, the number of HSCs is increased prior to compensatory growth and elevated marker gene expression indicates their activated state.
Prior to the onset of compensatory growth in prt/wnt2bb livers the number of hepatic stellate cells, a mesenchymal cell population with key roles in hepatic metabolism and injury-induced fibrosis, increased. Concomitant upregulation of indicators of stellate cell activation, acta2a, hgf and vegfaa, was surprising given the lack of injury, suggesting a more complex role for this ‘minor’ cell type in compensatory liver growth.
We propose that a link between vascular shear stress and hepatic stellate cell activation serves as a fine tuned size sensing mechanism in liver growth, indicating novel roles for mesenchymal stellate cells in organ homeostasis.

LAUREN SAUNDERS : Reverse genetics at single-cell resolution reveals lineage-specific programs in shared tissues

Lauren Saunders, Sanjay Srivatsan, Madeline Duran, Michael Dorrity, David Raible, Cecilia Moens, David Kimelman, Cole Trapnell

Single cell transcriptomics now enables comprehensive cellular atlases of whole developing embryos and offers immense promise for high-content phenotyping to dissect the genetic programs of development. However, cost and workflow complexity have limited efforts to profiling a handful of embryos and perturbations per experiment. Here we present a new experimental and analytical approach for high-resolution phenotyping of thousands of individually barcoded zebrafish embryos in response to dozens of genetic perturbations across development. Using this approach, we profile 3.2M cells: first we comprehensively map the zebrafish developmental landscape from 18 to 96 hours post-fertilization in 1,167 individually resolved embryos, and next, we profile 23 genetic perturbations across 645 embryos and 5 timepoints, comprising 98 conditions. The high degree of replication enables estimation of organism-wide variance in cell type abundances and to sensitively resolve perturbation-dependent cell type composition changes. Time-series profiling of classic notochord mutants revealed a cryptic population of brachyury-independent cells with a striking transcriptional resemblance to notochord sheath cells. Given the contribution from multiple embryonic sources — neural crest and mesoderm – to cranial development, this finding highlights parallel paths to cartilage development and offers new hypotheses about the evolutionary origins of the vertebrate skull. Our new approach for high-resolution phenotyping of whole developing organisms coupled with developmental genetics enables future studies to understand how complex phenotypes arise at the molecular and cellular level. And by integrating lineage tracing and cross-species studies, our new lab is exploring the plasticity of genetic networks and cellular behaviors underlying morphological diversity in vertebrates.

TANYA WHITFIELD : 3D cell shape changes during epithelial morphogenesis in the developing zebrafish inner ear

Haseeb K. Qureshi*, Ana A. Jones*, Nicholas J. van Hateren, Sarah Baxendale, Tanya T. Whitfield

Development of the semicircular canal ducts of the zebrafish inner ear involves a stereotyped sequence of morphogenetic events that fold, fuse and perforate the otic epithelium. The initial step in this process requires an inversion of epithelial curvature, converting the concave wall of the otic vesicle into the convex surface of an evaginating epithelial projection. The sites at which this event takes place are marked by the highly localised deposition of chondroitin sulphate, visible before any morphological deformation of the epithelium. Imaging of live and fixed samples reveals that inversion of epithelial curvature is accompanied by dynamic changes in individual 3D cell shape, including a pronounced doming of the apical cell membrane. Live imaging of transgenic cytoskeletal markers (Lifeact, EB3-GFP) indicates that apical doming is accompanied by an apical-to-basolateral shift of actomyosin, and a longitudinal-to-azimuthal shift of microtubule growth. Expression of some apical markers (ZO-1, Pard3) remains intact. These events contrast with formation of the endolymphatic duct, a short tube that invaginates from dorsal otic epithelium; here, there is no deposition of chondroitin sulphate, and cells undergo apical constriction as F-actin accumulates apically. These 3D cell shape changes allow a direct comparison of evagination and invagination events that generate tubular epithelial structures within the same sensory organ.

PASCALE BOMONT : Neurofilament dynamics in heath and neurodegenerative diseases

Kotaich F, Arias L and Bomont P

Neurofilaments (NFs) are neuronal Intermediate Filaments, a large family that forms the cytoskeleton of the cell together with the actin filaments and microtubules. Generally considered as the structural scaffold of the cell, NFs have been shown to exhibit essential functions in neurons and are key actors in neurodegeneration. NFs are not only a genetic cause of neuronal death in human, their abnormal aggregation is an early pathological hallmark in disease, and their genetic removal from axons has shown spectacular benefits in delaying disease onset, extending survival and restoring neurological functions in mouse models. Still, translation to human is impeded by the lack of appropriate biological systems and our poor knowledge on NF biology. Our laboratory exploits the advantages of the zebrafish species to scrutinize in vivo the dynamics of this cytoskeleton network, with the aim to monitor the behavior of NFs in a physiological context and to uncover the yet unknown mechanisms triggering neurodegeneration in disease. Combining novel NF zebrafish lines with state-of-the-art imaging and proteomic methodologies, we will present our recent advances on the dynamics of NFs in a physiological environment. Moreover, we reproduce NF-disease mutants to dissect in zebrafish and other systems the mechanisms underlying aggregation and neuronal dysfunctions in diseases. This project will shed light into the dynamics and signaling of NFs, a cytoskeleton network mostly seen as static, that can be targeted to develop effective therapies for human diseases.

KARUNA SAMPATH : Pinning down oocyte polarity and the germplasm

Andreas Zaucker, Sara Toral-Perez, Keerthi Baliga, Andre Pires Da Silva, Daniel Hebenstreit, Karuna Sampath

In many animals, the embryonic animal-vegetal axis is established during oogenesis. A key feature in many vertebrate oocytes is a membrane-less compartment, the Balbiani body (Bb), that contains many organelles and ribonucleoprotein complexes. The Bb first forms adjacent to the nucleus and subsequently, its position defines the vegetal pole of the oocyte. The molecular mechanisms that govern oocyte polarity, Bb and germ plasm distribution remain largely unknown. Through quantitative image analysis of germplasm dynamics and cytoskeletal reorganization in zebrafish eggs and embryos, we find that germ granule movements commence with furrow formation during early cleavage divisions. Analysis of zebrafish mutants affecting the RNA-binding protein Ybx1 (Y-box binding-protein 1) and a novel Ybx1 target called pinchado (pin), shows that the timing and dynamics of germ granule accumulation in the oocyte and blastoderm is a crucial factor for appropriate distribution of the complex to PGCs. Pin mRNA is detected in the Bb in early oocytes, in the cortex of late oocytes, and in cleavage furrows of 4-cell stage embryos. Loss of pin leads to defects in oogenesis and embryonic lethality. Maternal pin mutant eggs have increased numbers of micropyles such that mutant eggs appear punctured (‘pinchado’ in Spanish) and are radially symmetrical. The Bb is fragmented, dispersed in distribution and pin oocytes show loss of animal-vegetal polarity with abnormal expression of deleted in azoospermia-like (dazl), bucky ball (buc), and growth differentiation factor 3 (gdf3/vg1). Maternal ybx1 mutant embryos show reduced germplasm and mutant adults show biased adult sex ratios. Germplasm accumulation is ectopic in pinchado mutant oocytes, and reduced and ectopic aggregates form at the blastoderm margin of maternal ybx1 mutant embryos. In addition, germline gene expression is altered and there is increased expression of some somatic genes. Pin reporter fusions show dynamic localisation in early zebrafish embryos and Pin associates with the actin cytoskeleton at the cortex. Our findings suggest that pin functions in maintaining Bb integrity and anchoring of germplasm components. Thus, Pin and Ybx1 have crucial roles in regulation of oocyte polarity, Bb integrity, germplasm distribution and germline development.

BERTA ALSINA : Pioneer neurons and chemokines in inner ear axon guidance

Rumbo M, Bañón A and Alsina B

Auditory and vestibular sensory neurons of the statoacoustic ganglion (SAG) connect hair cells of the inner ear with secondary order neurons of the posterior brain. Yet, the mechanisms that regulate the establishment of proper connections in 3D during the development of the inner ear are not well understood. We have identified a population of pioneer neurons from non-otic origin that are required for the migration, and coalescence of otic delaminating zebrafish neuroblasts. Moreover, the pioneer neurons extend the first axons that innervate early-born hair cells, which are used as scaffolds for neuroblast migration and secondary axogenesis. High spatiotemporal imaging of these axons reveal their growth extension and refinement after targeting hair cells. Pioneer neuron ablation results in a misshaped SAG, indicative of their key role in coordinating the development and circuitry of the SAG. But how the pioneer axons find their targets? We have identified the chemokine Cxcl14 as a novel guidance molecule in the young inner ear. Cxcl14 is expressed in inner ear, but not lateral line, hair cells, as well as in two spots corresponding to hindbrain entry points of sensory axons. In Cxcl14 KO homozygous and heterozygous crispr mutants, the SAG pioneer axons show axon guidance and defasciculation defects, together with lack of posterior SAG formation. Interestingly, lateral line axons also present guidance errors, suggesting that a proper balance between cxcl12 and cxcl14 might be needed for the correct establishment of sensory innervation paths. Altogether, our work reveals the mechanism of how pioneer neurons shape the SAG and regulates the circuitry of the SAG.

SHAHAD ALBADRI : Redox signaling in retinal stem cell differentiation

Laura Belleri, Alice Pailleret, Sofia Petrucci, Gonzalo Rios Concepcion, Mayrone Mongellaz, Xia Tang, Jie He, Filippo Del Bene, Shahad Albadri

Signaling by Redox molecules, and H2O2 in particular, is emerging as an important new physiological player in stem cell biology, controlling the balance between cell proliferation and differentiation. Indeed, reactive oxygen species (ROS) may act as a “stem cell rheostat”, coordinating extrinsic cues and cellular responses to intrinsic programs. In this line, using the transgenic Tg(ubi:hyper) sensor line, we previously revealed the dynamics of H2O2, a major ROS metabolite and second messenger for cell metabolism, during zebrafish retinogenesis. Our previous work indeed demonstrated that H2O2 levels are tightly regulated during retinal development in zebrafish and orchestrate the proliferation to differentiation switch of retinal stem and progenitor cells (RSCs and RPCs). We demonstrated that this regulation notably occurs through the activity of the Catalase scavenging enzyme and thereby at the degradation step of H2O2. These findings identified a physiological role for redox signaling during vertebrate retinal development.
In order to assess the conservation and species-specific mechanisms implicated in this process, comparative single-cell transcriptomic analyses of zebrafish retinal cells and human iPSCs-derived retinal organoids were performed. Through this approach, we identified several redox-related genes differentially expressed in RPCs. We are mostly focus in assessing the roles of two of the identified proteins, the Cap’n’Collar Nrf3 transcription factor and the Peroxiredoxin 6 scavenger (Prdx6). Nrf3 is a member of the Cap’n’Collar (CNC) family, which are transcription factors known to confer cytoprotection against oxidative stress. However, their physiological role during retinogenesis remains unknown. Using a loss-of-function approach, we evaluated the influence of Nrf3 CNC factor that we found expressed in the ciliary marginal zone (CMZ), stem cell niche of the zebrafish retina, on (1) the expression of metabolic enzymes like Sod2 and Catalase and (2) on RPC differentiation/CMZ homeostasis. We show that the loss of Nrf3 function lead RPCs to fail exiting the cell cycle, as previously observed in the absence of Catalase. Interestingly, catalase expression in nrf3-/- mutant embryos is upregulated. We are currently investigating the relationship between Nrf3 and Catalase in the regulation of RPC switch from proliferation to differentiation.
Prdx6, another redox-members described as a target of CNC transcription factors is also expressed in RSCs and RPCs of the zebrafish CMZ as confirmed through multiplex in situ hybridization. Although the loss of Prdx6 antioxidant activity in human was shown to be associated with development of several ocular conditions, its function during retinal development which we aim to investigate though gain and loss of function remains currently unknown. We are doing so in combination with live-imaging both in vivo using zebrafish and human iPSC-derived retinal organoids as an in vitro model for human retinogenesis.
Together our work so far reveals a new function for Nrf3 CNC factor as part of the Redox signaling pathway for proper retinal development and explores the conservation of this mechanism in the context of human retinogenesis.

JULIEN VERMOT : Mechanosensitive cell hydraulics control endocardial morphogenesis

Christina Vagena-Pantoula, Sulaimaan Lim, Antoine Sanchez, Konstantinos Kalyviotis, Shuyi Feng, Igor Kondrychyn, Thomas Juan, Didier Stainier, Periklis Pantazis, Li-Kun Phng, Julien Vermot

Cell volume regulation is a fundamental mechanism in tissue morphogenesis. In zebrafish, endocardial cell (EdC) volume reduction is essential for atrioventricular valve formation; however, the underlying cellular and molecular processes remain unknown. Here, we demonstrate that the interplay of Piezo1, the Ca2+-binding protein calmodulin (CaM), and the aquaporin Aqp8a.1 water channel controls EdC volume. We show that Aqp8a.1 is required for EdC volume regulation, and aqp8a.1 mutant larvae display defective cardiac looping, valve malformation, disrupted cell polarity, and impaired F-actin remodelling. Using computational methods (image processing and biophysical modeling), we show that cell volume regulation is key to control endocardial morphogenesis. Mechanistically, we show that Piezo and CaM drive Aqp8a.1 localization to the plasma membrane to govern EdC volume dynamics. Altogether, these findings uncover a mechanosensitive mechanism for EdC volume regulation and heart morphogenesis, thereby advancing our understanding of early organogenesis.

DISEASE MODELING 1 3:00 PM -5:15 PM

ANTONELLA LAURI : Modelling rare brain disorders in zebrafish: functional genomics in vivo to assist clinical decisions and unravel druggable pathways.

Giulia Fasano, Martina Venditti, Graziamaria Paradisi, Catia Pedalino, Valeria Bonavolontà, Marco Tartaglia, Filippo Del Bene, Antonella Lauri (presenter)

Despite revolutionary Next Generation Sequencing (NGS) technologies becoming more affordable and implementable, yet numerous rare patients remain “invisible” with no clear diagnosis nor cure. To solve this huge burden, priority of EU health policies, the speed and accuracy reached in the identification of likely pathogenic variants in human genome must be matched by dedicated functional genomics approaches in valid model organisms to dissect the actual impact of the variants on whole-organism development and provide genotype-phenotype correlation. Such research pipelines in vivo can boost patients’ stratification, assist clinical decisions and generate tools for mechanism search across scales (from molecules to whole organism) towards early disease targets identification. They also offer a unique opportunity to discover gene functions, pathways and developmental processes. Here, I will present the overall goals and tools of our functional genomics pipeline recently established to tackle rare and ultra-rare encephalopathies using zebrafish as model organism and how it integrates within a large clinical and multi-approach research workflow. I will discuss results obtained in the last two years by multi-modal gene manipulation, reporter systems and morpho-functional characterization in fish that allowed us new disease-genes discovery, functional validation and mechanism investigation of novel disease genes and mutations affecting organelle homeostasis and causing malformations of cortical development (MCD), that we can now diagnose. I will also focus on our recent efforts combining CRISPR-Cas, super-resolution microscopy and patients comparison to model a rare and overlooked progressive motor neuron disease (MND) affecting cytoskeleton dynamics, map and validate the earliest signs of the disease and obtain addressable mechanistic insights for future treatment evaluations.

BETTINA SCHMID : A novel TDP-43 animal model to identify the first steps in ALS disease pathogenesis

Yiying Hu, Alexander Hruscha, Chenchen Pan, Martina Schifferer, Michael K Schmidt, Brigitte Nuscher, Martin Giera, Sarantos Kostidis, Özge Burhan, Frauke van Bebber, Dieter Edbauer, Thomas Arzberger, Christian Haass and Bettina Schmid

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that affects motor neurons, and the key pathological signature of ALS is the aggregation of the Tar-DNA binding protein of 43 kDa (TDP-43). TDP-43 is an RNA- and DNA-binding protein with diverse functions in RNA metabolism, which shuttles between the nucleus and cytoplasm. In disease, mis-localization of TDP-43 from the nucleus to the cytoplasm results in cytoplasmic TDP-43 aggregation and a nuclear clearance of TDP-43. The use of animal models to overexpress TDP-43 has been a major means of recapitulating features of ALS. Nevertheless, these models are likely to generate unspecific toxicity and only poorly recapitulate the disease state, since endogenous TDP-43 levels are very tightly regulated by autoregulatory feedback loops and high levels of wildtype and mutant TDP-43 are toxic.
The development of an animal model that incorporates both features of nuclear clearance of TDP-43 and physiological levels of endogenous cytoplasmic TDP-43 offers a valuable opportunity to gain further insights into TDP-43 function in vivo and to develop more effective treatments. The novel animal model, CytoTDP, was generated to identify mis-regulated pathways affected in early ALS and to optimize promising drug candidates and/or treatment in vivo by driving zebrafish endogenous TDP-43 from the nucleus to the cytoplasm. CytoTDP fish exhibit early larval phenotypes resembling clinical features of ALS, such as progressive motor defects, neurodegeneration and muscle atrophy. The cytoplasmic gain of function of endogenous TDP-43 leads to metabolic dysfunction in vivo reminiscent of early ALS clinical non-motor metabolic alterations. Thus, the CytoTDP zebrafish model offers a unique opportunity to identify mis-regulated targets for therapeutic intervention early in disease progression.

MIGUEL GODINHO FERREIRA : Organ Communication in aging of zebrafish

Miguel GODINHO FERREIRA

Telomere shortening of is a hallmark of aging and is counteracted by telomerase. Telomere shortening of is a hallmark of aging and is counteracted by telomerase. The intestine is one of the earliest organs to exhibit short telomeres and tissue dysfunction during normal zebrafish aging. This is recapitulated in the prematurely aged telomerase (tert) mutant. We show that gut-specific telomerase expression in tert zebrafish prevents premature aging. Induction of telomerase rescues gut senescence, apoptosis, inflammation and reduces gut permeability to wild type levels, also rescuing age-dependent gut microbiota dysbiosis. Remarkably, restoring gut dysfunction results in a systemic beneficial impact. Gut telomerase expression rescues premature aging markers (senescence) in remote organs, such as the reproductive (testis) and hematopoietic (kidney marrow) systems of tert-/- mutants. Functionally, it also rescues age-dependent loss of male fertility and testis atrophy. Finally, we show that gut-specific telomerase expression increases the lifespan of telomerase mutants. Our work demonstrates that delaying telomere shortening in the guts sufficient to systemically counteract aging in zebrafish.

YUKO NISHIWAKI : Genetic mutations of cone phototransduction gene pde6c cause cone degeneration through the elevation of cytoplasmic Ca2+ levels

Yuko Nishiwaki, Ichiro Masai

In human, mutations on cone phototransduction genes, gnat2, pde6c, cnga3 and cgnb3, cause photopic vision defects called achromatopsia. Among them, gnat2 mutations cause only achromatopsia, whereas pde6c and cnga3/b3 mutations cause cone dystrophy. It is interesting why these mutations show different symptoms, although these genes function in the same cone phototransduction pathway. In zebrafish, similarly, gnat2 mutations cause achromatopsia without cone degeneration, while pde6c mutations cause achromatopsia with cone degeneration.
First, we examined what factor mediates cone degeneration in zebrafish pde6c mutants. A candidate factor is the increase of intracellular concentration of cGMP, Ca2+, or both. We examined intracellular Ca2+ levels in zebrafish photoreceptors by confocal imaging using the GCaMP7a transgenic system. Cytosolic Ca2+ level was significantly elevated in pde6c mutant photoreceptors. On the other hand, immunohistochemical analysis showed that cGMP level was not altered.
Second, we examined whether cnga3 mutation can rescue cone degeneration in pde6c mutants. The elevation of cytosolic Ca2+ level and defects in structural integrity and maintenance of cone photoreceptors in pde6c mutants were significantly rescued by knock-down of cnga3. These data indicate that cone photoreceptor degeneration in the absence of PDE6C activity depends on abnormal elevation of cytosolic Ca2+ concentration, which may be caused by chronic opening of CNGA3. Third, we examined whether gnat2 mutation can rescue cone degeneration in pde6c mutants. We found that gnat2 mutation failed to rescue cone degeneration in pde6c mutants, suggesting that a simple blockade of phototransduction pathway does not induce photoreceptor degeneration, but that absence of PDE6C activity directly causes cone degeneration. These findings advance our understanding on pathological process of human photoreceptor degeneration linked to PDE6C mutations.

CAGHAN KIZIL : A novel mechanism of APOEε4-associated blood-brain barrier dysfunction in Alzheimer's disease

Prabesh Bhattarai, Elanur Yilmaz, Badri Vardarajan, Richard Mayeux, Caghan Kizil

The blood-brain barrier (BBB) plays a critical role in maintaining central nervous system homeostasis, and its dysfunction is a hallmark of Alzheimer's disease (AD), particularly in APOEε4 carriers. We found elevated accumulation of fibronectin, a key extracellular matrix (ECM) protein, at the BBB in APOEε4-related AD, implicating it in disease pathogenesis. Our research integrates human genomic data, zebrafish and mouse models combined with iPSC-derived human vascular cell types and advanced cerebrovascular cell culture systems to elucidate the pathogenic role of fibronectin and its potential as a therapeutic target.
We identified protective genetic variants in the FN1 gene through whole genome sequencing (WGS) in cohorts of cognitively healthy elderly APOEε4 carriers. Variants associated with reduced AD risk were enriched in ECM-related pathways, suggesting a protective role of ECM modulation in BBB integrity. Functional studies in zebrafish models with loss-of-function (LOF) mutations in fn1b, the ortholog for human FN1, demonstrated that fibronectin LOF reduced gliosis, enhanced gliovascular remodeling, and improved the microglial response. These findings suggest that excessive fibronectin deposition impairs toxic protein clearance at the BBB and contributes to APOEε4-mediated AD pathology. Our in vitro and in vivo studies revealed that fibronectin accumulation disrupts essential brain-vascular interactions and modulates homeostatic crosstalk mechanisms underlying BBB integrity. Transgenic and CRISPR functional genomics zebrafish models for AD pathology further clarified the mechanistic role of APOEε4 and Aβ42 in driving FN1 induction, mirroring results observed in 3D human iPSC-derived cerebrovascular models and postmortem human brains. Validation in independent human cohorts underscored the significance of FN1 variants in delaying AD onset and reducing APOEε4-related BBB pathology. Homozygous APOEε4 carriers without dementia showed lower fibronectin deposition and decreased reactive gliosis compared to APOEe4 carriers with AD, aligning with zebrafish studies that highlight fibronectin's pathological role. Moreover, protective FN1 variants correlated with reduced ECM dysregulation, pointing to the therapeutic potential of targeting ECM components to restore BBB homeostasis.
In this talk, I will present the detailed downstream mechanisms we identified that underlie FN1-related pathology at the BBB, shedding light on how these mechanisms may explain the pathological effects of APOEε4 in driving BBB dysfunction in Alzheimer's disease. I will also mention our pharmacological intervention studies for ameliorating BBB dysfunction. Our work provides a clinical and basic science framework for therapeutic strategies targeting fibronectin and the ECM to mitigate Alzheimer's disease risk.

<u>CORINNE HOUART</u> : Dual functions of a single gene coordinates Fate Decisions And Metabolism

Hannah Bruce, Clinton Monfries, Oniz Suleyman, Fursham Hamid, & Corinne Houart

Modulation of mitochondrial function is at the core of cell fate decisions and tissue homeostasis, yet the mechanisms that govern their activity are not understood. Here, we provide evidence that mitochondrial activity is controlled in a tissue-specific manner through a non-canonical cytoplasmic function of the transcription factor FOXG1. Using zebrafish and human models of the neurodevelopmental disorder, FOXG1 Syndrome, we found that FOXG1 mutations inducing a premature stop codon unexpectedly lead to the production of a short C-terminal peptide. The expression of this truncated protein is responsible for an excess of excitatory neurons and a structural, functional, and translational mitochondrial phenotype in mutants. We demonstrate that this activity is a gain of function, normally carried out by a cleavage product in wildtype. Both peptides promote the translation of mitochondrially-encoded transcripts, are preferentially transported to the mitochondria, and interact with mito- ribosomal proteins. These findings unveil a mechanism that integrates cell fate decisions with metabolic output. Adjusting the dosage of the mutant peptide rescues aspects of FOXG1 Syndrome, offering a new therapeutic avenue for the treatment of disorders involving mitochondrial dysfunctions.

<u>JAN PHILIPP JUNKER</u> : Transcriptional diversity and cellular plasticity in neuroblastoma

Nora Fresmann, Julia Köppke, Anton Gauert, Pedro Olivares-Chauvet, Bastiaan Spanjaard, Anja Heeren-Hagemann, Jan Philipp Junker

Transcriptional heterogeneity and phenotypic plasticity are increasingly recognized as drivers of tumor progression, metastasis and treatment evasion. While tumor heterogeneity can be measured with single cell transcriptomics, major biological questions remain unresolved since we cannot readily follow cells over time: In particular, how plastic are gene expression programs of tumor cells, and to which degree are gene expression states determined by the cell of origin or the local environment?
Neuroblastoma is a neural crest derived malignancy of the peripheral nervous system and is the most common and deadliest tumor of infancy. Neuroblastoma is characterized by high phenotypic heterogeneity but low genetic diversity. Here, we combined zebrafish models of neuroblastoma, single-cell transcriptomics, massively parallel lineage tracing, and transplantation of tumor cells to i) systematically dissect intra- and inter-tumor transcriptional heterogeneity, ii) to experimentally measure the plasticity of tumor states, and iii) clarify to which degree tumor states are determined by cell of origin or local environment. We discovered a large spectrum of distinct neuroblastoma states in zebrafish, which can be broadly classified according to physiological states or cell of origin. Importantly, the tumor states in zebrafish reflect the transcriptional diversity of patient samples, including an alk-expressing and a ribosomal gene expression program, both of which are associated with poor prognosis in patients. Analysis of CRISPR/Cas9- inserted lineage barcodes revealed a gradient of plasticity across tumor states, with lower plasticity in tumor states related to cell of origin compared to tumor states associated with physiological processes. Transplantation of zebrafish tumors into embryos showed that even the least plastic tumor states can be reprogrammed upon exposure to a different signaling environment, an observation which has important consequences for future therapeutic strategies.
Together, we present a comprehensive dataset integrating computational dissection of tumor expression programs, lineage tracing, and transplantation of tumor cells, to measure tumor cell plasticity and elucidate how cell of origin and local environment cooperate to shape tumor expression profiles.

DAY 2 - APRIL 3, 2025

NEUROBIOLOGY 9:00 AM -10:30 AM

INBAL SHAINER : Transcriptomic neuron types vary topographically in function and morphology

Inbal Shainer, Johannes Kapper, Herwig Baier

Neuronal phenotypic traits such as morphology, connectivity, and function are dictated, to a large extent, by a specific combination of differentially expressed genes. Clusters of neurons in transcriptomic space correspond to distinct cell types and in some cases (e. g., C. elegans neurons1 and retinal ganglion cells2–4) have been shown to share morphology and function. The zebrafish optic tectum is composed of a spatial array of neurons that transforms visual inputs into motor outputs. While the visuotopic map is continuous, subregions of the tectum are functionally specialized5,6. To uncover the cell-type architecture of the tectum, we transcriptionally profiled its neurons, revealing more than 60 cell types that are organized in distinct anatomical layers. We then measured the visual responses of thousands of tectal neurons by two-photon calcium imaging and matched them with their transcriptional profile. Furthermore, we characterized the morphologies of transcriptionally identified neurons using specific transgenic lines. Notably, we found that neurons that are transcriptionally similar can diverge functionally and morphologically. Incorporating the spatial coordinates of neurons within the tectal volume revealed functionally and morphologically defined anatomical subclusters within individual transcriptomic clusters. Our findings demonstrate that position-dependent factors expand the phenotypic repertoire of genetically similar neurons.

ROBERT HINDGES : Early visual experience elicits cellular and functional plasticity in the retina and alters behaviour

Phoebe Reynolds, Davide Marchi, Yan To Ling, Katja Slangewal, Max Capelle, Zhaklin Chalakova, Armin Bahl & Robert Hindges

Our interaction with the surrounding environment shapes how our brain processes sensory information and drives adaptive behaviour. This plasticity allows the brain to rewire in response to specific sensory experiences. For instance, early manipulation of visual inputs profoundly impacts brain plasticity, which is crucial for functions like size perception, object recognition, and visuospatial processing. While neuronal plasticity has been detected in visual target structures such as the colliculus, thalamus, and cortex, it remains unclear if the retina, the primary sensory organ, undergoes significant plasticity. Here, we show that the zebrafish retina demonstrates pronounced plastic transformations in response to alterations of the visual environment during development, which ultimately modifies the detection of oriented visual stimuli. We demonstrate that orientation-selective amacrine cells undergo profound morphological changes in animals exposed to distinct visual environments during development. We further find that the functional orientation-selective output from the retina is altered in a manner consistent with the visual environment in which the animals are raised and that these changes are persistent. Finally, animals tested in a virtual reality system show that early exposure to different visual environments changes their innate preference for specifically oriented patterns. Our findings unveil a developmental form of sensory organ plasticity with continuing structural and functional consequences.

JASON RIHEL : A cell-intrinsic circadian clock enhances wake-related brain clearance

Goble, Talya; Kundu, Tanushree; Meyer, Leticia; Hawkins, Tom; Rihel, Jason

Brain Lymphatic Endothelial Cells (BLECs; also known as muLECs or FGPs) are a recently discovered scavenger cell that resides in the zebrafish meninges. These cells are in contact with the cerebrospinal fluid and are capable of internalizing a variety of cargos, suggesting that these cells may participate in clearing toxic byproducts derived from the brain. To investigate the function of BLECs, we have developed an in vivo, quantitative assay to assess the uptake rates for a variety of macromolecular cargos. Rate measurements across a 24-hour day-night cycle revealed that BLEC uptake rates for proteins, lipids, and sugars are all maximal during the waking day period and minimal during night-time. A combination of sleep deprivation and circadian clock-break experiments demonstrated that the daily fluctuation in protein internalization is wholly controlled by a BLEC-autonomous circadian oscillator and not by sleep-wake state, while the diurnal peak in lipid uptake is not regulated by the circadian clock. Laser ablation of BLECs led to an increase in sleep duration, tying BLEC activity to behavioral consequences. Together, these data suggest a model in which BLEC scavenger function peaks during day-time waking behaviors when macromolecular by-products from the active brain are maximally accumulating, and failure to curb the build-up of this waste alters signals involved in sleep-wake regulation.

ALESSANDRO FILOSA : Neuronal circuits mediating the modulatory action of a cytokine on acute stress

Alessandro Filosa

The brain is constantly engaged in bidirectional communications with other organs. For example, signals from the central nervous system can modulate functional properties of the immune system. More recently, it has become evident that immune signaling molecules can also influence several brain processes. Some of these influences are integral aspects of normal physiological processes regulating brain development and activity. However, the immune system also plays an important role in neuropathological and psychiatric diseases. For example, it was shown that people experiencing stress or affected by stress-related psychiatric conditions, such as depression and anxiety, have altered circulating cytokine levels. Moreover, interfering with cytokine signaling in animal models altered their responses to stressors.
We have used the zebrafish larva to study the circuit mechanisms regulating the action of immune signaling molecules on neuronal substrates of stress. In order to do this, we have focused our attention on the role of the cytokine Interleukin 4 (Il4) in modulation of acute stress. We found that inducible overexpression of Il4 decreases behavioral and physiological responses to acute application of a stressor. By using a combination of post-mortem staining of neuronal activity markers, and in vivo calcium imaging, we identified specific brain structures in which neuronal activity in response to a stressor is altered by Il4. Finally, by using an mRNA sequencing approach, we discovered molecular pathways in the brain affected by Il4 signaling.

EMRE YAKSI : Thalamocortical-like circuits transform and integrate sensory information in the zebrafish telencephalon

Anh-Tuan Trinh, Bjørn André Bredesen-Aa, Emre Yaksi

In vertebrates, cortical regions are defined by distinct thalamic innervations that carry sensory and cortico-thalamic information. The zebrafish telencephalon, considered an ancestral homolog of the vertebrate cortex, supports complex behaviors like navigation and social interactions. How the zebrafish telencephalon receives and processes sensory information, and how these processes relate to other vertebrate cortices, remains unclear.
Through anatomical tracing, electrophysiological circuit mapping, calcium imaging, and comparative transcriptomics, we characterized thalamocortical systems in zebrafish. We found that the preglomerular nucleus (PG) is the primary source of visual and auditory inputs to the telencephalon. We demonstrated that PG neurons and their axonal innervations in the telencephalon exhibit topographically organized, sensory-specific responses. In contrast, the sensory responses of telencephalic neurons reveal multiple layers of topographically organized hierarchies, from simple sensory-specific responses to multi-modal and coincidence-detecting non-linear responses.
Overall, we observed an increasing complexity in the hierarchy of telecehalic sensory computations, which is topographically organized into distinct nuclei from posterior to anterior. We mapped these individual nuclei to distinct cortico-limbic cell types in other vertebrates, showing that zebrafish telencephalic neurons analogous to the vertebrate neocortex are at the top of the sensory computation hierarchy. We are currently further investigating the role of these neocortex-like neurons in relaying telencephalic computations to the rest of the brain.

GIL LEVKOWITZ : Neuropeptide oxytocin facilitates its own brain-to-periphery uptake

Preethi Rajamannar, Oren Raz and Gil Levkowitz

The hypothalamo-neurohypophyseal system is an important neuroendocrine brain-to-blood conduit through which the neurohormones oxytocin and arginine-vasopressin are released from the brain into the general circulation to affect peripheral physiological functions such as salt balance, metabolism and reproduction. However, the mechanism, which executes fast and efficient neurohormone release to the periphery remains unsolved. We show, using live imaging in zebrafish, that a hyperosmotic physiological challenge elicits a local increase in neurohypophyseal blood flow velocities and a change in capillary diameter, which is dictated by the geometry of the hypophyseal vascular microcircuit. Genetic ablation of oxytocin neurons and inhibition of oxytocin receptor signaling attenuated changes in capillary blood flow and diameter. Optogenetic stimulation of oxytocin neurons resulted in an oxytocin receptor-dependent increase in blood flow velocities. Lastly, both osmotic challenge and oxytocin neuronal activation elicited a local rise in neurohypophyseal capillary permeability in an oxytocin signaling-dependent manner. Our study demonstrates that physiologically elicited changes in neurohypophyseal blood flow and permeability are regulated by oxytocin. We propose that oxytocin-dependent neuro-vascular coupling facilitates its efficient uptake into the blood circulation, suggesting a self-perpetuating mechanism of peripheral hormone transfer.

PEDRO HERNANDEZ CERDA : Interleukin-22 in enteroendocrine cells controls early-life gut motility through interactions with the microbiota in zebrafish

Soraya Rabahi, Lucie Maurin, Emiliano Marachlian, Fabian Guendel, Aya Mikdache, Keinis Quintero-Castillo, Vincenzo Di Donato, Jessica Riou-Ramon, Alvaro Banderas, Akshai Janardhana Kurup, Yazan Salloum, Gwendoline Gros, Patricia Diabangouaya, Camila Garcia-Baudino, Ignacio Medina-Yáñez, Pascal Hersen, Jean-Pierre Levraud, Georges Lutfalla, Filippo Del Bene, Carmen G. Feijoo, Gerard Eberl, German Sumbre, Jos Boekhorst, Sylvia Brugman, Pedro P. Hernandez

The gut microbiota, immune system, and enteric nervous system tightly interact to regulate adult gut physiology. Yet the mechanisms establishing gut physiology during development remain unknown. In zebrafish, although mature lymphocytes appear at 2-3 weeks of age, ingestion and exposure of the gut to environmental cues, including microbes, start as early as four days post-fertilization. How intestinal physiology and homeostasis, typically modulated by microbiota-lymphocyte interactions in adulthood, are established in developing animals still lacking a mature immune system remains unknown.
Here, we report that in developing zebrafish, enteroendocrine cells produce IL-22 in response to microbial signals before lymphocytes populate the gut. In larvae, IL-22 is crucial to set gut microbiota composition and ghrelin hormone expression to promote gut motility. IL-22 developmental function depends on its ability to modulate gut microbiota, as bacterial transfer from wild-type zebrafish restored gut motility in il22-/- by reestablishing ghrelin hormone expression.
Additionally, we identified Lactobacilli as a mediator of microbiota-dependent gut motility. Monocolonization with L. plantarum gut motility and ghrelin protein levels in il22-/- larvae. Furthermore, blocking the ghrelin receptor during monocolonization abolished motility rescue in IL-22-deficient larvae. Furthermore, IL-22-deficient mice show impaired gut motility and reduced ghrelin expression in early life, indicating a conserved function. Altogether, we identify a circuit where enteroendocrine cells regulate gut function via cytokine control of the microbiota, showing how gut physiology is set prior to immune system maturation.

ANNA BARRON : Metabolic Reprogramming as a Driver of Microglial Differentiation in Brain Development and Disease

Wei Jing Chong, Kei Onn Lai, Jia Hui Wong, Kiat Boon Tan, Suresh Jesuthasan, Anna M. Barron

The tissue-resident macrophages of the brain, microglia, play critical roles in brain development, function and pathology. Microglia are unique compared to other tissue-resident macrophages because they derive embryonically from the yolk sac and are the only primitive macrophages that persist into adulthood. Understanding how these self-renewing cells differentiate and mature is critical for treating microglial dysfunction in brain disease. Here, we demonstrate that metabolic reprogramming—dynamic changes in cellular metabolism—can drive microglial differentiation into distinct morphological and functional phenotypes during embryogenesis. Using embryonic zebrafish, we show that microglia transition between glycolysis and oxidative phosphorylation, two key metabolic pathways, as they mature from primitive macrophages into specialized microglial cells. Further, we find that morphologically and functionally distinct microglia exhibit corresponding metabolic differences: ameboid, phagocytic microglia exhibit a higher dependence on oxidative phosphorylation compared to ramified, surveilling microglia. To test the causal role of metabolic reprogramming in microglial differentiation, we inhibited glycolysis to promote oxidative phosphorylation. Inhibition of glycolysis enhanced differentiation of microglia into ramified, surveilling phenotypes in the embryonic zebrafish brain. While developmental changes in metabolic reprogramming were once thought to simply reflect changes in energetic demand, our findings indicate that metabolic programming is a defining feature of microglial identity, playing a crucial role in shaping their functional differentiation during brain development. Understanding these metabolic mechanisms promises novel insights into microglial biology and potential therapeutic targets for brain disorders associated with microglial dysfunction.

JEAN-PIERRE LEVRAUD : Direct and immune-mediated impact of neuroinvasive viruses on the developing brain

Hannah Wigget, Maroun Abi Younes, Valerio Laghi, Ingrid Colin, Michaël Demarque, Jean-Pierre Levraud

Viral encephalitis has devastating consequences; even when promptly treated with antivirals, the rate of serious neurological sequelae is very high, particularly when infection occurs in infants. Besides antivirals, which are not always available, the only treatment consists in standard critical care. A better understanding of the mechanisms that underpin the acute and long-term deleterious effects of viral encephalitis on the developing brain is required to rationally design novel therapies.
To address this issue, we have developed an encephalitis model in zebrafish larvae using the alphavirus Sindbis virus (SINV), a mosquito-borne virus which is also used to study encephalitis in mammals. We have generated reporter viruses with several colors. We previously showed that SINV is highly neuroinvasive in zebrafish larvae relies on axonal transport to invade the brain from peripheral tissues (Passoni et al., DMM 2017, doi.org/10.1242/dmm.029231). We have also compared various sites of inoculation, analyzed entry mechanisms and modeled the host-virus interactions (Laghi et al., BioRXiv 2023, doi.org/10.1101/2024.05.19.594871). Intravenous injections of SINV yields the most variable pattern. Intramuscular injections in caudal somites consistently results in spinal cord invasion, with dorsal root ganglion sensory neurons providing the main entry route. By contrast, pericardial injections systematically result in hindbrain invasion, presumably via the vagal nerve. Statistical analysis of multicolor virus injections indicate that CNS invasion typically results from 2 or 3 independent entry events. After initial CNS entry, SINV usually disseminates to more CNS areas and establishes a persistent infection. By contrast, infection of peripheral tissues is transient.
SINV infection elicits a strong immune response, eliciting a rapid increase in expression of type I interferons (IFNs) and downstream ISGs (IFN-stimulated genes). This response plays a key role, as IFN-deficient mutants are highly susceptible to the virus. We have generated reporter transgenic zebrafish for both groups of IFNs and for the ISG MXA. The IFN response is stronger and more widespread in the periphery than in the CNS, providing an explanation for the differential persistence in these two compartments. Mathematical modelling also suggest that neural tissue is less responsive to IFNs, presumably to limit its side effects. Neutrophils and macrophages also respond to the infection.
To tease apart the direct effect of the virus and of the host response on the developing brain, we have also studied the effect of recombinant zebrafish IFNs on the brain. We identified neuron-specific ISGs, and, after multiple injections, behavioural alterations reminiscent of the depressive syndrome which is a well-known side effect of IFN therapy in humans. Work is ongoing to link these observations and to better characterize the functional changes of virus-infected neurons.

FILIPA SIMOES : Decoding immune-related spatial heterogeneity in the regenerating heart

Ehsan Razaghi, Selin Tüzüner, Trishalee Gungoosingh, Kerem Çil and Filipa C. Simões

Zebrafish have the remarkable ability to regenerate their heart after injury. Macrophages are critical in this process: they clear up dead cells and debris, participate in fibrosis but also contribute to regeneration through interactions with their tissue microenvironment. However, little is known about the precise regulation shaping macrophage identity and function in response to cardiac injury. By combining single-cell and spatial transcriptomics, we discovered the composition and activation states of not only macrophages, but also other immune cell populations found in distinct spatial territories of the regenerating and homeostatic heart. We uncovered information about macrophage, dendritic, B and NK cell transcriptomes and their correspondent location within the cardiac tissue, including the epicardium, the outflow tract, as well as the injury-zone cardiomyocyte microenvironment. By reconstructing immune-related cellular niches across the regenerating and the homeostatic heart, we were able to discover the molecular signatures mediating such specific cell-cell communication, therefore contributing towards dissecting the regulatory programmes driving macrophage pro-fibrotic and pro-regenerative phenotypes. Further analysis is underway to understand how specific microenvironmental signalling interactions may be enhanced or disrupted to support tissue regeneration. Our findings reveal how knowledge of cardiac niche-specific immune interactions could guide more effective pro-regenerative and anti-fibrotic therapies.

<u>TOMASZ PRAJSNAR</u> : Streptococcus pneumoniae is targeted by two non-canonical autophagy pathways within zebrafish macrophages

Bartosz J. Michno, Niedharsan Pooranachandran, Tonisha C. Smith, Erin Faught, Andrew K. Fenton, Annemarie H. Meijer, Tomasz K. Prajsnar

Streptococcus pneumoniae is an opportunistic pathogen responsible for several life-threatening diseases such as pneumonia and meningitis, causing over 1 million deaths worldwide each year. The pulmonary defense against S. pneumoniae is mostly mediated by alveolar macrophages, which can effectively internalize and degrade bacteria. Recent studies have implicated canonical and non-canonical autophagy-related processes, such as xenophagy and LC3-associated phagocytosis (LAP), in bacterial clearance.
Here, we utilize a well-established in vivo zebrafish larval infection model to investigate the role of autophagy in host defense against pneumococcal infection. Using a transgenic autophagy reporter CMV:GFP-Lc3 line, we tracked the autophagic response to internalized bacteria within zebrafish macrophages. Our findings reveal that the autophagy marker Lc3 is rapidly and abundantly recruited to bacteria-containing vesicles within macrophages. The genetic inhibition of core autophagy genes (atg5 and atg16l1) led to loss of Lc3 associations with phagosomes containing internalized pneumococci and their impaired acidification, significantly delaying bacterial clearance. This Lc3 recruitment is partially mediated by LAP, as knockdown cyba and rubcn, moderately reduced Lc3 association with phagosomes and diminished pneumococcal degradation, confirming the host protective function of this process. Interestingly, we observed no involvement of xenophagy components in S. pneumoniae-infected macrophages, suggesting the activation of an other non-canonical autophagy pathway, distinct from LAP, that targets pneumococci-containing phagosomes. Instead, we found that production of pneumococcal pore-forming toxin - pneumolysin induces LAP-independent Lc3 lipidation. This Lc3 decoration of vesicles containing pneumolysin-positive pneumococci can be abolished by knockdown of tecpr1a (tectonin beta-propeller repeat-containing 1a) indicating the involvement of another non-canonical autophagy pathway called sphingomyelin–TECPR1-induced LC3 lipidation (STIL).
Collectively, our observations shed new light on the host immune response against S. pneumoniae, demonstrating that non-canonical autophagy pathways play a supportive role in bacterial degradation by macrophages and providing a potential target for the development of novel therapeutic approaches to combat pneumococcal infections.

<u>SURESH JESUTHASAN</u> : Solitary chemosensory cells in zebrafish skin: evidence for a role in host-microbiota interactions

Kathleen Cheow, Vaishnavi Chandramouli, Julia Peloggia, Karen Guillemin, Tatjana Piotrowski and Suresh Jesuthasan

The skin of many species of fish is peppered with solitary chemosensory cells (SCCs) of unknown function. These cells are characterised by an actin-rich apical protrusion that is in contact with the external environment, while the soma is surrounded by afferent neurites. In some species, SCCs have been observed to respond to an undefined component of fish mucus and human saliva, based on recording of the facial nerve. Here, we use the zebrafish to test the hypothesis that SCCs mediate an interaction with bacteria, which are known to reside in these substances. A feature of SCCs, which are found in larval, juvenile and adult stages, is the high content of serotonin. Analysis of single cell transcriptome data from larval skin identified one cluster of cells that expresses genes involved in the synthesis of serotonin. This cluster is characterised by the presence of several neuropeptides including the gastrin-related peptide (grp). In situ hybridization indicates that all solitary chemosensory cells express grp. The cluster contains the htr3al receptor and in situ hybridization confirms that this gene is expressed in SCCs. Time-lapse imaging demonstrates that SCCs respond to a transient exposure of external serotonin with a sustained rise of intracellular calcium. Additionally, ERK is phosphorylated in goblet cells, which secrete mucus and thereby boost skin defence, in response to external serotonin. Given that serotonin is a bacterial signalling molecule, we propose that SCCs mediate bi-directional communication between the host and bacteria, influencing host behaviour and mucus secretion. SCCs are thus a previously unrecognised component of neuro-immune interactions in the fish.

JUSTYNA ZMORZYNSKA : Modeling TSC-associated neuropsychiatric disorders using zebrafish

Olga Doszyń, Magdalena Kędra, Justyna Zmorzyńska*

Tuberous Sclerosis Complex (TSC) is a rare genetic disease that manifests with early symptoms, including childhood epilepsy and TSC-associated neuropsychiatric disorders (TANDs). The latter comprise anxiety, autism spectrum disorder, and intellectual disability, among others. We showed before that the tsc2vu242/vu242 zebrafish recapitulate TSC pathology in human patients as we observed heterotopias and hyperactivation of the mTorC1 pathway in the pallial brain regions, commissural thinning responsible for brain dysconnectivity with delayed axon development and aberrant tract fasciculation, epileptogenesis that resulted in non-motor seizures, and anxiety-like behavior. We showed that lack of light preference of the tsc2vu242/vu242 is caused by aberrant processing of light stimuli in the left dorsal habenula and tsc2vu242/vu242 fish have impaired function of the left dorsal habenula, in which neurons exhibited higher activity and lacked habituation to the light stimuli. These characteristics were rescued by rapamycin. We thus discovered that hyperactive mTorC1 caused aberrant habenula function resulting in lack of light preference. Our results suggest that mTORC1 hyperactivity contributes to atypical reactivity to sensory stimuli in autism spectrum disorder.
However, as TSC patients are often heterozygotes, we analyzed also heterozygotic siblings of the accepted TSC zebrafish model. We discovered that the tsc2vu242/+ mutants did not suffer from early epileptogenesis and seizures, yet they showed hyperactivation of the mTorC1 pathway in the pallial brain regions and commissural thinning responsible for brain dysconnectivity. Notwithstanding, the heterozygous tsc2vu242/+ mutants also exhibited increased anxiety-like behaviour, decreased learning and memory, and aberrant development of social behaviour, suggesting that loss of one allele of the tsc2 gene is enough to cause TANDs-like phenotypes in the zebrafish model of TSC. Our results are in line with the hypothesis that the majority of symptoms in TSC are inherited in an autosomal dominant manner – including TANDs – but for seizure development the loss of heterozygosity is needed.

JENS KROLL : Modeling Diabetes and Diabetes-induced microvascular organ complications in zebrafish

Lucas Wiggenhauser, Shu Li, Bowen Lou, Haozhe Qi, Elisabeth Lodd & Jens Kroll

Background: Diabetic retinopathy and diabetic nephropathy are common microvascular complications of diabetes mellitus. We established a series of new genetic zebrafish models to study the onset, progression and formation of diabetes and its related organ complications.
Methods: pdx1-deficient zebrafish were generated and analyzed in the eyes and kidneys. We further established zebrafish mutants for detoxifying enzyme systems, which remove reactive metabolites spontaneously formed during glycolysis and fatty acid oxidation. These are Glyoxalase 1 (Glo1), Aldehyde-dehydrogenase 3a1 (ALDH3a1) and Aldo-keto-reductase 1a1a (AKR1a1a). Zebrafish were sacrificed for blood sugar measurements, analysis of metabolomics, expression profiles and vascular, retinal and kidney morphology.
Results: pdx1-/- mutants showed increased retinal angiogenesis and thickening of the glomerular basement membrane. Knockout of glo1, aldh3a1 and akr1a1a led to the accumulation of the dangerous reactive metabolites methylglyoxal, 4-HNE and acrolein, respectively, before the onset of hyperglycemia. They induced hallmarks of type 1 and type 2 diabetes, including pancreatic beta cell loss accompanied by loss of insulin expression, reduced expression and activity of insulin receptors and its downstream signaling molecules leading to insulin resistance. As a consequence, all mutants developed subsequently hyperglycemia and hallmarks of diabetic retinopathy and nephropathy.
Conclusion: Zebrafish recapitulate important hallmark of human diseases related to diabetes. We further identified internally and spontaneously produced metabolites, such as methylglyoxal, 4-HNE and acrolein, and if not detoxified by their corresponding detoxifying enzyme systems, act upstream of hyperglycemia and thereby inducing diabetes and its organ complication.

MARCO SCHIAVONE : RNA sequencing and living zebrafish biosensors reveal the cascade of events underlying DMD pathogenesis

Cannone Elena, La Spina Martina, Castagnaro Silvia, Gnutti Barbara, Tobia Chiara, Luca La Via, Sabatelli Patrizia, Faldini Cesare, Fiume Giuseppe, Finazzi Dario, Gennarelli Massimo, Magri Chiara, Schiavone Marco

Duchenne muscular dystrophy (DMD) is a severe muscle disorder characterized by progressive muscle wasting and massive replacement of muscle fibers with adipose tissue. Several in vitro and in vivo studies deepened our understanding of the dysregulated mechanisms underlying DMD progression, but the early pathogenic processes following dystrophin loss have yet to be defined. To reach this aim, we performed an in-depth transcriptomic analysis of both sapje dystrophic zebrafish (one of the most severe vertebrate models of human DMD) at different stages of disease development, and human DMD cell lines. We found: i) down-regulation of pax3 target genes together with up-regulation of myog, and ii) down-regulation of genes involved in the regulation of Ca2+ homeostasis at the first stages of disease pathogenesis. By integrating RNA seq results with in vivo imaging, we confirmed the occurrence of both mitochondrial dysfunction and defects in muscle differentiation at the onset of DMD. Our results support the new emerging hypothesis that DMD pathogenesis begins with USCs hyperactivation which generates weak muscle fibers and promotes muscle fibrosis. Finally, our data suggest that pharmacological approaches targeting the USCs could represent a valid strategy to hinder DMD progression.

CHIARA GABELLINI : Relevance of ATM-dependent regulation of autophagy in Ataxia Telangiectasia: integrating patient-derived cells and zebrafish disease models

Rosa Scarpitta, Francesca Luongo, Francesca Di Lorenzo, Annalina Caroli, Maria Marchese, Giuseppe Neri, Laura Coculo, Michela Puccello, Daniela Trisciuoglio, Francesca Barco, Tommaso Butini, Francesco d'Errico, Venturina Stagni, Chiara Gabellini

Ataxia-telangiectasia (AT) is a multisystem disorder characterized by cerebellar ataxia, immunodeficiency, hypersensitivity to ionizing radiations and increased cancer risk. It is caused by mutations in the ATM gene, which encodes a multifunctional kinase that regulates DNA damage response, cell cycle checkpoint arrest and apoptosis. Over 3,000 unique mutations were identified into the whole gene, with phenotype severity depending on residual protein activity. The broad clinical spectrum in AT patients is unlikely to depend exclusively on defects in DNA damage response. Growing evidence indeed supports new ATM cytoplasmic roles including regulation autophagy and redox homeostasis.
Our preliminary data identified a new signaling pathway that connects ATM to autophagy. In particular, ATM sustains the expression of the autophagic gene ATG4C, whose upregulation is able to rescue some defects in AT patient-derived lymphoblastoid cells, like reduced cell growth and sensitivity to oxidative stress. Recent evidence suggests that ATM regulates also the expression of another autophagic gene, ATG4D, which encodes for a member of the same protease family that includes ATG4C, through a mechanism that seems to be dependent on the transcription factor NRF2.
Our aim is to investigate ATM-dependent autophagy dysregulation in AT integrating in vitro models with in vivo models based on zebrafish. Interestingly, zebrafish partially mimic AT phenotypes when ATM orthologue is mutated. Indeed, marked similarities exist between zebrafish and human ATM gene and protein, including the kinase domain. We have already demonstrated that in zebrafish atm mRNA has a maternal origin and is expressed during early development.
Here, we propose atm loss of function zebrafish models, generated using CRISPR/Cas9 gene editing system or specifically inhibiting atm kinase activity by KU-55933 treatment, to characterize the consequences of atm function impairment on autophagy and redox homeostasis. Interestingly, atm loss of function determined a hyperlocomotory phenotype in zebrafish embryos and larvae, suggesting that atm deficiency induces an easily monitorable early-stage embryonic alteration.
Recently, the accumulation of unrepaired DNA damage due to sleep deprivation has been associated to a hyperlocomotory phenotype in zebrafish. To test if the same phenotype observed in our models is due to the persistent DNA damage in atm loss of function models, we evaluated the effects on zebrafish development of UV-B radiation, as a well-known DNA damage agent. Zebrafish embryos and larvae at different developmental stages were exposed to UV-B at 30-70-100 mJ/cm2 dosages. Results revealed dose-dependent phenotypical alterations like teratogenic effects and fin skin reduction.
If confirmed as a proof of concept of AT disease modelling, zebrafish-based atm loss of function models may represent an extremely useful tool to evaluate the possible effect of small molecules with autophagy-inducing or autophagy-inhibiting activity in rescuing phenotypical, behavioural, cellular and molecular alteration induced by atm function impairment. In particular, hyperlocomotory behaviour could represent the output phenotype to test small molecules in high-throughput experimental approaches, to confirm autophagy modulation as possible therapeutic strategy for AT patients.

JEROEN DEN HERTOG : RASopathies are associated with defects in lymphangiogenesis

Jeroen den Hertog, Jelmer Hoeksma, Tieme Bijlsma, Mariska Dijkers, Yuanyuan Liu, Daniëlle Woutersen

RASopathies form a group of syndromes that have partially overlapping symptoms and are characterized by activated RAS/MAPK signaling. Many factors of the RAS/MAPK signaling pathway have been found to be associated with RASopathies, including the protein-tyrosine phosphatase, SHP2, and the guanine nucleotide exchange factors, SOS1 and SOS2. RASopathy symptoms include short stature and defects in heart function, hematopoiesis and craniofacial development as well as defects in lymphangiogenesis. We use zebrafish to study RASopathies in vivo and we have generated knock-outs lacking functional Shp2, Sos1 and Sos2. Moreover, we have generated knock-in lines using CRISPR/Cas9 technology and homology-directed repair, expressing variants of Shp2 and Sos1 or Sos2 that were identified in human patients. Recent work focusses on defects in lymphangiogenesis. We found that Shp2 and Sos function are required for normal lymphangiogenesis and that our zebrafish Noonan Syndrome models phenocopy defects observed in human patients. These observations and pharmacological interventions to ameliorate the defects in zebrafish models will be discussed.

JATIN NAGPAL : Mechanistic dissection of microbiome-brain communication using zebrafish as a model system

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Changes in the microbiota are associated with alterations in nervous system structure-function and behaviour and have been implicated in the aetiology of neuropsychiatric and neurodegenerative disorders. Most of these studies have centred on mammalian models due to their phylogenetic proximity to humans. Indeed, the germ-free mouse has been a particularly useful model organism for investigating microbiota-brain interactions. However, microbiota-brain axis research on simpler genetic model organisms with a vast and diverse scientific toolkit (zebrafish, Drosophila melanogaster, and Caenorhabditis elegans) is now also coming of age (Nagpal and Cryan, 2021; Lynch, Nagpal et al., 2024). Advantages of the cross-species mechanistic approach to unravel the microbiota-brain communication, specifically using zebrafish, will be discussed. Furthermore, we have established a state-of-the-art zebrafish facility at University College Cork thereby nucleating an Irish zebrafish community and have embarked on several projects leveraging the zebrafish model systems focusing on stress (Nagpal et al., 2024), behaviour (Diaz et al., in preparation), neurodevelopment, myelination (Lynch et al; in submission), diet (Bouffard et al., in preparation) as well as serving as an in-vivo platform to evaluate the effects of microbiome-modulating substances on brain and behaviour (Valderrama et al, 2025). Results from these diverse arrays of studies will be shared. One such study aims at identifying bacterial strains, termed psychobiotics, that can modulate neuro-signaling pathways (Valderrama et al, 2025). Herein, we introduce an innovative framework that combines in-silico, in-vitro, and in-vivo methodologies to systematically identify and characterize psychobiotic candidates. As a proof of concept, we focused on the characterization of a strain of Lactiplantibacillus plantarum (Valderrama et al. 2024). Our findings demonstrate that this bacterial strain can effectively metabolize prebiotics, such as fructooligosaccharides (FOS), galactooligosaccharides (GOS), and inositol, and produce neuroactive molecules, including GABA, tryptophan, and acetate. Moreover, our in-vivo experiments using zebrafish larvae revealed that the administration of this strain's supernatants significantly modulated the expression of key genes in the GABAergic pathway (gad1 and gabra1) and resulted in notable alterations in anxiety-like behavior.

MICHAEL DORRITY : Quantitative analysis of developmental timing in whole embryos

The genetic program of embryonic development is remarkably robust, but temperature stress can degrade its ability to generate animals with invariant anatomy. While the stereotyped, consistent phenotypes associated with environmental stress during vertebrate development suggest that some cell types are more sensitive to stress than others, the basis of this sensitivity is unknown. We characterize hundreds of individual zebrafish embryos under temperature stress using whole-animal single cell RNA-seq to identify cell types and molecular programs that drive phenotypic variability. We build quantitative models of embryogenesis and find that temperature perturbs the normal proportions and gene expression programs of numerous cell types and introduces asynchrony in their development, driven by non-uniform acceleration of developmental tempo. Current work in our group is exploring how developmental tempo is linked to robustness across different species, leveraging single cell genomics as a tool for comparative analysis of developmental trajectories across cell types. We focus on cell type-specific differentiation dynamics in a “slow” species (medaka) vs a “fast” species (zebrafish) that show similar thermal habits. We develop new tools to directly compare these species despite their differing developmental tempos, constructing and aligning developmental trajectories to identify cell type-specific timing, or heterochrony, between medaka and zebrafish.

HERWIG BAIER : The shell-dwelling cichlid Lamprologus ocellatus: a new model system for neuroethology, behavioral ecology, and cognitive neuroscience

Herwig Baier, Swantje Grätsch, Ash Parker, Alessandro Dorigo, Peter Jägers, Vaishnavi Agarwal

Our group is introducing a new model organism for behavioral neuroscience, the shell-dwelling cichlid Lamprologus ocellatus (LO). This species has evolved exclusively in Lake Tanganyika as part of the explosive radiation of cichlids in the lake over the past 9 million years. LO are about the size of zebrafish and grow to adulthood in about 4-5 months. The ecology of LO has been extensively studied by Alex Jordan and others in the field, and its genome has been sequenced by Walter Salzburger and colleagues. We have imported this species into our laboratory and have established prolific breeding colonies, molecular imaging protocols, and transgenesis methods. The life of an LO individual revolves around one or more empty snail shells, which they find in abundance on the lake floor (or, under more controlled conditions, in our aquariums). Instinctively, an adult LO seeks to conquer a small number of shells and, once successful, builds an elaborate nest around one of them. The shell serves as a shelter to hide, rest, sleep, and raise progeny, and is vigorously defended by its owner. A single LO male cohabitates with multiple females in a territory that is fiercely protected from all sorts of intruders, which can be other fish, but also human divers (or the hands of human experimenters). Following a complex courtship ritual, LO females lay 15 to 50 eggs into their own shell, where they are fertilized by the male. LO embryos, larvae, and juveniles grow up inside the shell, before they emerge to forage and, eventually, leave for good to capture a shell of their own. Behavioral maturation follows a rigid developmental schedule set by intrinsic timers that coordinate the behaviors of both the young and their caregiving parents (Parker et al., Current Biology 2025). In our laboratory, we study the fish’s cognitive abilities that we expect to have evolved as adaptations to the species-specific demands. We have dissected the behavioral motifs organizing nest building (Grätsch et al., submitted), collected evidence for spatial memory and shell caching (Jägers et al., in preparation), and devised preference assays to study shell object recognition (Agarwal et al., in preparation). While this research program is still in its infancy, we have already provided evidence that the impressive cognitive capacities of this teleost fish can not only be probed with behavioral tests, but also mapped onto brain circuitry with immediate-early gene in situ hybridization techniques. If selected for a talk, we will focus on recent results that illustrate the promise of this new model system.

<u>GERMAN SUMBRE</u> : Evolutionary repurposing of the visual optic tectum in blind cavefish

Ehud Vinepinsky and German Sumbre

The optic tectum, homologous to the superior culliculus in mammals, is the main visual center in fish. Its functional role is mainly visual. It responds to the different physical properties of the stimuli (position, movement direction and size), and generates goal directed behaviors (swim towards or away from the stimulus).
Despite the loss of its major input, the optic tectum of pachón and molino cavefish did not degenerate and its functional connectivity is not significantly different from that of surface fish. This suggests that in cavefish, evolution repurposed the optic tectum for a functional role different from vision processing. Using two-photon fluorescence microscopy in combination with transgenic Astyanax larvae expressing the genetically encoded Ca2+ indicator (GCaMP6s), we showed that a small portion of the optic tectum is used to exclusively process vocalizations. A larger portion of the cavefish optic tectum, generally responding to visual stimuli in surface fish, did not respond to other sensory stimuli such us acoustic, lateral line or somatosensory stimuli. However, we found that strong responses across large portions of the optic tectum emerge during spontaneous or stimuli-induced behaviors. Our preliminary results suggests that evolution has repurposed the optic tectum of cavefish to process motor behavior. Further experiments will shed light whether the optic tectum represents proprioception to improve navigation in the absence of vision.

<u>LUTGARDE ARCKENS</u> : Functional integration of new neurons following traumatic brain injury in the African turquoise killifish: a viral vector and behavioral study

Valerie Mariën, Anouk Maes, Marialuisa Tognolina, Caroline Zandecki, Jolien Van houcke, Emre Yaksi and Lutgarde Arckens

Aging is an inevitable and irreversible process that every organism experiences. As a vertebrate model, the African turquoise killifish holds the record for fast aging. The combination with a high regenerative ability makes the killifish ideally suited to study the effect of aging on regeneration capacity. We already uncovered that, morphologically, at a young adult age, the telencephalon of killifish can fully recover after stab wound injury, while in aged fish permanent and mammalian-like glial scarring occurs. Nevertheless, aged fish still respond to the injury by generating newborn neurons, albeit fewer and slower than young fish. The question remains if such new neurons fully mature and functionally integrate into the existing circuitry. To this end, we optimized a viral vector labeling technique to permanently trace the newborn cells upon injury. The injection of a gamma-retroviral vector into the telencephalic ventricle enables the specific labeling of only the dividing stem cells at the time of the injury. By incorporating the viral vector genes (GFP) in their DNA, and upon differentiation and maturation into neurons, their full cellular morphology becomes visible. As a first indication of maturation and integration, we applied Sholl-analysis and immunofluorescent synaptic marker stainings. Already at 23 days post-injury the newborn neurons are surrounded by SV2+ (synaptic-vesicle 2, pre-synaptic marker) contacts, indicative of circuit integration. Morphologically they also resemble resident, naive neurons based on Sholl analysis. To judge functional integration, we performed ex vivo patch-clamp of such GFP-positive newborn neurons, comparing firing rates between resident and newborn neurons and analyzing the number and frequency of post-synaptic currents. To allow behavioral assessment of functional recovery, we specifically chose to injure the Dm zone, the homolog of the mammalian amygdala, which is involved in fear learning and memory. After training fish in a conditioned place avoidance test, where they learned to avoid the dark zone of the maze due to coupling visits to an electric shock, we bilaterally injured the Dm zone. One to four days post-injury, the fish no longer showed dark avoidance like before injury, indicating the loss of both memory and learning due to physical damage to the Dm zone. From 42 days post-injury, fish regained their Dm function since they relearned avoiding the conditioned dark arm. In sum, our data prove that when injury is inflicted in young adult killifish, the neuronal circuits can fully recover morphologically and functionally in four to six weeks. We are now ready to uncover the molecular machinery behind functionally relevant neurorepair to hopefully one day translate to human patients suffering from traumatic brain injury.

NADIA MERCADER : A paternal cardiac lesion induces cardiac adaptation in offspring

Benedetta Coppe, María Galardi-Castilla, Andrés Sanz-Morejón, Prateek Arora, Javier Lucas, Laura Lalaguna, Enrique Lara Pezzi, Ignacio Flores and Nadia Mercader

Organisms are exposed continuously to environmental stimuli that activate genetic and epigenetic responses in the cells. When such alterations are established in the gametes, they might escape embryonic reprogramming and be transmitted from one generation to another, giving rise to new phenotypic traits. Diet alteration, chemical exposure, and early life traumatic events experienced by ancestors have been described as events influencing the health and behavior of the subsequent generations. Here we investigated if a cardiac injury and transitory dysfunction can also influence the immediate progeny by using two animal models that regenerate the heart after an injury: the zebrafish and the neonatal mouse. Our results point to mild changes in cardiac remodeling and improved heart function in the offspring of injured fathers, as analyzed by histological stainings, echocardiography, and bulk- and single-nuclei RNA-seq. Additionally, our observations suggest that an inflammatory response occurs in male gametes in response to cardiac damage and possibly influences spermatozoa information. Overall, our results disclose the intergenerational transmission of cardiac damage “memory” from the father to the offspring in two vertebrate animal models

MAXIMILIAN FURTHAUER : De novo induction of zebrafish chiral morphogenesis by unconventional type 1 Myosins

Amélie Le Parc, Thibaut Artus, Charlotte Sidney, Delphine Leroux, Farid Salmy & Maximilian Fürthauer

The establishment of animal Left-Right (LR) asymmetry has long been thought to rely on species-specific mechanisms that range from cilia-driven fluid flows to chiral cell rearrangements. Only relatively recently has work form a number of groups including ours identified the unconventional type I Myosin Myo1D as an evolutionarily conserved regulator of LR asymmetry. Our previous work revealed that zebrafish Myo1D controls the orientation of motile cilia orientation and the establishment of a symmetry-breaking fluid flow in Kupffer’s Vesicle which acts as a central LR Organizer (Juan et al, Nat.Com. 2018). While it is presently clear that Myo1D is essential for organismal chirality in flies, fish, frogs and humans, it remains to be understood how the same molecule is implicated in both cilia-dependent (in vertebrates) and cilia-independent (in flies) mechanisms of symmetry breaking.
In addition to Myo1D, the zebrafish genome encodes the closely related protein Myo1G. Although myo1g and myo1d mutations both impair embryonic laterality, our recent work revealed that Myo1G exerts a function that is distinct from Myo1D by regulating the transfer of laterality information by Nodal ligands (Kurup et al, Nat.Com. 2024). While these findings further substantiate an important function of Myosin1 proteins as central regulators of LR asymmetry, the fact that neither cilia nor Nodal ligands are involved in symmetry breaking in Drosophila raises the question whether a common, Myosin1-dependent cellular mechanism that underlies the establishment of LR asymmetry in different animal species still remains to be identified.
Our recent work focuses on the importance of Myosin1 proteins for the chiral deformation of embryonic tissues, a morphological event required for all lateralized morphogenesis. Of particular interest, our work reveals that - in addition to being required for endogenous LR asymmetry - fish and fly Myosin1 proteins possess the unique capacity to induce chiral zebrafish morphogenesis de novo, in tissues that would normally undergo symmetric development. Here we will present our current analysis of this novel asymmetry-inducing function of Myosin1 proteins.

DEVELOPMENT 2 9:00 AM -10:30 AM

SOOJIN RYU : Preparing for future stress- how early Glucocorticoid exposure alters hypothalamic cells

Min-K Choi, Anna Tochwin, Soojin Ryu

Excess stress exposure during early life can affect sensitivity to subsequent stress both in humans and animal models. Studies show that Glucocorticoids (GCs) play pivotal role in mediating the long-lasting effect of early life stress on adult function. The hypothalamus represent an important target region of GCs. However, it is currently not known whether and how early GC exposure alters hypothalamic cells to shape their adulthood response to stress. To tackle this knowledge gap, we recently developed a double-hit stress zebrafish model, which combines exposure to high level of GC during development (hdGC) and acute stress exposure in adulthood. In this model, we observed that zebrafish exposed to hdGC exhibit an exaggerated stress response in adulthood, as evidenced by changes at the endocrine, behavioral, and transcriptional levels. To characterize potential differences at the hypothalamic level in fish that have been exposed to hdGC, we performed single nucleus multiome analyses of the neurosecretory preoptic area (NPO), zebrafish equivalent of mammalian paraventricular hypothalamic nucleus (PVN). Our analyses identified distinct neuroendocrine cell clusters based on their transcriptome and open chromatin profiles. Using those profiles, we identified differences in the gene expressions of key neuropeptides as well transcriptional regulators in neuroendocrine cell clusters exposed to hdGC. Moreover, we identified differential transcriptional responses of genes associated with GC signalling, neuronal signaling, and epigenetic modifications in some neuroendocrine cell clusters upon acute stress exposure in adulthood. Our results suggest that hdGC exposure alters functional identities of neuroendocrine cells in adulthood and in subset of neuroendocrine cell clusters exposed to hdGC, distinct transcriptional regulation occur upon acute stress exposure. Together they suggest potential mechanisms underlying altered stress responsivity in adulthood following early stress exposure.

PATRICK MULLER : Probing the duality of temperature and osmotic strength on developmental tempo using deep learning

Brunnhuber T, Önder O, Safroshkin M, Arutyunov G, Dimmler L, Čapek D, Müller P

The tempo of embryonic development is highly sensitive to environmental conditions. Recent influential work has proposed a duality of temperature and osmotic strength on cellular function, and suggested that solvent thermodynamics driven by changes in water availability regulate protein activity and macromolecular interactions. However, this hypothesis has not been tested in the context of complex developing multicellular systems, and the relative contributions of temperature and osmotic strength on the regulation of developmental tempo remain unclear. To address this question, we developed zMorphoNet, a deep learning-based tool for detailed morphometric analyses across developmental stages. zMorphoNet combines semantic and instance segmentation for accurate tissue identification and was trained and validated using manually annotated zebrafish datasets. Our tool enables detailed morphometric analyses across developmental stages and precisely delineates embryonic structures, including the yolk, yolk extension, cells, embryonic body, and eyes. By applying zMorphoNet to embryos exposed to varying environmental conditions, we quantified tissue-specific growth rates and morphological changes with unprecedented precision. We found that temperature consistently dominated the regulation of developmental tempo in zebrafish embryos, while modulating osmotic strength had more subtle effects on tissue boundary integrity and morphogenetic processes. These findings suggest that temperature-driven solvent thermodynamics play a primary role in regulating the biochemical activity essential for developmental progression. By combining deep learning with experimental manipulations, our study underscores the utility of zMorphoNet for quantifying tissue dynamics and developmental tempo in complex biological systems.

ZHAOXIA SUN : Zebrafish Motile Cilia Mutants Reveal co-Translational Assembly of Axonemal Dynein Heavy Chains

Yuanyuan Li, Wenyan Xu and Zhaoxia Sun

Motile cilia beat rhythmically to propel cell movement or drive extracellular fluid flow. The functional importance of cilia motility in human health is highlighted by primary ciliary dyskinesia (PCD), a genetic disease caused by cilia motility defects. Patients with PCD display left-right asymmetry defect, reduced fertility, and progressive lung disease. Currently there is no specific therapy for PCD and management of symptoms has been the main approach. The dynein arms that power cilia motility comprise multiple components that are pre-assembled in the cytosol; and many genes associated with PCD encode components of these dynein arms. In addition, a separate group of PCD genes encode proteins that reside in the cytosol and appear to be involved in the assembly of dynein arm subunits and they are called dynein axonemal assembly factors (DNAAFs). Interestingly, multiple DNAAFs are localized in droplet shaped cytosolic foci that was proposed to form through liquid-liquid phase separation (LLPS). However, the precise function of these foci and most DNAAFs at a molecular level remains poorly understood.

We previously identified a collection of zebrafish mutants with almost identical cilia-associated phenotypes. Using these mutants, we subsequently showed that Ruvbl1/Pontin and Ruvbl2/Reptin function as co-chaperones for dynein arm assembly. In addition, we have observed that endogenous Pontin, Reptin and the DNAAF protein Lrrc6 are enriched and colocalize in droplet shaped foci, suggestive of LLPS, in the cytosol of motile ciliated cells. However, our recent results demonstrate that mRNAs encoding interacting heavy chains (HCs) of outer dynein arms co-localize in cytosolic foci, along with nascent HCs. We observe that the previously identified Lrrc6, Ruvbl1 and Ruvbl2 foci co-localize with these HC foci. We additionally show that Ruvbl1 is required for the recruitment of Lrrc6 into the HC foci and that both proteins function co-translationally. We propose that these HC-DNAAF foci are novel membraneless cytosolic assemblages anchored by stable interactions between translating HCs, ribosomes and encoding mRNAs, followed by co-translational molecular condensation of co-chaperones and assembly factors, distinct from LLPS mediated by numerous weak interactions. They function as assembly hubs to coordinate the translation, folding and assembly of axonemal dynein arm components at scale.

NATHALIE JURISCH YAKSI : The neuromodulatory function of cerebrospinal fluid

Percival D’Gama, Inyoung Jeong, Mert Ege, Emre Yaksi, Nathalie Jurisch-Yaksi

Cerebrospinal fluid (CSF) is a clear liquid found within the brain and spinal cord of vertebrates. New evidence suggests that CSF may modulate brain physiology through volume transmission, and thereby contribute to brain disorders. Yet, we still lack a good understanding of how CSF is produced and circulated, how it modulates the brain and how CSF disruption leads to brain disorders. To address these questions, we use the zebrafish as a model as it allows us to monitor and manipulate cellular and neurological processes in vivo in an intact brain. Using genetics, imaging and histology, we first identified that the zebrafish has an evolutionary conserved ventricular system, consisting of interconnected ventricles lined by ciliated ependymal cells and CSF-producing choroid plexus. Next, we observed that several physiological factors regulate CSF dynamics and solute transport in the brain ventricles, similarly to mammals. This includes ependymal cilia, the cardiac cycle, bodily movements, diffusion and CSF secretion by the choroid plexus. Using genetic tools to manipulate cilia and choroid plexus biology, we identified that CSF dynamics are critical for brain physiology through modulation of neural and astroglial networks. We are now identifying the underlying molecular mechanisms leading to altered brain activity and maturation of neuronal circuits, and the behavioral relevance of our findings. Our long-term goal is to elucidate how CSF modulates the brain in healthy and pathological conditions.

<u>CAROLINE HILL</u> : Shaping Fgf/Erk signalling dynamics via the cell cycle

Scott Wilcockson and Caroline S. Hill

Erk signalling dynamics can elicit distinct cellular responses and the early zebrafish embryo is an ideal model to explore the role of Erk signalling dynamics in an in vivo context. In these embryos a well characterised gradient of Fgf signalling at the blastula embryonic margin both patterns the dorsal–ventral axis, primarily through the induction of phosphorylated Erk (pErk) and regulates the specification of mesodermal and endodermal lineages in a short temporal window during the late blastula/early gastrula period. To investigate the dynamics of Fgf/pErk signalling, we have generated a highly specific Erk biosensor and used it to monitor the growth of the Fgf signalling gradient in vivo. In doing so, we discovered the phenomenon of mitotic erasure, whereby pErk signalling is extinguished as cells undergo mitosis and then is restored with variable dynamics as cells re-enter G1. This introduces short variable periods of Erk inactivity in an asynchronously dividing population of cells. We show that not only is pErk dephosphorylated during mitosis, but Erk targets are too. We have explored this phenomenon more thoroughly using inhibitors of key regulators of the cell cycle. We have shown that cells arrested at the end of G1 or G2 exhibit higher levels of Erk activity, whilst cells treated with a Wee1 inhibitor that prematurely drives cells into mitosis reduces levels of Erk activity. The consequences of this for mesoderm and endoderm specification will be discussed. Finally, we have investigated whether the rate of cell proliferation varies within the developing embryo. We show that cells at the margin proliferate more slowly than those at the animal pole, and this is regulated by Fgf signalling. Taken together, our work reveals a mechanism of developmental signalling regulation that couples cell fate decisions to tissue growth.

<u>ALEXANDER SCHIER</u> : Single-cell multiomics and spatial transcriptomics for embryogenesis

The interplay between transcription factors and chromatin accessibility regulates spatiotemporal gene expression and cell type diversification. However, comprehensive atlases and gene regulatory networks underlying these processes have remained elusive. I will present our recent efforts in single-cell multiomics and spatial transcriptomics to address this challenge. We generated a single-cell multiomics atlas of RNA expression and chromatin accessibility during zebrafish embryogenesis and developed DeepDanio, a deep learning model to dissect cis-regulatory interactions. In parallel, we developed a whole-embryo imaging platform to quantify the expression of hundreds of genes at subcellular resolution. Integration with the single-cell multiomics data generated an atlas detailing the expression of ~25,000 genes and the accessibility of ~300,00 chromatin regions, as well as the online browser MERFISHEYES. I will discuss how exploration of the atlas and the gene regulatory network identified instant differentiation as a novel mode of cell differentiation and how changes in gene expression generate sharp boundaries during gastrulation.

URS BOHM : Optical recording of spinal cord dynamics with voltage imaging

U. L. Böhm, Y. Kimura, T. Kawashima, M. B. Ahrens, S. Higashijima, F. Engert, A. E. Cohen, B. Judkewitz

Spinal cord activity underlies all locomotor output, but due to the difficulty of recording activity in intact, behaving animals, the diversity of both motor and sensory activities during complex behaviors has been largely inaccessible. Voltage-imaging holds great potential to noninvasively record the membrane potential of many spinal cord neurons in parallel and overcome some of these limitations.
By combining voltage-imaging and fictive behavior in a virtual environment, we recently demonstrated the potential of this technology by measuring the activity of all glutamatergic neurons in the larval zebrafish spinal cord and characterized a previously undescribed subpopulation of tonic-spiking ventral V3 neurons.
We furthermore developed a new approach for fast and light efficient remote focusing that enables high-speed volumetric voltage imaging at 500 volumes/s, enough to image the entire volume of a section of zebrafish spinal cord and record from >100 spontaneously active neurons in parallel.
In the future these new approaches will allow us to describe the diversity of spinal cord network activities that occur during specific behaviors as well as the millisecond timing precision of spinal cord neurons during locomotion. By investigating both the cellular and functional diversity of the entire intact spinal cord circuitry, our work will ultimately lead to a deeper understanding of what activity drives different behaviors and how neural networks generate dynamic but stable motor outputs.

THOMAS JUAN : A recombinase-activated ribozyme to knock down endogenous gene expression in zebrafish.

Thomas Juan, Tonatiuh Molina, Lihan Xie, Sofia Papadopoulou, Bárbara Cardoso, Shivam Govind Jha, and Didier Y.R. Stainier

Precise regulation of gene expression is essential to understand a wide range of biological processes. Control over gene expression can be achieved using site-directed recombinases and endonucleases. However, their efficiency is variable and dependent on the genomic context. Here, we develop a self-cleaving ribozyme-based tool to control mRNA levels of endogenous targets in zebrafish. Using an in vivo reporter strategy, we first show that inserting the T3H48 self-cleaving ribozyme in an intron enables rapid pre-mRNA cleavage, with up to 20-fold reduction in expression, and that this ribozyme displays superior activity compared with other ribozymes. We then inserted the ribozyme in the second intron of the albino gene using a CRISPR/Cas9 strategy and observed a pigmentation phenotype similar to that in the mutant. Using a base-editing strategy to inactivate the ribozyme, we show that this phenotype is reversible, illustrating the specificity of the approach. In addition, we generated a Flippase- and Cre-activatable version of the T3H48 ribozyme, called RiboFlip, to control the mRNA levels of the albino gene. RiboFlip activation induces mRNA knockdown and also recapitulates the albino mutant phenotype. Furthermore, we show that a Cre- and Dre-controllable Gal4/UAS reporter in the RiboFlip cassette can label knocked-down cells independently of the expression of the target gene. Altogether, we introduce the RiboFlip cassette as a flexible tool to control endogenous gene expression in a vertebrate model and as an alternative to existing conditional knockdown strategies.

MONICA BELTRAME : TICK, a temperature-inducible K+ channel, as a new tool to induce behavioral changes in zebrafish

Chiara Mercurio1, Alessandro Ronzoni1, Giulia Faini2, Matilde Pallini1, Filippo Del Bene2, Anna Moroni1, Monica Beltrame1. 1 Dip. Bioscienze, Università degli Studi di Milano, Italy; 2 Institut de la Vision, Paris, France

We present here the engineering and in vivo validation of temperature-inducible K+ channels (TICK), whose activity is modulated by small temperature changes. TICKs exploit the minimal size of the viral Kcv channels, the smallest and more primitive form of potassium (K+) channels, characterized only by the pore domain, that was fused to a bacterial C-terminal cytoplasmic domain (CTD), conferring thermal sensitivity. A folded CTD acts as a constraint for Kcv and inhibits K+ flow. Unfolding of the CTD, beyond a certain temperature, releases inhibition. A prototype TICK was characterized in mammalian cells through electrophysiological analyses. Further mutagenesis allowed us to identify a constitutively inactive form of TICK and a more refined active version, with tighter thermal control. Moreover, additional elements were introduced to optimize intracellular trafficking and TICK membrane expression in mammalian cells.
Codon-optimized versions of these TICK constructs were broadly expressed in zebrafish, through microinjection of RNAs at 1-2 cell stage. Embryonic and larval development were not affected at 28.5 °C, consistent with TICK channels being closed at this T. Short exposure of 2 dpf embryos at 35-39 °C caused inhibition of the touch-evoked escape response in a reproducible fraction of TICK-injected embryos. Inhibition of escape response was reversible, after embryos were returned to lower T, and was not elicited by the constitutively inactive form of TICK. Moreover, transgenic expression of TICK in neurons modified Ca2+ spikes in zebrafish larvae upon thermal stimulus. These data show that thermal-induction of the engineered K+ channels specifically affects neuronal circuits in vivo and paves the way for further studies in mammals.

SUMEET PAL SINGH : CellCousin: A Novel Ablation Tool Reveals Transdifferentiation as a Key Mechanism of Liver Regeneration During Growth Spurts

Sema Elif Eski, Jiarui Mi, Macarena Pozo-Morales, Gabriel Garnik Hovhannisyan, Camille Perazzolo, Rita Manco, Imane Ez-Zammoury, Dev Barbhaya, Anne Lefort, Frédérick Libert, Federico Marini, Esteban N. Gurzov, Olov Andersson, Sumeet Pal Singh

The liver’s extraordinary regenerative capacity relies on diverse cellular mechanisms to restore tissue integrity. Current paradigms emphasize hepatocyte self-duplication as the primary mode of regeneration following minor injuries in adults. However, our recent findings challenge this view, revealing that during growth spurts, the rapid demands of organ expansion necessitate an alternative strategy: cholangiocyte-to-hepatocyte transdifferentiation. While this secondary mechanism of liver regeneration is typically associated with chronic liver injury, our work demonstrates that it is not confined to pathological contexts. Instead, it emerges as a vital and dominant process during developmental growth. Despite its critical role, the molecular drivers and physiological triggers enabling this plasticity remain poorly understood, presenting an untapped opportunity to advance our understanding of organ regeneration and its therapeutic applications.
To address these knowledge gaps, we developed CellCousin, a novel transgenic tool that enables partial ablation of specific cell populations and distinguishes between spared and newly generated cells in zebrafish. By using this tool, we investigated how hepatocytes derived from self-duplication differ from those generated through transdifferentiation during growth-associated liver regeneration. Following partial ablation and partial hepatectomy (PHx) in zebrafish larvae, we observed that hepatocyte recovery is mediated both by spared hepatocyte self-duplication and ductal-to-hepatocyte transdifferentiation. Surprisingly, this transdifferentiation occurred even in the presence of healthy, proliferating hepatocytes, underscoring its critical role in developmental growth.
Single-cell RNA sequencing at various regeneration stages revealed distinct molecular signatures between de novo hepatocytes and spared hepatocytes. Newly formed hepatocytes exhibited residual ductal traits, lower functional capacity, and a higher cell-cycle signature at early time points, suggesting a division of labor: spared hepatocytes maintain liver function, while transdifferentiated cells drive tissue restoration. After two weeks, ductal traits partially resolved, raising questions about the long-term fate and functional integration of transdifferentiated hepatocytes.
Our study further identified nutrition-induced mTOR signaling as a key regulator of ductal-to-hepatocyte transdifferentiation. Pharmacological inhibition of mTOR pathway in the uninjured liver suppressed transdifferentiation post-injury, linking metabolic and growth-related cues to regenerative outcomes. These findings highlight that transdifferentiation is not merely a compensatory response to injury but a fundamental mechanism aligned with the physiological demands of growth.
By integrating insights from molecular signaling, lineage tracing, and regenerative biology, this study underscores the utility of CellCousin in uncovering cellular and molecular mechanisms of regeneration. It establishes cholangiocyte-to-hepatocyte transdifferentiation as a pivotal strategy during growth spurts, paving the way for therapeutic applications in liver diseases and regenerative medicine.

OR SHAHAR : Cell-type-specific protein dynamics during seizures

Or Shahar

Epilepsy is a common neurological disorder with a high rate of pharmacological resistance. It can lead to psychiatric disorders, cognitive deficits, and increased mortality from the direct or indirect effects of seizures. Although little is known about the molecular mechanisms regulating the development and severity of seizures and epilepsy, it has been shown that protein synthesis plays a role. For example, alterations in protein translation mediated by the eukaryotic elongation factor 2 kinase (eEF2K) can affect the balance of excitatory and inhibitory synaptic transmission, leading to changes in seizure susceptibility and intensity. Additionally, seizures have been shown to disrupt protein synthesis, thereby impacting brain metabolism. The specific proteins and cell types that mediate these processes remain unknown. To study the complex interplay between protein synthesis and epileptic seizures we apply a technique for cell-type-specific labeling of nascent proteins during and following induced seizures in the zebrafish animal model. The brain's immense complexity has so far interfered with our ability to study its protein dynamics in an unbiased manner. Various cell types and long neuronal processes are tightly entangled within the tissue, and neurons with their long axons and dendrites cannot be surgically dissected to allow cell-type-specific proteomic analysis. To overcome this challenge, we developed employ an approach, based on non-canonical amino acid tagging and click chemistry, to label nascent proteins in a cell-type-specific manner in zebrafish larvae. Zebrafish larvae exhibit seizures following various chemical and genetic manipulations. I will present our ability to induce seizures in zebrafish larvae, combined with the technique for cell-type-specific labeling of nascent proteins. This approach provides an ideal framework to identify the proteins synthesized in neurons and glia, measure global and local levels of nascent protein during seizures, and detect potential proteomic changes that occur at later stages following seizures.

GIACOMO MISEROCCHI : 3D culture transplantation in zebrafish embryos: an innovative approach to study in vivo tumor dynamics

Michele Zanoni, Michela Cortesi, Chiara Liverani, Alessandro De Vita, Chiara Spadazzi, Claudia Cocchi, Silvia Vanni, Chiara Calabrese, Sofia Gabellone, Sofia Dellavalle, Paola Ulivi, Marco Schiavone and Giacomo Miserocchi

Introduction : Cancer is a heterogeneous and dynamic disease in continuous crosstalk with surrounding and distant tissues. The reproduction of these complex interactions is still utopic for the currently available in vitro systems. Therefore, in vivo models represent the most realistic approach for the study of cancer processes. However, the tumor masses developed in animals remain difficult to control and characterized. In this context, zebrafish xenograft is an excellent option except for the lack of reproducibility of single cell suspensions injected, which does not reproduce spatio-temporal distribution, and the conservation of cellular and acellular compositions of cancer tissues. On the other hand, the injection of digested ex vivo samples does not guarantee the recovery of whole cell populations while transplantation of undigested tissue fragments represents a low reproducibility approach. A strategy to overcome part of these methodologic limits is the integration of different validated and characterized models.
Hence, our proposal is to perform the transplantation of 3D cultures in zebrafish embryos in order to combine biomimetic systems and high-throughput in vivo models to better understand the biology of cancer diseases.
Methods : We developed spheroids using the MCF7 breast ductal carcinoma cell line only or in combination with 3T3 fibroblast cells. Cell lines were stained with different non-toxic fluorescent dyes and cultured for 2 days on low attachment 384 well plates before obtaining implantable spheroids. 3D cultures were transplanted into the yolk sack of 48 hpf Tg(Fli1:EGFP) zebrafish embryos. The yolk membrane was damaged with the tip of a glass capillary and spheroids were forced to get into the wound using ultra fine forceps. The images acquired with confocal microscopy were used to obtain 3D pictures of spheroids and embryos vasculature.
Results : Several cell concentrations were used to develop spheroids of different sizes. Spheroids originated from 50 cells showed a dimension of 150 µm, a relative size of 1/3 to ½ the yolk sack size. The transplantation process resulted supportable by larvae that do not display abnormal alterations during embryogenesis. At 24 hpi, vascular abnormal sprouts were detected along the spheroids' area, demonstrating the capability of the 3D culture to induce neoangiogenetic processes. This technique allows also the recovery of cancer cells through the damage of the yolk membrane.
Conclusions :Here, we obtained and described an innovative model that integrate two well-characterized in vitro and in vivo systems. Indeed, 3D cultures transplanted into zebrafish embryos can be used for the development of high- throughput platforms to improve the data translational value obtained through common xenograft techniques. In conclusion, this method provides a tool to better understand the pathophysiological tumor processes in order to accelerate information's transfer from bench side to clinical practice.

ELIM HONG : Cholinergic signaling promotes functional organization in the habenula of zebrafish larvae

Soumaiya Imarraine, Erika Bullier-Marchandin, Hervé Le Corronc, Margherita Zaupa, Jean-Pierre Coutanceau, Alice Rousseau, Daniele Avitabile, Delphine Salort, Benoit Perthame, Jean-Marie Mangin, Raphael Candelier, Elim Hong

The habenula is a major cholinergic region in the brain implicated in mediating aversive behaviors. However, the role of its intrinsic cholinergic signaling in neural circuit activity remains poorly understood. Here, we investigate whether cholinergic signaling plays a role in the functional organization of habenular neurons during spontaneous activity and in response to external stimuli. Volumetric calcium imaging of spontaneous neuronal activity reveals that the habenula is organized into spatially segregated, tightly organized neuronal clusters. Distinct neuronal clusters located adjacent to one another display anti-correlated activity, suggesting the presence of lateral inhibition between the clusters. External aversive stimuli induce similar spatial organization of the neuronal clusters with increased anti-correlated activity. Genetic perturbation of cholinergic signaling in vachtb mutants reveals preferential attenuation of the inhibitory response during both spontaneous activity and upon aversive stimuli, resulting in reduced distinction between the spatially-defined functional clusters. Acetylcholine perfusion in brain explants is sufficient to induce anti-correlated activity, suggesting a direct effect of acetylcholine in the functional organization of neuronal clusters. Finally, we provide a minimal mathematical model of acetylcholine-mediated anti-correlated activity via lateral inhibition between two neuronal clusters. Our study highlights the pivotal role of cholinergic signaling in orchestrating the functional patterning of the habenular neural circuitry.

THOMAS FRANK : A Brain-Wide Map of Neuronal Dynamics in Chemosensory Processing and Behavior

Bethan Jenkins, Thomas Offner, Thomas Frank

The olfactory system is essential for a wide range of behaviors, yet how the brain processes odor information to drive and modulate behavior remains poorly understood. Odor information is transmitted from the olfactory bulb (OB) to distributed, parallel circuits in the higher olfactory system, which route it to decision-making and motor coordination centers via largely uncharacterized pathways. Using larval zebrafish, a model with unique advantages such as optical transparency and advanced genetic tools, we investigated brain-wide neuronal dynamics and behaviors in response to appetitive and aversive chemosensory stimuli. Our findings reveal reliable stimulus-evoked behaviors despite inter-individual variability, conserved in partially immobilized fish. Brain-wide responses were strongest in the forebrain but extended to mid- and hindbrain areas. Within the higher olfactory system, distinct target areas exhibited specialized responses, with the posterior tuberculum showing the most pronounced transformations of OB inputs. Notably, valence-encoding neurons were concentrated in the forebrain, forming networks potentially independent of behavioral execution. Neurons correlated with behavioral output were predominantly located in the mid- and hindbrain. This study provides a comprehensive map of neuronal activity relate to the processing of behaviorally relevant odors, providing critical insights into distributed processing strategies in the brain.

ARMIN BAHL : Behavioral algorithms of ontogenetic switching in larval and juvenile zebrafish brightness preferences

Max Capelle & Armin Bahl

Animals show major behavioral changes throughout their ontogenetic development. However, the cognitive computations and neural mechanisms controlling this process remain elusive. Here, we use a combination of multiple complementary phototaxis assays and high-throughput behavioral tracking to explore how young zebrafish adjust their brightness preferences while growing from larval to juvenile stage. We observed that larvae are attracted to luminance but repelled by changes in luminance, whereas juveniles are becoming attracted to darkness but remain repelled by luminance changes. Using the observed swim event statistics, we build a library of generative agent-based models, with unique parameter sets for each fish. We validate these models by their predictive power of animal behavior in more complex visual environments. The behavior of both larvae and juveniles can be captured best by a superposition of two competing elements: one element senses the current global luminance level, while the other processes information regarding eye-specific luminance change. We think that the implementation of phototaxis through a competitive arrangement of these two processing streams allows animals to flexibly adapt their behavior in dynamic visual environments, based on their internal state, and their changing behavioral goals during development. Our rigorous model-based dissection approach is a novel way to identify the algorithmic and cognitive changes during ontogeny. To explore the mechanistic implementations of the adjustments in the brain, we will now leverage the rich molecular genetic toolkit and whole-brain single-cell-resolution imaging techniques available for zebrafish, at both stages of development.

GEORGES DEBREGEAS : Stabilizing swimming speed in the presence of sensorimotor delays

Leonardo Demarchi, Monica Coraggioso, Volker Bormuth, Georges Debrégeas

To maintain their course, flying and swimming animals rely on visual cues to adjust their speed and compensate for external perturbations such as winds or currents. This process requires dynamically adapting the visuomotor gain according to the distance to the visual scene. It has been proposed that animals achieve this feat by minimizing prediction errors, i.e. by learning the relationship between their movements and the sensory feedback.
We investigated this hypothesis by analyzing the visuomotor behavior of Danionella cerebrum (DC) larvae swimming fictively against external currents. This miniature freshwater fish, closely related Zebrafish, retains a small and quasi-transparent brain throughout its life, making it uniquely suited for brain-wide functional imaging at all developmental stages. We observed that DC's countercurrent swimming exhibited persistent speed oscillations at approximately 1 Hz. By systematically manipulating the visuomotor feedback, we demonstrated that these oscillations reflect delays in the sensorimotor loop and can arise either from internal noise or as limit cycles, depending on the system's parameter regime.
Remarkably, we found that the fish can adapt its response without requiring prior learning of the visuomotor gain. This adaptability is a direct consequence of the logarithmic nonlinearities of the sensory (Weber-Fechner law) and motor (Henneman's size principle) systems. These nonlinear transformations, conserved across species and sensory modalities, have been associated with efficient coding. Our findings suggest an additional advantage: tthey contribute to ensuring stability in the presence of sensorimotor delays and incomplete sensory information.
Additionally, we performed calcium imaging during these virtual reality experiments and identified neural populations across the brain whose activities correlate with specific aspects of both behavior and visual stimulation.

ETHAN SCOTT : Brain-wide circuitry underlying altered auditory habituation in zebrafish models of autism

Sarah Josephine Stednitz , Andrew Lesak , Adeline L Fecker, Peregrine Painter , Philip E Washbourne , Luca Mazzucato, Ethan K Scott

Auditory processing is widely understood to occur differently in autism, though the precise network-level changes underlying these differences are not well understood. The diversity of autism also means there may be multiple ways the network can change to produce a similar behavioural output. We used larval zebrafish to investigate auditory habituation in four genetic models of autism: fmr1, mecp2, scn1lab and cntnap2. In free-swimming behavioural tests, we found all four autism model lines had different habituation characteristics from their wild-type siblings. We then used light-sheet microscopy and GCaMP to reveal activity across the brain at cellular resolution as habituation occurred. Using correlations to auditory stimulus timings and fish movements, we identified auditory and motor responses throughout the brain. While we did not see any pronounced differences in responses in primary auditory areas, differences were apparent in brain regions associated with sensory integration and sensorimotor gating. The scn1lab mutants had the strongest behavioural phenotype, and had uniquely drastic reductions in habenular activity. We found an overlapping phenotype of anticorrelation to motor activity in the granule cells of the cerebellum in scn1lab and mecp2 fish, indicating some shared network dynamics underlying their behavioural phenotypes. We also found a bilateral nucleus in the hindbrain which responded very strongly in the beginning of the habituation period in fmr1 and cntnap2 mutants. These results indicate distinct but overlapping circuit changes underlying the different habituation phenotypes in the four different genetic lines. These results, encompassing just a few of the hundreds of genes associated with autism, illustrate the complex relationships that exist among genetics, sensory processing, and behaviour.

TAKESHI YOSHIMATSU : Visual comptetion between the central and peripheral visions

Takeshi Yoshimatsu

Retinal circuits transform the pixel representation of a visual scene, captured by an array of photoreceptors, into the diverse visual feature representations in retinal ganglion cells. Circuit mechanisms underlying the extraction of visual features have been extensively studied. Furthermore, genetic ablations of specific retinal ganglion cell populations have shown that each visual feature signal could be directly linked to specific behaviors. However, how visual information about different visual features is combined or competes for behavioral outcomes is less understood. Here, this study focuses on two competing visual behaviors in larval zebrafish: eye fixation and optokinetic response (OKR), and investigates how visual signal processing in the early visual system influences the selection of behavioral outcomes.

Eye fixation and OKR are mediated by different retinal regions. Many animals, including humans and zebrafish, fixate a high-acuity area (the fovea in humans) on objects that require current attention. This HAA vision or the central vision enables animals to see the details of the objects. In contrast, OKR is triggered by wide-field vision, primarily captured by the retinal periphery. I first examined the behaviors of larval zebrafish when presented with visual stimuli that induce eye fixation and OKR, either separately or together. Stimuli conditions were chosen based on previous research. Specifically, prey-like stimuli (a small moving dot in ultraviolet) were presented in the HAA visual field to induce eye fixation, while OKR was induced by moving red gratings presented in the peripheral visual field. These experiments revealed that larval zebrafish can suppress OKR during eye fixation: the performance of eye fixation (the rate of eye fixation per prey-like stimulus presentation) was unchanged with and without OKR-inducing stimuli. Next, to gain insights into the neural mechanisms underlying the suppression of OKR during eye fixation, I used volumetric two-photon imaging to record neural activity in the brain and the retina under prey-like and/or moving grating stimuli. By examining the population size of activated neurons from the outer to the inner retina and to the retinal recipient areas in the brain, these experiments revealed how visual signals evoked by pre-like stimuli, which would activate only a handful of cone photoreceptors, transform into robust signals in order to suppress OKR.

In humans, eye fixation and the suppression of OKR are mainly controlled by visual attention. This research uncovered that larval zebrafish are also capable of performing attentive eye fixation. With their optical accessibility, this study highlights larval zebrafish as a powerful system for investigating how various visual feature signals collectively influence visual behaviors.

VOLKER BORMUTH : Biomechanics and Neural Substrates Underlying Dual Postural Control Strategies in Larval Zebrafish

Sharbatanu Chatterjee*, Natalia Beiza-Canelo*, Muntasir Callachand, Georges Debrégeas, Volker Bormuth+

Vertebrate postural control relies on the vestibular system but is frequently impaired in neurological disorders. Larval zebrafish, with their streamlined body plan and transparency, offer a uniquely accessible model for studying the biomechanical and neural foundations of this process. We focused on two behavioral strategies under roll-axis vestibular stimulation: a continuous vestibular bending reflex (VBR) and discrete swim-like tail movements.
Kinematic analyses of the VBR revealed transient and tonic response components modulated by stimulus amplitude, response saturation at 15° beyond a threshold roll angle of 20°, and a ~200 ms response delay. A viscoelastic model successfully captured these features and explained frequency-dependent responses. When the VBR response saturated, fish initiated discret bouts, initiated by a prolonged swim bout—lasting up to ten times longer than normal bouts—followed by shorter bouts with inter-bout intervals as brief as 50 ms at 90° roll observed. Additionally, asymmetric bouts were strongly biased contralaterally to the stimulus direction with increasing postural destabilization.
Brain-wide imaging revealed that over 70% of hindbrain V2a neurons were activated during the VBR, highlighting a prominent vestibulospinal pathway underscoring the critical role of V2a neurons in postural control. By integrating behavioral, kinematic, and neural activity measurements, our results provide a framework for quantifying postural control deficits in zebrafish models of neurological disorders.

JUSTIN KENNEY : Neural basis for individual differences in fear memory recall in adult zebrafish

Barbara D. Fontana, Neha Rajput, Jacob Hudock, Dea Kanani, and Justin W. Kenney

Fear is a fundamental emotional state that is highly conserved across the animal kingdom. Despite the intrinsic importance of fear for survival, its behavioral manifestation varies between individuals where the choice of response can be the difference between life and death. However, we know little about the biological basis for these individual differences in fear behavior. Zebrafish were trained to associate a new environment with fear by exposing them to conspecific alarm substance (CAS), an ethologically relevant chemical stimulus released from the epithelial cells of injured fish to alert nearby animals to danger. After tracking with DeepLabCut, we trained a random forest machine learning model to identify different behaviors (e.g., freezing, bursting, and erratic movements) to greater than 95% accuracy. We collected data from over 400 animals from four different inbred strains (AB, TU, TL, and WIK) and both sexes. We used an unsupervised machine learning approach to identify four distinct behavioral clusters: (1) low fear responsivity, (2) erratic behavior, (3) high freezing interspersed with erratic behavior, and (4) high freezing interspersed with normal swimming. We found that both background strain and sex had an influence on the type of fear behavior exhibited. Finally, we performed whole-brain activity mapping to identify the neural basis individual differences in fear behavior. To do this, we used a combination of in situ hybridization chain reaction for c-fos, tissue clearing, light-sheet microscopy, and image registration to the adult zebrafish brain atlas. We used partial least squares to identify brain regions that covary with freezing and evasive behaviors. We found that freezing strongly engages the cerebellum and parts of the dorsal and ventral telencephalon whereas evasive behavior is characterized by elevated activity in the nucleus isthmi.

STEFANIA NICOLI : Elevated Mitochondrial Activity during Embryogenesis Increases Adult Cerebrovascular Anomalies

Ivan Fan Xia1,2, Anupama Hemalatha1,2,3, Jared Hintzen1,2, Paola Carneiro1,2, Gabriel Baldissera1,2, Siyuan Cheng1,2, Nicole J. Lake1,2, Valentina Greco1,2,3 and Stefania Nicoli1,2.

Cerebrovascular diseases are leading causes of death worldwide. A network of arteries called the Circle of Willis (CoW) ensures blood supply to the brain. About 50% of the adult population has incomplete CoW, increasing the risk for cerebrovascular diseases. Genetic and environmental factors influence CoW variations in adults, but it is unclear whether incomplete CoW is an inherited developmental anomaly. Understanding this could reveal how embryos are programmed for lifelong brain health. We found that zebrafish lacking microRNA-miR-125a show common incomplete CoW variations, such as narrowed or absent posterior arteries. This heritable condition results from hyper-angiogenesis of endothelial cells forming posterior CoW vessels during embryonic development. Mechanistically, miR-125a inhibits the mitochondrial biogenesis regulator ppargc1a/PGC-1a. Inhibiting ppargc1a or modulating mitochondrial activity in miR-125a mutants restored complete CoW formation in adults and reduced stroke risk during hypertension. Thus, mitochondrial activity during embryogenesis determines CoW anatomy and lifelong cerebrovascular health.

FELIX GUNAWAN : The basement membrane regulates morphogenesis and biomechanical features of the cardiac valves

Newsha Mortazavi, Saskia Baum, Dagmar Zeuschner, Karina Mildner, Sara Wickstrom, Felix Gunawan

Patterning of the heart require tightly orchestrated movements and differentiation of cardiac cells, which occur under strong biomechanical pressure from heartbeat and blood flow. Cardiac valves, complex structures that prevent retrograde blood flow, arise from specialized endocardial cells experiencing the highest levels of flow. The endocardial cells migrate, proliferate and differentiate into fibroblast-like interstitial cells. The cardiac extracellular matrix (ECM) provides physical space for endocardial cell migration and differentiation, but functional analyses of different ECM compartments during this process have not been performed. One major ECM compartment, the basement membrane, lines endothelial cells to establish barriers and a signaling platform for growth and differentiation. However, the precise cellular and molecular processes regulated by the basement membrane during cardiac morphogenesis are not fully understood.
Here, our work uncovers requirements for the basement membrane during cardiac valve morphogenesis. We performed single-cell RNA sequencing from purified endocardial cells and found strong enrichment of genes encoding basement membrane proteins, including core basement membrane constituents Laminins. Imaging of a Laminin reporter line and anti-laminin immunostained hearts show high deposition of the cardiac basement membrane around valve endocardial cells. Through analysis of several ECM mutants, we uncovered an indispensable role of laminin alpha5 (lama5) in promoting cardiac valve formation. Interestingly, although overall cardiac volumes and shapes are mostly unaltered in lama5 mutants, loss of lama5 leads to significant enlargement of the cardiac valve tissue and extracellular space. Total cell numbers and rate of cell proliferation in lama5 mutant valves significantly increased, but the endocardial cells fail to fully differentiate into valve interstitial cells, indicating that the basement membrane restricts valve overgrowth and promotes endothelial-to-fibroblastic differentiation. Focal adhesion activation was significantly reduced in the valve interstitial cells of lama5 mutants. Using Brillouin microscopy and immunostaining against cellular tension markers, we found reduced biomechanical tension in the cardiac valves of lama5 mutants. These defects lead to failure of valves to close the cardiac lumen and significantly higher rates of retrograde blood flow in lama5 mutant hearts. Finally, although Lama5-containing basement membrane regulates valve morphogenesis, slowing down cardiac contractions led to an ectopic deposition of the basement membrane throughout the heart.
Our investigation uncovers the function of the basement membrane in promoting endothelial-to-fibroblast cell differentiation and mechanical rigidity of cardiac valves, while also restricting valve tissue growth. Our results indicate that crosstalk between heartbeat-induced mechanical forces and the cardiac ECM keep the basement membrane at its endogenous level. Ultimately, our work provides deeper understanding into how the extracellular environment and external biomechanical forces synergistically inform the patterning of the contractile heart, with implications for how complex tissues are patterned under physical pressure.

RASHMI PRIYA : Break to Build: ECM Fractures Pattern the Developing Myocardium

Organogenesis is a remarkably robust process, as it is critical for organismal growth and life. Yet, our understanding of how developing embryos reproducibly build organs with the right shape, size, and function remains limited. As the zebrafish embryo grows, to sustain its increasing physiological demands, the embryonic myocardial wall of the heart transforms into an intricate 3D architecture, composed of an outer compact layer enveloping an inner layer of multicellular trabecular ridges. How these tissue layers acquire their characteristic form suited for their function remains an open question. Combining 4D live imaging, controlled perturbations, morphometrics and theoretical modelling, we now reveal that a multiscale coupling between tissue geometry, mechanics and organ function builds a functional beating heart. Trabecular cells are seeded through stochastic single-cell delamination based on differences in local actomyosin tension (PMID: 33208950). Notably, this delamination is spatially constrained in the outer curvature of the heart by mechanical fracturing of the underlying extracellular matrix (ECM). Further, single-celled trabecular seeds recruit outer compact layer cells to mature into clonally heterogeneous multicellular ridges, thereby amplifying cardiac contractile forces. In response, the remaining compact layer cells are stretched, which impedes their further recruitment, thereby constraining trabecular ridge density. Concomitantly, Notch-dependent actomyosin dampening triggers a sharp transition in the myocardial tissue area, activating rapid organ growth that expands blood-filling capacity (bioRxiv 2024.07.24.604962). In this talk, I will be discussing some of these recent findings. The long-term goal of my lab is to reveal design rules underlying the emergence of robust functional organs during embryogenesis.

WIEBKE HERZOG : Mechanisms of Wnt and S1P Signaling Pathway Interactions during Brain Angiogenesis

Romana Scheffel, Hannes Drexler, Wiebke Herzog

The brain is a heavily protected site with the brain vasculature contributing to formation of the blood-brain barrier. Therefore brain vessels have specialised properties which also affect their angiogenesis. While multiple signaling pathways are involved in generating and maintaining the BBB, we could show that Wnt and Sphingosine 1-phosphate (S1p) signaling functionally oppose each other by direct interaction during BBB development (Hübner et al 2018). Yet the downstream mechanisms of how these pathways fine tune barrier tightness remain elusive. We found that interrupting Wnt signaling results in pre-mature S1p receptor 1 (S1pr1) and downstream of it Rac1 activation and in turn severe defects in vascular development. However, we are focusing on how the interaction is mediated and which downstream components will balance it.
We are analysing these interactions using zebrafish embryos as well as human cerebral microvascular cells (hCMEC/d3). Isolating zebrafish brain capillaries, we analysed transcriptome changes downstream of Wnt signaling, while using the hCMEC cell line we analysed phospho-proteomic changes downstream of S1p signaling and identified a large set of differentially regulated genes or differentially phosphorylated proteins. Of these, we have characterised the role of various GAP proteins involved in brain angiogenesis and specifically in the regulation of Rac1 activity, downstream of either Wnt signaling or S1p signaling.

DEBORAH YELON : Regionalized regulation of actomyosin organization influences cardiomyocyte cell shape changes during chamber curvature formation

Dena M. Leerberg, Gabriel Avillion, Rashmi Priya, Didier Y.R. Stainier, and Deborah Yelon

Cardiac chambers emerge from a heart tube that balloons and bends to create expanded ventricular and atrial structures, each containing a convex outer curvature (OC) and a recessed inner curvature (IC). The distinct identities that the curvatures subsequently attain play a crucial role in cardiac function; however, a comprehensive understanding of the cellular and molecular mechanisms underlying curvature formation remains lacking. Here, we demonstrate in zebrafish that the initially similar populations of OC and IC ventricular cardiomyocytes diverge in the organization of their actomyosin cytoskeleton. This divergence is followed by the acquisition of distinct OC and IC cell morphologies. Altering actomyosin dynamics hinders these cell shape changes, especially in the OC, and mosaic analyses indicate that actomyosin regulates cardiomyocyte shape in a cell-autonomous manner. Additionally, both blood flow and the transcription factor Tbx5a are influential in establishing the basal enrichment of actomyosin and squamous cell morphologies in the OC. Together, our findings suggest that intrinsic and extrinsic factors intersect to control actomyosin organization in OC cardiomyocytes, which in turn promotes the cell shape changes that drive curvature morphogenesis.

NAOKI MOCHIZUKI : Cadherin-6-dependent zippering of endothelial cells during the connection of endocardial and venous sheets

Moe Fukumoto and Naoki Mochizuki

The connection between the heart and great vessels established during embryogenesis is essential for circulation. However, it remains unclear how great venous endothelial cells (ECs) adhere to ECs of endocardium. The endocardial sheet (End sheet) outside of the beating heart is connected to the EC sheets of bilateral common cardinal vein (CCV sheets). Here, using zebrafish, we demonstrate that the End sheet and CCV sheets are sealed in a zipper-closing manner outside the heart to form an inflow tract. The gradual elongation of the endocardium driven by convergent extension during embryogenesis induced the zipper-like closure by pulling CCV sheet along the anterior-posterior axis. Moreover, manipulation of heart rate show that heartbeat affected the endocardial elongation-dependent force. We developed Cadherin-6 tagged with EGFP knock-in fish and visualized endogenous Cadherin-6 localization between the End sheet and CCV sheets. By time-lapse imaging of Cadherin-6-dependent adherens junctions that might counterbalance mechanical forces, we found a specific contribution of Cadherin-6 instead of Cadherin-5 to sensing endocardium-specific mechanical force. This specificity was confirmed by the depletion of Cadherin-6. Collectively, we propose Cadherin-6 mediated EC-zippering as a novel mechanism that updates the understanding of Cadherin usage in dynamic morphogenesis.

KASKA KOLTOWSKA : Deciphering the chromatin landscape and regulatory logic behind lymphatic endothelial cell fate using a multi-omics approach.

Virginia Panara*, Hannah Arnold*, Marleen Gloger*, Renae Skoczylas, Victoria Vidal Gutierrez, Anna Johansson, Agata Smialowska, Katarzyna Koltowska

Switching genes on and off is a fundamental part of development, ensuring that a unique molecular code is set up to orchestrate tissue morphogenesis. Changes in chromatin organisation dictate accessibility to gene regulatory elements and control gene expression. Several molecular factors regulating lymphatic endothelial cell (LEC) specification and differentiation have been identified. However, how chromatin organisation differs between lymphatic and blood endothelium and how these differences are reflected in gene expression remains to be determined.
In this study, we combined ATAC-sequencing and Hi-C to characterise chromatin accessibility and 3D architecture in LECs and blood endothelial cells (BECs). We uncovered cell type-specific chromatin organisation at a global level, revealing predominant interactions outside of promoter regions in LEC and with promoters in BECs. By combining ATAC-sequencing and Hi-C with single-cell RNA-sequencing we defined the regulatory logic for nine genes whose expression is enriched in LECs. By transgenic approach we validated ten candidate sequences and confirmed that short- and long-range enhancers can drive expression confined to LECs. In addition, we identified their potential to topographically restrict gene expression. We focused on the known lymphatic regulator mafba and reconstructed its tissue-specific regulatory networks and identified a genetic interaction with tfe3a in vivo necessary to limit ectopic vessel formation. Overall, our work provides a powerful resource of multi-omic data sets, that can be used to systematically determine the regulatory networks governing LEC identity and genes linked to lymphatic disease.

SNEZANA KOJIC : The ankrd1a participates in the regulation of muscle cell differentiation during adult zebrafish skeletal muscle repair zebrafish

Snezana Kojic, Mirjana Novkovic, Jovana Jasnic, Andjela Milicevic, Emilija Milosevic, Srdjan Boskovic

Zebrafish repair skeletal muscle injury through an evolutionary conserved multi-step process that involves activation of satellite-like cells, differentiation of progenitor cells into myocytes, and their fusion into myotubes, followed by myotube maturation and myofiber hypertrophy. Coordination and timely regulation of these events are essential for functional muscle recovery. Here we identify ankrd1a, a gene responsive to muscle stress, as a new player in the repair of adult zebrafish skeletal muscle and show its involvement in modulating molecular mechanisms of myogenic cell differentiation. To assess the function of ankrd1a in muscle healing we used the ankrd1a loss of the function mutant line generated by CRISPR/Cas9 genome editing. It exhibited 4 bp deletion in exon 4, leading to the formation of a premature stop codon. The predicted protein product lacks ankyrin repeats, which are essential for its function. We showed that loss of ankrd1a function affected muscle repair in adult zebrafish. Although both ankrd1a mutant and wt were actively repairing the injury, the wounded area in mutant appeared smaller than the injured area in wt at 7 dpi. Moreover, the maturation of newly forming muscle fibers in the ankrd1a mutant was delayed according to the expression of maturation marker myoz3a. In injured wt zebrafish, the myoz3a expression profile resembled the one seen during the mouse muscle repair. In the mutant, myoz3a was not responding to the injury in the same way, suggesting that ankrd1a regulates the expression of at least this maturation marker. Next, by transcriptome profiling, we identified potential targets of ankrd1a involved in skeletal muscle repair. RNA-seq was performed on samples collected at 5 dpi, in the stage of myogenic differentiation marked by upregulation of myogenin expression in both injured ankrd1a mutant and wt muscle to a similar level. Loss of ankrd1a function caused changes in the expression of genes related to muscle contraction, muscle cell differentiation, myocyte fusion, including actin cytoskeleton organization and cell adhesion, and MAPK signaling pathway. All this suggested accelerated myogenic differentiation and faster muscle injury repair in ankrd1a mutant compared to wt. This hypothesis was tested by comparing birefringence intensity in the larvae's injured (and adjacent uninjured) somites. On the fourth day post-injury, mutant larvae recovered more injured muscle than wt larvae. Taken together, our results imply that in injured skeletal muscle ankrd1a protects repairing muscle tissue from premature differentiation of newly forming myofibers, which may affect functional muscle recovery.

CANCER 9:00 AM -10:00 AM

YI FENG : Oncogenic Ras activation in permissive somatic cells triggers rapid onset phenotypic plasticity and elicits a tumour-promoting neutrophil response

Abigail M. Elliot, Isabel Ribeiro Bravo, Jeanette Astorga Johansson, Esme Hutton, Richard Cunningham, Henna Myllymäki, Kai Yee Chang, Justyna Cholewa-Waclaw, Yiyi Zhao, Mariana Beltran, Ross Dobie, Amy Lewis, Philip M. Elks, Carsten Gram Hansen, Neil Henderson, Yi Feng

Oncogenic driver mutations are frequently found in normal tissues, indicating that additional non-genetic factors are necessary for tumourigenesis. Phenotypic plasticity is a crucial gateway to malignancy, and inflammation can fuel tumourigenesis; however, little is known about the timing and mechanisms by which these hallmarks first emerge. Using single-cell transcriptomics and in vivo live imaging, we characterised the immediate cell-intrinsic and innate immune responses during the first 24 hours following oncogenic Ras activation in an inducible zebrafish model of HRASG12V-mediated skin tumour initiation. We found that only a subset of basal keratinocytes, but not superficial keratinocytes, are susceptible to RAS-driven phenotypic plasticity. These preneoplastic cells undergo dedifferentiation and partial EMT, resembling malignant cells observed in human squamous cell carcinoma (SCC). Strikingly, the same subset of cells initiates the development of tumour-promoting neutrophils, which in turn enhance preneoplastic cell proliferation. Our findings demonstrate that the effects of oncogenic Ras are primarily determined by the cell of origin. We reveal a link between the unlocking of phenotypic plasticity and the onset of tumour-promoting inflammation.

MARIANNE VOZ : Unraveling the Distinct Pathways of Pancreatic Cancer Progression from Acinar and Ductal Cells through Single-Cell RNA Sequencing

Chiara Goossens, Colin Lolos, Maurijn Kessel, Elisa Deom, Marianne Voz

Introduction : Pancreatic ductal adenocarcinoma (PDAC) stands as one of the most lethal cancers, primarily attributed to late detection and ineffective therapeutic approaches. Two main cellular origins, acinar and ductal, have been described but today it is still not possible to classify human PDAC based on their cellular origin. Comprehending the impact of cellular origin on pancreatic tumor formation is vital for gaining a deeper understanding of the disease and potentially developing tailored therapies in the future.
Methods :We created two zebrafish models of PDAC by targeting KrasG12D expression either in acinar or ductal cells. We performed single cell RNAseq (scRNaseq) to profile the transcriptional changes occurring at different stages of tumorigenesis. Subsequently, we compared the transcriptional paths taken by acinar and ductal cells to identify both common and distinctive signatures. We also compared zebrafish transcriptomic profiles with scRNAseq datasets from mammalian models to pinpoint factors that are evolutionarily conserved across distant species, suggesting their probable involvement in the tumorigenesis process.
Results : In acinar cells, the induction of KrasG12D triggers an acino-ductal metaplasia remarkably akin to the murine and human process. Like in mammals, zebrafish metaplastic cells reactivate the pancreatic developmental program by inducing key transcriptional factors such as Sox9, Hnf1b and Onecut1. Our scRNAseq identifies also numerous other pancreatic progenitor factors, expressed sequentially during metaplasia, whose roles merit exploration.
In ductal cells, the induction of KrasG12D results in tumors with distinctive histological characteristics compared to those originating from acinar cells. Ductal-derived tumors also exhibit much greater aggressiveness, with approximately 70% invading the spleen. Moreover, the trajectory followed by these cells is markedly different from that observed in the acinar model. Consequently, these ductal-derived tumors express specific markers such as Agr2, previously identified by Ferreira et al. (2017) as a highly specific marker for murine ductal-derived tumors.
Conclusion: The acinar- and ductal- derived tumors display specific signatures that should enable to distinguish the cell of origin among the human PDAC patients , shedding light on the proportion of acinar versus ductal-derived PDAC in humans—an aspect that remains elusive.

MARINA MIONE : Leveraging Single cell RNA sequencing to elucidate novel pathological mechanisms in tumor models in zebrafish

Vittorio Bontempi, Francesca Lorenzini, Irene Pecchini, Marina Mione

Single cell RNA sequencing (scRNA-Seq) offers a great potential for studying zebrafish tumor models allowing the analysis of both cancer cells and tumor microenvironment (TME) at unmatched resolution. Zebrafish serve as a great model to develop tumor models thanks to the ease in genetic manipulation and rapid growth. We leveraged single cell RNA sequencing to characterize in depth tumor models previously developed in the lab, specifically models of uveal melanoma and glioblastoma (GBM). In uveal melanoma models, we validated a novel pathological mechanism involving the interaction of neutrophils in the TME and cancer cells, responsible for the upregulation of Yap oncogenic signaling. In GBM models, we were able to dissect intratumoral heterogeneity of cancer cells according to markers of different telomere maintenance mechanisms, thus revealing the importance of these mechanisms for stemness, proliferation and differentiation of cancer cells. Finally, scRNA-Seq allowed us to identify different subtypes of immune cells present in different tumors, providing a novel approach to dissect the contributions of the immune system to cancer development or to anti-cancer immunity in zebrafish cancer models.

MARTA POPOVIC : The repair of DNA-protein crosslinks: Creating new disease models and identifying molecular causes underlying human diseases

Ivan Anticevic, Cecile Otten, Marin Kutnjak, Marta Popovic*

DNA-protein crosslinks (DPCs) are the second most common DNA lesions which obstruct replication and transcription and deficient repair of these toxic lesions is associated with the onset of cancer, neurodegeneration and aging. Induction of DPCs is used in medicine to treat many cancers and understanding the repair at the organismal level could provide an impetus for the development of new drugs and combination therapies. In addition, the accumulation of DPCs leads to aging and neurological disorders, so it is important to understand the mechanisms behind the crosslink repair. In recent years, we and other groups have gained mechanistic insights into DPC repair (DPCR) factors. However, the function of repair factors at the organism level is still largely unknown. In my group, we use the zebrafish model and CRISPR-Cas gene editing to study the interplay of DPCR factors and sub-pathways including proteolysis-, nuclease- and tyrosyl-DNA phosphodiesterase-dependent repair at the biochemical and cellular level. I will present our recent discoveries using three new zebrafish strains generated with the CRISPR-Cas system: a catalytic mutant and a C-terminal mutant of the ACRC protease involved in DPCR and a transgenic strain with the inactive DPCR factor, tyrosyl-DNA phosphodiesterase 1 (TDP1). We found that ACRC is an essential protease in vertebrate development, as a catalytic mutation leads to early embryonic lethality and the cause of early embryonic lethality is the accumulation of specific DPCs including histones, topoisomerases and Parp1. We also show that TDP1 is required for the resolution of topoisomerase 1- and histone-DPCs at the organismal level and in human cells, and we characterise a novel TDP1-mediated repair pathway for histone-DPC repair. Furthermore, in combination with gene silencing of other target genes, we recently identified a novel DPC repair pathway (nucleophagy) at the crossroad of autophagy and DNA repair in the nucleus. Our results provide insights into the complex DPCR pathways and their implications for human disease and cancer treatment.

ERIC LIAO : Comprehensive genome wide identification and functional study of epithelial-mesenchymal transition genes that regulate cleft pathogenesis and regeneration

Sogand Shaffer, MD, Shannon Carroll, PhD, Feng Wang, PhD, Yongjun Li, PhD, Caroline Caetano, PhD, Scott Tucker, PhD, Yi Xing PhD and Eric C. Liao, MD, PhD.

Introduction :Plastic surgeons work on epithelial structures. When we use skin grafts to resurface burn injured anatomy or release simple polydactyly – we are surgically modifying epithelial derived tissues and structures. Esrp1/2 are epithelial-specific RNA splicing regulators thar govern epithelial mesenchymal transition (EMT), a central cell biological process observed in carcinogenesis, embryologic morphogenesis and tissue regeneration, as epithelial cells transdifferentiate into mesenchymal cells to mediate these canonical processes. Esrp1/2 mediate these processes by regulating alternative splicing (AS) of mRNA to generate isoforms where titration of specific isoforms confer epithelial vs. mesenchymal phenotypes. Loss of Esrp1/2 results in global alteration of mRNA isoforms and causes orofacial cleft malformation and poor wound healing humans, mouse and zebrafish. Given the central role of Esrp1/2 to regulate EMT, there is an urgent scientific and clinical unmet need to understand its role isoform level resolution and specificity across vertebrate species.
Methods : We applied state-of-art advances in long read sequencing and RNA computation analysis to comprehensively identify RNA isoforms genome wide, between wild-type and Esrp1/2 mouse and zebrafish mutants. Differentially expressed isoforms were prioritized based on biological pathw ay, relative abundance and isoform differences between wildtype and Esrp1/2 genotypes
Results : We found that >60% of isoform switches identified involved complex or combinatorial AS patterns, which are missed by standard short-read RNA-seq. We also identified numerous novel isoforms, that were absent from existing Ensembl annotations, including in genes with well-established important functions in epithelial mesenchymal transition (EMT). We discovered that a key transcription factor tp63 is regulated by esrp. Over-expression of tp63 isoforms was sufficient to rescue esrp1/2 mutant clefts, demonstrating for the first time that isoform specificity of a developmental process. Other key regulators in Wnt signaling and tight junctions such as GSK3b and CTNND1 were dysregulated, with aberrant expression of isoforms of these genes in the periderm.
Conclusions : These results showcase the ability of long-read RNA-seq technology to identify novel isoforms where short-read RNA-seq and conventional methods fall short. We discovered that tp63 isoforms require esrp1/2 function, and that expression of specific tp63 isoforms is sufficient to rescue cleft palate of the esrp1/2 mutant. These results provide the scientific basis of a gene therapy strategy to exploit modulation of exon usage to mitigate clefts before they form during embryonic development. This work also enables us to generate a EMT long-read RNAseq atlas that will inform broad research questions in cancer biology, embryology and tissue regeneration where EMT is the underpinning mechanism.

JAN KASLIN : Hydrodynamic forces promote neural stem cell activity in the regenerating zebrafish spinal cord

Samuel H. Crossman1, Jingyi Wang1, Alon M. Douek1, Jan Kaslin1, 2*

Regenerative vertebrates respond to spinal trauma by activating dormant ependymal radial glial cells (ERGs), which proliferate after injury to replenish damaged tissue. Beyond their role as neural progenitors, ERGs also circulate cerebrospinal fluid (CSF) via specialised flow-generating motile cilia. Here, using zebrafish spinal cord lesion models and high-speed live imaging, we show that injury-associated inflammatory signals trigger increased cilia motility at the injury site. This creates regional alterations in fluid flow that activate mechanically sensitive neurons. Upon activation, these neurons promote progenitor activity by modulating the calcium-regulated histone modifying enzymes in ERGs. Together, these findings uncover a hydrodynamic signalling relay operating in the regenerating spinal cord and highlight the importance of the local mechanical microenvironment in promoting neurogenesis after injury.

<u>DONGHUN SHIN</u> : The role of FGF signaling in liver progenitor cell-mediated liver regeneration

Donghun Shin and Juhoon So

The liver is a highly regenerative organ, but its regenerative capacity is greatly compromised in severely damaged livers. Promoting liver regeneration can be beneficial to patients with severe liver diseases. In search of small molecules that can augment liver regeneration in diseased livers, we have established two zebrafish models for liver progenitor cell (LPC)-mediated liver regeneration. Using these models, we have identified the FGFR inhibitor BGJ398 as such a molecule. Inhibiting FGFR signaling promoted LPC-mediated liver regeneration, specifically the differentiation of LPCs into hepatocytes, in regenerating zebrafish larvae. fgf10a and fgfr2 mutants also exhibited the enhanced LPC-to-hepatocyte differentiation, as observed in BGJ398-treated, regenerating larvae. Consistent with these loss-of-function data, the overactivation of FGFR signaling suppressed LPC-to-hepatocyte differentiation. Mechanistically, Fgf10a-Fgfr2 signaling regulates LPC-to-hepatocyte differentiation via the Mek-Erk-Etv5b axis. Mek Inhibition promoted LPC-to-hepatocyte differentiation, as observed with FGFR inhibition. The Fgf10a-Fgfr2-Mek-Erk axis induces the expression of etv5b in regenerating livers, and Etv5b suppresses LPC-to-hepatocyte differentiation. etv5b mutants exhibited the enhanced LPC-to-hepatocyte differentiation, as observed in fgf10a and fgfr2 mutants. The overexpression of Etv5b in LPCs suppressed their differentiation into hepatocytes while promoting their differentiation into biliary epithelial cells. Collectively, our findings reveal that the Fgf10a-Fgfr2-Mek-Erk-Etv5b axis suppresses LPC-mediated liver regeneration, specifically LPC-to-hepatocyte differentiation, and suggest FGFR2 inhibitors as potential therapeutics that can augment innate liver regeneration in patients with severe liver diseases.

<u>ARICA BEISAW</u> : Investigating mechanisms of cardiomyocyte invasion of injured tissue during cardiac regeneration

Florian Constanty, Bailin Wu, Ke-Hsuan Wei, I-Ting Lin, Julia Dallmann, Stefan Guenther, Till Lautenschlaeger, Rashmi Priya, Shih-Lei Lai, Didier Y.R. Stainier, and Arica Beisaw

Despite numerous advances in our understanding of zebrafish cardiac regeneration, an aspect that remains less studied is how regenerating cardiomyocytes invade, and eventually replace, the collagen-containing fibrotic tissue following injury. Here, we provide an in-depth analysis of the process of cardiomyocyte invasion using live-imaging and histological approaches. We observed close interactions between protruding cardiomyocytes and macrophages at the wound border zone, and macrophage-deficient irf8 mutant zebrafish exhibited defects in extracellular matrix (ECM) remodeling and cardiomyocyte protrusion into the injured area. Using a resident macrophage ablation model, we show that defects in ECM remodeling at the border zone and subsequent cardiomyocyte protrusion can be partly attributed to a population of resident macrophages. Single-cell RNA-sequencing analysis of cells at the wound border revealed a population of cardiomyocytes and macrophages with fibroblast-like gene expression signatures, including the expression of genes encoding ECM structural proteins and ECM-remodeling proteins. The expression of mmp14b, which encodes a membrane-anchored matrix metalloproteinase, was restricted to cells in the border zone, including cardiomyocytes, macrophages, fibroblasts, and endocardial/endothelial cells. Genetic deletion of mmp14b led to a decrease in 1) macrophage recruitment to the border zone, 2) collagen degradation at the border zone, and 3) subsequent cardiomyocyte invasion. Furthermore, cardiomyocyte-specific overexpression of mmp14b was sufficient to enhance cardiomyocyte invasion into the injured tissue and along the apical surface of the wound. Notably, depletion of macrophages in the presence of cardiomyocyte-specific mmp14b overexpression dampened the effects of mmp14b overexpression, suggesting that macrophages are essential for efficient cardiomyocyte invasion. Altogether, our data shed important insights into the process of cardiomyocyte invasion of the collagen-containing injured tissue during cardiac regeneration. We have generated spatial proteomic datasets from the border zone of regenerating zebrafish hearts, and are currently investigating the role of candidate proteins in regulating cardiomyocyte invasion and cell:ECM interactions that stimulate cardiac regeneration.

LIEVE MOONS : Fueling CNS repair: the evolutionary role of local glycolysis in axonal regeneration

Lieve Moons, Anyi Zhang, Steven Bergmans, Karl Farrow, Lies De Groef, Luca Masin

The limited regenerative capacity of the adult mammalian central nervous system (CNS) is a significant barrier to functional recovery following injury. Energy deficits, primarily caused by mitochondrial dysfunction, hinder axonal regrowth. In contrast, the adult zebrafish CNS exhibits a robust capacity for regeneration, driven by efficient metabolic adaptations and gene expression reprogramming. Comparative analyses between these species offer valuable insights into conserved mechanisms underlying axonal regeneration and potential therapeutic strategies for mammalian CNS repair.
Using the optic nerve crush (ONC) model in zebrafish, our prior studies demonstrated a pivotal redistribution of mitochondria from the somatodendritic compartment to the axon during early regeneration1. This was postulated to compensate for local energy deficits caused by mitochondrial depolarization and support successful axonal regrowth. Transcriptomic analyses of fluorescence-activated cell-sorted zebrafish retinal ganglion cells (RGCs) further revealed substantial metabolic reprogramming during regeneration. Early stages were characterized by downregulation of oxidative phosphorylation (OXPHOS) genes and upregulation of genes related to mitochondrial transport, glycolysis, the thioredoxin antioxidant pathway, and the pentose phosphate pathway (PPP). These metabolic shifts appear crucial for neutralizing reactive oxygen species (ROS) and providing energy for axonal repair. Further functional validation using ex vivo explant cultures and in vivo inhibition experiments demonstrated that blocking key enzymes in glycolysis or the thioredoxin pathway significantly impaired regeneration and/or delayed optic tectum reinnervation in zebrafish. These findings establish that both pathways, via ROS neutralization and boosting of energy production, are required to create the correct biochemical milieu for injury-induced axonal regrowth.
To investigate evolutionary conservation, we compared our zebrafish findings with the murine Pten and Socs3 co-deletion (cdKO) model, which shows induced axonal regeneration2. A pseudobulk analysis of transcriptomic data from this model revealed significant overlap in the upregulated pathways between zebrafish and regeneration-competent mice. Notably, axonal regeneration in cdKO neurons was associated with enhanced mitochondrial transport within axons and local upregulation of glycolysis in distal axonal compartments, mirroring the metabolic adaptations observed in zebrafish3.
In summary, this study emphasizes glycolysis, along with mitochondrial transport, as a core- evolutionary conserved mechanism in axonal regeneration. Targeting these metabolic pathways and redirecting intraneuronal metabolism, we may unlock the regenerative potential of the mammalian CNS, paving the way for novel treatments for neurodegenerative diseases and traumatic CNS injuries.

LAURE BALLY-CUIF : Linking individual cell heterogeneities and population dynamics for adult neural stem cell maintenance

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Neural stem cell (NSC) populations ensure the production of neurons and astroglial cells in the vertebrate adult brain. NSC activity persists at long-term in zebrafish, but exhausts rapidly in mouse from the young adult stage. To decrypt NSC lineage progression in the zebrafish adult pallium (dorsal telencephalon), we used long-term genetic clonal tracing and intravital imaging and revealed that two distinct NSC sub-populations cooperate to ensure population maintenance and neurogenesis (1, 2). These sub-populations are endowed with self-renewal vs neurogenesis capacity and are organized in a functional hierarchy. Individual NSCs of the self-renewing population divide systematically asymmetrically, and appear molecularly homologous to mouse striatal astrocytes, which are physiologically not neurogenic (3). We are focusing on this population to understand both the mechanisms of self-renewing NSC trajectories and the evolution of adult neurogenesis.
Using sc-omics, we and others observed that this sub-population is itself molecularly heterogeneous (3–5). The biological significance and regulation of these heterogeneities are unknown. We are taking in situ approaches (notably multiplexed whole-mount smFISH and NSC time-stamping) to decipher individual NSC trajectories and their coordination within the transcriptomic and physical spaces. We also use genetic and pharmacological gain- and loss-of-function assays in vivo to understand the regulation of these trajectories. We could show that the extended quiescence phase of self-renewing NSCs (up to several months) is promoted by Notch and mTORC1 signaling and includes a sub-state of deep quiescence additionally promoted by the transcription factor Nr2f1b. Finally, upon quiescence exit, systematic asymmetric division appears environment- and Notch-independent and can be read by 4.5kb of the deltaA regulatory elements (2), which we are now screening for potential regulators. I will present our latest results on these aspects, which together should shed light on the mechanisms ensuring the spatiotemporal homeostasis of adult NSC populations.

MICHAEL BRAND : Combining unbiased single-cell RNA sequencing and spatial transcriptomics to study the regeneration response after zebrafish traumatic brain injury

Sebastian Eguiguren, Volker Kroehne, Fabian Rost, Julieta Aprea, Michael Brand

In contrast to mammals, zebrafish show remarkable plasticity and regeneration capacity upon injury to several organs, including the central nervous system. The widespread and life-long maintenance of radial glia, acting as neural stem cells in the zebrafish forebrain, has proven to underlie regeneration upon lesion. Almost immediately after traumatic brain injury, radial glia undergo reactive proliferation and reactive neurogenesis. Subsequent neuronal migration to the area of the lesioned hemisphere results in the generation and long-term maintenance of mature neurons. The general molecular mechanisms that control neurogenesis in the adult zebrafish telencephalon during homeostasis and regeneration have been explored, yet are not fully understood. By using an unbiased approach for single-cell RNA sequencing (yielding >41000 cells), distinct cell types and transcriptomic states involved in inflammation, neurogenesis, and axon regeneration were identified. We established a bioinformatics pipeline that distinctively defines cell types and states, and utilized this information to design a 480 gene panel for in-situ sequencing using a spatial transcriptomics approach (profiling >160000 cells). By combining spatial transcriptomics with long term cell tracing after a short pulse of EdU at peak of reactive proliferation of the neural progenitors, we determine the identity of the neuronal cell types that are restored and maintained in the regenerated brain. Our study provides an unbiased bottom-up approach in zebrafish for transcriptome exploration at the single cell level, and validation of the discovered genes in-situ by using a large gene panel design. It provides a comprehensive and integrative view of the zebrafish brain regeneration response to traumatic brain injury, and reveals novel neural cell types and states from the adult zebrafish telencephalon.

BEN SHI-LEI LAI : Harnesssing immune response to promote cardiac regeneration

Kaushik Chowdhury, I-Ting Lin, Ke-Hsuan Wei, Yao-Ming Chang, Hsing-Wei Liu, Chia-Lin Huang, Jun-Chung Zheng, Yu-Jen Hung, Khai-Lone Lim, and Ben Shih-Lei Lai

Myocardial infarction in humans causes irreversible cardiomyocyte loss and fibrosis, ultimately leading to heart failure and death. In contrast, zebrafish (Danio rerio) can regenerate their hearts after a similar injury. Comparative analyses between regenerative zebrafish and non-regenerative medaka (Oryzias latipes) revealed that the differential immune response is a key to successful heart regeneration. Interestingly, immune modulation by administration of poly I:C, a synthetic dsRNA analog/damage-associated molecular pattern, can promote de novo heart regeneration in medaka, despite the underlying mechanism remains elusive.

To investigate how poly I:C promotes heart regeneration, we performed comparative analyses on poly I:C-treated vs. PBS-control medaka post cryoinjury. Temporal RNAseq analysis revealed that poly I:C promoted phagocytosis-related gene activation, a process involving cell debris engulfment and degradation after tissue injury. Validating these findings by TUNEL assay and phalloidin staining to detect apoptotic cells and necrotic debris further revealed that poly I:C treatment reduced cell death and promoted faster debris clearance in medaka, similar to the dynamics in regenerative zebrafish. Coincidently, inflammatory resolution also occurs faster in zebrafish and poly I:C-treated medaka following injury. Moreover, inhibiting phagocytosis by o-phospho-l-serine (L-SOP) administration impeded debris clearance in poly I:C-treated medaka hearts and abolished the regenerative effect. To understand how poly I:C stimulates phagocytic activity, we characterize and compare the heterogeneity and function of phagocytes in poly I:C-treated vs. control medaka hearts by single-cell RNA sequencing. Our results identified unique macrophage subsets enriched in poly I:C-treated hearts and activated genes associated with phagocytosis and tissue repair, including granulins. Functionally, we found that recombinant Granulin administration can promote cardiomyocyte proliferation in vitro and enhance cardiac repair and fish survival in vivo.

Altogether, these results highlight how immune modulation may promote de novo cardiac regeneration by enhancing debris clearance, inflammatory resolution, and cardiomyocyte proliferation and hint at a potential therapeutic strategy.