Abstract Book

Heidelberg, Germany

October 16-18 2026

Center for Organismal Studies
University of Heidelberg

Scientific Committee

Sepand Rastegar - KIT Karlsruhe
Daniela Panakova - University Hospital Schleswig Holstein Kiel
Joaquin Navajas Acedo - Biozentrum University of Basel
Rima Siauciunaite - KIT Karlsruhe
Nicolas Simon Foulkes - IBCS-BIP, KIT Karlsruhe
Laetitia Préau - KIT Karlsruhe
Christoph Englert - Leibniz Institute of Aging
Tamara Tal - UFZ Leipzig
Sven Reischauer - Justus Liebig University Giessen

Organising Committee

Sepand Rastegar - KIT Karlsruhe
Lauren Saunders - COS, University of Heidelberg
Joaquin Navajas Acedo - Biozentrum University of Basel
Arica Beisaw - Inst. of Experimental Cardiology, Univ. Heidelberg
Nicolas Rohner - University of Muenster
Stefan Schulte Merker - University of Muenster
Véronique Neuzeret - ViewPoint Behavior Technology

Thank you to our sponsors and exhibitors

Partner and Co-Organiser

ViewPoint
Behavior Technology

Granted by

The Company of Biologists

European Zebrafish Society
Supporting Fish Models for Biomedical Research

European Zebrafish Resource Center

Sponsored by

Exhibitors

bionomous

LXA Fishlab

European Zebrafish Resource Center

F·S·T

FINE SCIENCE TOOLS

MI

MOLECULAR INSTRUMENTS

TECNIPLAST

tefor

Paris-Saclay

Ternaria Biosciences

UNION BIOMETRICA

Large Particle Analysis and Sorting

ViewPoint

Behavior Technology

wFluidx, Inc.

Know Your Fish

ZEISS

Wednesday September 16th

Time	Session
09:00 - 12:00	**Workshop** • Bruker Fluorescence Microscopy Solutions for Zebrafish Imaging - Room 001 • Molecular Instruments The HCR - Room 005
	Tour of the fish facility Müller & Pfeger GmbH
12:00 - 13:00	**Registration**
13:00 - 13:15	**Opening & welcome**
13:15 - 15:11	**Session 1 — Development, Patterning & Signaling** Guest Speaker : Laetitia Préau - KIT Karlsruhe - Vegf signalling at the neurovascular interface: From development to pathophysiology
14:22–14:32	Short break (stretch)
15:13–15:43	**Coffee break**
15:43–16:50	**Session 2 — Emerging Technologies & Methods** Guest Speaker : Lucie Zilova - Cos Heidelberg Development Without Constraints: Fish Organoids as Test beds for Alternative Developmental Pathways
16:53–17:08	Short break
17:08–18:08	**KEYNOTE : Ferenc Muller** - How does transcription initiation control define mRNA diversity and mRNA fate in development and cancer
18:08–19:08	**'From Postdoc to PI' session**
19:10–21:00	**Poster Session 1**

Session 1 — Development, Patterning & Signaling

Deung-Dae Park - University of Ulm

Title: Smarce1-dependent chromatin remodeling controls cardiomyocyte proliferation through Stat3 signaling in the zebrafish heart

Deung-Dae Park, Tillman Dahme, Alena Boos1, Sujin Kim, Leonie Krieg, Antonia Hermann, Wolfgang Rottbauer and Steffen Just

Cardiomyocyte proliferation is essential for cardiac development and represents a central mechanism underlying heart regeneration. Unlike adult mammals, zebrafish retain robust myocardial growth and regenerative capacity, making them an ideal model to uncover conserved regulators of cardiomyocyte cell-cycle control. However, the epigenetic mechanisms that fine-tune cardiomyocyte proliferation remain incompletely understood.
Here, we identify Smarce1, a subunit of the SWI/SNF chromatin-remodeling complex, as a critical regulator of cardiomyocyte proliferation in the zebrafish heart. Using an ENU mutagenesis screen, we isolated the embryonic-lethal heart of stone (hos) mutant, which displays severe ventricular wall thickening at 96 hpf. Quantitative analyses revealed that this phenotype is caused by cardiomyocyte hyperplasia rather than hypertrophy, as hos mutant hearts exhibit increased ventricular cardiomyocyte numbers and enhanced EdU- and pH3-positive proliferative activity without increased cardiomyocyte size. Genetic mapping identified a loss-of-function mutation in smarce1, resulting in reduced smarce1 transcript levels and loss of Smarce1 protein. Morpholino-mediated smarce1 knockdown phenocopied the hos phenotype, whereas smarce1 mRNA injection rescued excessive cardiomyocyte proliferation.

Conversely, global and myocardium-specific inducible overexpression of smarce1 suppressed cardiomyocyte proliferation, demonstrating that Smarce1 acts as a cell-autonomous negative regulator of cardiomyocyte cell-cycle activity.
To define the underlying mechanism, we performed heart-specific RNA-seq, single-cell RNA-seq, and ATAC-seq. Smarce1-deficient hearts showed increased chromatin accessibility and transcriptional activation of cell-cycle and Jak/Stat3 pathway components, including jak1, stat3, il11ra, il6st, and ccnd1. Moreover, Smarce1 deficiency increased Stat3 phosphorylation, and genetic and pharmacological modulation of Stat3 signaling demonstrated that this pathway functionally mediates Smarce1-dependent cardiomyocyte proliferation.
Together, our findings establish a Smarce1-Stat3 regulatory axis linking SWI/SNF-dependent chromatin remodeling to cardiomyocyte proliferation. By identifying Smarce1 as an epigenetic regulator that restrains cardiac growth, this work provides new insight into chromatin-based mechanisms relevant to myocardial regeneration.

Julia Ganz - Michigan State University, USA

Title: Gene regulatory programs in zebrafish enteric nervous system development and disease

Eryl Bevan, Mujahid Shah , Brooke Jeffery , Elina Fourli , Tiffany Heanue , Vassilis Pachnis, Dylan R. Farnsworth, Julia Ganz

A large proportion of the Western population suffer from unsolved gastrointestinal (GI) symptoms drastically affecting quality of life. Many GI disorders are associated with enteric nervous system (ENS) dysfunction. The neural crest-derived ENS is the intrinsic gut nervous system and consists of a complex network of neurons and glial cells. Despite the ENS's essential role in regulating gut function, we lack a comprehensive understanding of the gene regulatory programs that control ENS differentiation. Consequently, the genetic basis of many ENS disorders and GI diseases with an ENS component remains poorly understood. Our work centers on understanding the gene regulatory programs that control neuronal and glial development in the ENS. We used a single-cell RNA-sequencing (scRNA-seq) dataset of ENS cells extracted for a whole embryo atlas spanning 1 to 7 days post fertilization to capture both non-neuronal and neuronal ENS cell types and ENS-specific ATAC-sequencing to identify putative enhancers across ENS development. Based on the scRNA-seq data set, we present a comprehensive characterization of non-neuronal ENS cell types as well as a model of enteric neuronal fate specification. We find that enteric progenitor cell marker genes are expressed throughout non-neuronal cell clusters in a "salt and pepper" expression pattern resulting in transcriptional heterogeneity within clusters. Differentially expressed genes reveal specialized cell types such as neural stem cells and multiple glial cell populations. Using developmental trajectory analysis, we propose a new model of glial and neuronal differentiation across ENS development. Based on our ATAC-sequencing data, we have identified putative enhancers of key developmental regulator genes of ENS neuronal development.

We are testing their function using reporter constructs and CRISPR based enhancer knockouts.
Together, our findings provide insights into the gene regulatory programs important for ENS development, which is critical for a better understanding of ENS development, and the genetic mechanisms of GI disorders associated with ENS dysfunction.

Rupal Gehlot - MPI for Heart and Lung Research, Bad Nauheim

Title: Hand2 is required cell-autonomously in endocardial cells for cardiac valve formation

Rupal Gehlot1, Yanli Xu, Douglas Adamoski, Marga Albu, Stefan Günther, Didier Y.R. Stainier

Cardiogenesis requires the coordination of several morphogenetic processes including valve formation, and disruption of these processes is a leading cause of congenital heart diseases. The bHLH transcription factor HAND2 has been shown to play a role in cardiac valve formation in mouse; yet, little is known about its mechanism of action. To get a mechanistic understanding of the role of Hand2 during cardiac valve formation, we used the zebrafish model, which offers easy genomic manipulation and accessibility for live imaging. Since the early and severe cardiac defects in zebrafish hand2 mutants have prevented the lineage-specific and spatiotemporal study of its function, we generated a floxed hand2 allele to investigate its function specifically in the endothelial/endocardial cells, particularly in areas of high shear stress such as the atrioventricular (AV) canal. Consistently, loss of cardiac contractility and blood flow leads to hand2 downregulation, indicating that mechanical forces actively promote hand2 expression and identifying Hand2 as another mechanosensitive transcription factor gene in the endocardium.
Knocking out hand2 in kdrl expressing cells (i.e., endothelial cells) resulted in a complete loss of valve interstitial cells (VICs), a decrease in valve endocardial cells (VECs), and increased extracellular matrix (ECM) deposition in the AVC, suggesting that Hand2 is required for endothelial to mesenchymal transition (EndoMT) and ECM remodeling during cardiac valve morphogenesis.

In contrast, hand2 overexpression in endothelial cells led to an increased number of VECs, further supporting a critical role of hand2 expression levels during cardiac valve formation. Endothelial-specific hand2 KO embryos do not survive to adulthood, likely due to progressive heart deformities and periocular and intestinal edema suggestive of broad lymphatic dysfunction, highlighting the importance of Hand2 in these processes. Transcriptomic analysis of hand2 KO and overexpression hearts revealed a number of disrupted pathways and identified two candidate effector genes, id2b and haln1b, and rescue experiments with the respective stable lines are ongoing to establish their contribution to the observed phenotypes. Additionally, live imaging of a Notch signaling reporter line in hand2 KO hearts revealed changes in Notch signaling, a known regulator of EndoMT and cardiac valve morphogenesis, suggesting that Hand2 plays a role in modulating Notch signaling during cardiac valve cell fate decisions.
To further dissect the regulatory landscape of Hand2, we are generating ATAC-seq data from the hand2 endothelial KO hearts to identify Hand2 dependent chromatin accessibility changes, as well as a knock-in line carrying an endogenous ALFA-tagged Hand2 to enable mapping of Hand2 genomic targets in the endocardium. Altogether, our data support a model in which mechanical forces induce hand2 expression in endocardial cells, where it directs EndoMT and ECM remodeling through effector genes such as id2b and hapln1b, with important implications towards our understanding of the mechanosensitive regulation of cardiac valve formation.

Tanishta Bhattacharya -MPI for Heart and Lung Research,

Title: Understanding the specification and maturation of cardiac pacemaker

Tanishta Bhattacharya, Marco Tarasco, Florian Reuter, Thomas Juan, Didier Y. R. Stainier

Cardiac arrhythmia, caused by abnormal heart pacing, affects up to 5% of the population. The cardiac conduction system plays a crucial role in ensuring rhythmic contraction. Although several key players involved in the development of the cardiac pacemaker, the sinoatrial node (SAN), have been identified, little is known about the proteins and signaling pathways that lead to SAN maturation. This project aims to identify genes and signaling pathways affecting SAN formation and maturation while also modeling arrhythmias and aiding in the development of therapeutic interventions.
We have identified using single-cell RNA sequencing data from zebrafish hearts across different time points, several candidate genes that are upregulated in the SAN region during early embryonic stages (i.e., after heart looping at 36 hours post-fertilization (hpf)) conserved from zebrafish to humans. We then performed a crispant (i.e., mosaic crispr) screen to identify genes that when mutated would lead to changes in heart rate and SAN specification for further evaluation using stable mutant lines. Among these candidate genes is sema3aa (semaphorin-3a), an axonal chemorepellent, important for cardiac innervation. We show that loss of sema3aa in zebrafish leads to hyperinnervation of the heart, particularly at the sinoatrial node (SAN), consistent with its role as a spatially restricted axonal chemorepellent. Using the Tg(chata:GAL4-VP16; UAS:mCherry-MA) reporter line, we observed significantly increased cholinergic innervation density at 100 hpf, with a subset of mutants showing premature axonal projections as early as 75 hpf.

This hyperinnervation is accompanied by increased atrial chamber volume at 100 hpf and reduced heart rate at 120 hpf, suggesting that excessive parasympathetic input disrupts cardiac morphology and pacing,
providing novel insights into the molecular basis of neuro-cardiac crosstalk during cardiac development.
Another candidate, fgf13a (fibroblast growth factor 13a), is highly expressed in the SAN region and is known to regulate Na+ channel activity. Loss of fgf13a in zebrafish results in a reduction in shox2-expressing pacemaker cells at 48 and 72 hpf, as assessed using the Pt(shox2:Gal4; UAS:GFP) reporter line, suggesting a role for fgf13a in pacemaker cell specification or maintenance. Conversely, mosaic atrial-specific overexpression of fgf13a drives expansion of the shox2+ cell population, demonstrating that fgf13a levels bidirectionally regulate pacemaker cell number. fgf13a$^{-/-}$ mutants display reduced heart rate at 72 hpf, which is further exacerbated by flecainide treatment, suggesting that fgf13a regulates cardiac conduction at least in part, through the modulation of sodium channel activity. To further understand the molecular mechanisms underlying these phenotypes, we are performing transcriptomic analyses to identify dysregulated pathways and downstream targets that govern the establishment of the cardiac conduction system.

Marco Podobnik - Monash University, Australia

Title: Identifying mechanisms of skeletal muscle growth in danionins as models for comparative functional genetics and genomics

Yansong Lu, Marco Podobnik & Peter D. Currie

How organ growth is coordinated in the body is largely unknown for any animal. Closely related species often differ in their growth capacity despite a shared evolutionary history, providing a platform for defining the molecular basis for this divergence. Danionin fishes are emerging as important models for comparative functional genetics and genomics. Giant danio (Devario malabaricus) grows larger than zebrafish (Danio rerio) and Danionella cerebrum is a progenetic miniature. Using these three differently sized danionins as model organisms we have started to characterize differential growth at the level of skeletal muscle as model organ. Quantitative morphometric analysis of skeletal muscle using the newly developed tool fishROI revealed divergence in spatial hyperplastic patterning between species. While juvenile giant danio and zebrafish undergo increased mosaic hyperplasia, post-embryonic Danionella cerebrum entirely lacks hyperplasic growth in the deep musculature. This evolutionary loss represents a newly documented developmental truncation in the broadly progenetic species, which may serve as a new model for investigating the post-natal loss of hyperplasia in mammals. Investigating the decline in hyperplastic growth toward adulthood, species-integrated single-cell RNA sequencing data analysis identified upregulation of extracellular matrix (ECM) pathway genes in muscle stem cells (MuSCs) as a conserved program in adults relative to juveniles. Skeletal muscle lineage-specific CRISPR/Cas9-mediated mutagenesis of candidate collagen genes resulted in changes in MuSC number in zebrafish. Together, our analyses suggest that MuSCs actively remodel their niche by differential ECM deposition during skeletal muscle growth.

Such stem cell-based mechanisms may also be required for growth of other organs to reach the final body mass. Future studies will aim to identify genes and pathways that diverged to contribute to differential organ growth in danionins as tractable vertebrate models.

Melina Köhler - KIT, IBCS-BIP, Karlsruhe

Title: Deciphering the gene regulatory network governing the differentiation of intraspinal serotonergic neurons

Melina Köhler, Fushun Chen, Mehmet Ilyas Cosacak and Sepand Rastegar

Damage to the vertebrate central nervous system, whether caused by injury or by neurodegenerative and neurodevelopmental diseases, severely impairs its function. Therefore, elucidating the mechanisms underlying neurogenesis is essential for the development of regenerative therapeutic strategies. Single-cell transcriptomics revealed that sox1a, a known marker of neuronal progenitors and mature neurons, is also expressed in late-born intraspinal serotonergic neurons (ISNs), a population implicated in locomotor control and axon regeneration following spinal cord injury in zebrafish larvae. However, the progenitor population and gene regulatory network governing ISN differentiation remain unknown in zebrafish spinal cord. Using transcriptomic data, we recently identified the lateral floor plate (LFP), a region homologous to the mouse p3 domain, as the progenitor population giving rise to ISNs. We further characterized intermediate ISN precursors (ISN-pre) that co-express markers of both progenitors and mature ISNs, providing insight into the transition between these states. Among the genes enriched in this population, several transcription factors previously implicated in serotonergic neuron development in the rodent hindbrain emerged as key candidates.
To investigate their functional relevance, we generated CRISPR/Cas9 knockouts of gata2a, gata3, lmx1bb, lmx1ba and fev, and analyzed the resulting phenotypes and downstream gene expression using hybridization chain reaction (HCR) RNA FISH. Our results indicate that gata2a, gata3, lmx1bb and fev, but not lmx1ba, act upstream of tph2, which encodes the rate-limiting enzyme for serotonin synthesis.

Furthermore, our data suggest that gata3 and fev function downstream of gata2a, revealing a hierarchical regulatory cascade controlling ISN differentiation.
Deciphering the genetic program underlying ISN development will not only advance our understanding of spinal cord neurogenesis but may also provide a foundation for future regenerative strategies targeting spinal cord repair.

Wiebke Herzog - Friedrich-Alexander Universität Erlangen

Title: Mechanisms of Wnt and S1P Signaling Pathway Interactions during Brain Angiogenesis and regulation of Blood-Brain Barrier tightness

Romana Scheffel, Markus Freise, 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 specialized 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 focussing on how the interaction is mediated and which downstream components will balance it. Using comparative transcriptomics on Kaede converted FACS isolated central arteries with and without Wnt-Signaling inhibition, we isolated Arhgap28 as a RAC1 activity regulating factor downstream of Wnt signaling.
We can show that Arhgap28 deficiency phenocopies loss of Wnt signaling and that it is sufficient to mediate the effect of Wnt-signaling on the S1p-signaling pathway.
Additionally we analyzed the downstream effectors of S1p-Signaling using human cerebral microvascular cells (hCMEC/d3) as an in vitro Model of BBB endothelial cells. These cells respond to S1p stimulation, with a short fast opening of the barrier followed by a slower tightening. Through phospho-proteomics we isolated a novel S1p signaling target, which not only seems to be involved in driving activated Rac1 to modulate cytoskeletal rearrangements, but is also capable of interacting with Wnt-ß-catenin signaling.

Our data provide novel insight into the molecular interaction of the Wnt- and S1p Signaling pathways and the balancing of BBB tightness versus brain vessel angiogenesis.

Session 2 — Emerging Technologies & Methods

Jean-Pierre Levraud - TEFOR Paris Saclay

Title: Phantom: a transparent triple-mutant zebrafish lacking all three pigments, generated in a single step using CRISPR gRNA multiplexing

Axel Benchetrit, Pierre Affaticati, Jean-Pierre levraud

Transparency is a key experimental advantage of embryonic zebrafish, and extending this property to later developmental stages is highly desirable. Zebrafish possess three main pigment cell types: melanophores containing black melanin, xanthophores containing yellow pterins, and iridophores containing silvery guanine crystals — all three pigments are also present in the retinal pigmented epithelium. Several recessive mutations have been described that result in the loss of body and/or eye pigmentation into adulthood. Their combination gave rise to the widely used double mutant casper strain, characterized by a transparent body but black eyes, and more recently to the triple mutant crystal strain, which displays transparency in both body and eyes. However, these strains still produce yellow pigments responsible for significant autofluorescence. In addition, introducing fluorescent reporter transgenes into these genetic backgrounds requires laborious crossing steps.
Here, we describe a novel triple mutant strain, which we named phantom, combining the loss of melanin through a tyr mutation, the loss of iridophores through a ltk mutation, and the loss of yellow pigments through an slc2a11b mutation. Phantom zebrafish could develop to adulthood and were fertile. The absence of yellow pigments markedly enhances the detection of cells expressing low levels of GFP under the widefield microscopy.

We have developed a combination of guide RNAs enabling the efficient generation of phantom triple CRISPants. On the AB genetic background, we achieved nearly complete pigment loss in 58.3 ± 17.5% of viable larvae, making this a practical and efficient approach to “phantomize” any existing transgenic line in a single generation.

Reinhard Köster - Technische Universität Braunschweig

Title: Rescue of Dyrk1a overexpressing zebrafish cerebellar Purkinje cells by quantum dot-delivered Leucettinib-21

Luiza Araújo Gusmão, Annemarie Metzke, Laurent Meijer, Antonio Claudio Tedesco, Reinhard W. Köster

Purkinje cells (PCs) of the zebrafish cerebellum express Dyrk1A, and altering dyrk1a expression levels affects the morphology and function of these neurons. For example, transgenic zebrafish with overexpression of human Dyrk1A in PCs displayed a significant decline in the number of synapses formed by the PC population. We have established sparse expression of either human Dyrk1a or a mutant with an inactive kinase domain in individual PCs. In vivo confocal microscopy revealed a reduced outgrowth of PC dendrites and a reduction in dendrite branching in Dyrk1A expressing PCs, whereas PCs expressing the kinase inactive variant of Dyrk1A were indistinguishable from wildtype PCs. This shows that dendrite maturation defects in PCs with Dyrk1A hyperactivity is caused in a cell-autonomous manner and dependent on the kinase activity of Dyrk1A.

Leucettinib-21 (LCTB21) is a potent, high-affinity inhibitor of the Dyrk1A kinase activity, but, based on predictions, with mediocre brain penetration properties. We therefore tested, whether nanoparticle carriers could promote the passage of LCTB21 across the blood brain barrier (BBB). We have synthesized nitrogen-doped graphene quantum dots (NGQDs) that are organic fluorescent nanoparticles with high bio-tolerance in zebrafish embryos. Furthermore, injections into the vasculature revealed that these NGQDs, unlike conventional quantum dots composed from semiconductor material, rapidly leave the blood vessels and spread into the surrounding tissues, including passage through the BBB.

In the brain, these NGQDs were internalized by neurons, but showed no accumulation in the nervous system suggesting that NGQDs are metabolized or excreted over time.
When LCTB21 was administrated into the vasculature of zebrafish overexpressing human Dyrk1A in cerebellar Purkinje neurons, it was able to rescue compromised dendrite maturation leading to larger and more complex dendritic trees. This confirms that dendrite defects in PCs were caused by the hyperactivity of the kinase domain of Dyrk1A. Importantly, when LCTB21 was conjugated to NGQDs, such a rescue of PC dendrite formation could be achieved at a ten-fold lower LCTB21 concentration, at which LCTB21 alone was not yet effective. Therefore, NGQDs could promote bioavailability of compounds to cells of the central nervous system, likely by facilitating the passage of compounds across the BBB. This reduction in therapeutic compound concentration will help to reduce potential side effects, furthermore NGQDs themselves can be harnessed as therapeutics, for example because of their aggregation inhibiting activity.

Onur Onder - Konstanz University

Title: zMorphoNet: Deep learning-based quantification of developmental dynamics

Onur Önder, Tim Brunnhuber, Matvey Safroshkin, Grigory Arutyunov, Natalie Klein, Lisa Dimmler, Coralie Bentele, Daniel Čapek, Laura Tejada Heidenhain, Cedric Wolfer, Patrick Müller

Embryonic development is a highly dynamic process in which cells, tissues, and body structures change continuously over time. Quantifying these changes is essential to understand how embryos progress through development and how intrinsic and extrinsic factors alter developmental timing and morphology. However, classical microscopic analysis often relies on manual inspection, which is time-consuming, subjective, and difficult to scale to large time-lapse datasets. Here, we present zMorphoNet, a deep learning-based segmentation framework for automated analysis of early zebrafish development. zMorphoNet identifies and quantifies major embryonic structures from time-lapse microscopy images, enabling objective measurement of morphological changes throughout development. By converting image data into time-resolved quantitative features, the framework allows developmental progression to be compared across embryos and experimental conditions. We demonstrate the utility of zMorphoNet in three case studies addressing environmental, intrinsic, and cross-species applications. First, we apply zMorphoNet to embryos raised under varying temperature and osmolarity. We find that temperature is the major driving force of developmental speed, while osmolarity modulates but does not dominate developmental timing. This temperature sensitivity extends to metabolic perturbations: embryos treated with rotenone, a mitochondrial Complex I inhibitor, exhibit a dose-dependent developmental delay that can be counteracted by temperature. Second, we extend the framework to intrinsic perturbations involving signaling pathway inhibitors and gene knockouts.

Although trained on wild-type embryos, zMorphoNet detects morphological signatures of pathway perturbations. Building on this, we show that elevated salinity can modulate the rescue of temperature-dependent sqt knockout phenotypes, linking an environmental variable to a genetic condition. Third, we demonstrate that zMorphoNet is adaptable to non-model species through transfer learning, applying it to Perca fluviatilis embryos raised at different temperatures to characterize their developmental progression. Together, these results establish zMorphoNet as a versatile framework for linking image-based morphological quantification to developmental and environmental variables across conditions, perturbations, and species.

Vanessa Fee Saalmann - Helmholtz Centre for Environmental Research - UFZ, Leipzig

Title: RetiNAM: Identification of Biomarkers for the Disruption of the Retinoic Acid Signaling Pathway Using a Zebrafish Embryo Model

Vanessa Saalmann, Éva Fetter, Jürgen Arning, Gerd Maack, Susanne Walter-Rohde, Wibke Busch, Tamara Tal,Stefan Scholz

Endocrine-disrupting chemicals are a major concern in ecotoxicology due to their ability to interfere with hormone signaling across species. Therefore, current regulations for chemicals such as REACH, have a particular focus on the identification of endocrine disruptors (ED). Standardized test methods for endocrine disruption focus on EATS (estrogen, androgen, thyroid, steroidogenesis) modalities. The retinoic acid (RA) pathway is another highly conserved endocrine signaling pathway, essential for physiological and cellular processes including neurodevelopment and morphogenesis. As RA pathway disruption can affect organisms at population-relevant levels, regulatory interest in the retinoid system has grown, with OECD efforts in 2012 and 2021 to review and harmonize regulations, focusing on key organ systems and highlighting the need for validated assays. However, there is a lack of validated methods to detect disruption of the RA signaling pathway. In line with the EU “Roadmap to phase out animal testing”, new approach methods (NAMs) are needed to fill ED-related testing gaps. One strategy to identify RA disrupting chemicals is the identification of biomarkers for key events leading to apical adverse outcomes. The RetiNAM project aims to identify and quantify sensitive and specific biomarkers for RA pathway disruption in zebrafish embryos with a focus on chemicals that act via binding to the retinoid X receptor (RXR) or retinoic acid receptors (RAR), and

We demonstrated that exposure to RAR and RXR agonists and antagonists induced specific effects, including craniofacial, fin and tail malformations. Acute neuroactivity testing indicated diverse neuroactive effects across multiple endpoints.
Developmental neurotoxicity testing resulted in altered visual and acoustic startle responses. Additionally, widespread hyper- or hypoactivity were observed, respectively. These results suggest that the zebrafish embryo model can be used to diagnose RA pathway perturbations. Ongoing transcriptomic analyses will identify key RA pathway target genes, anchoring the phenotypic effects to the underlying molecular mechanisms. By integrating morphological assessment, behavioral profiling and transcriptomic analysis, this study will address current regulatory gaps in the evaluation of RAR or RXR–mediated endocrine disruption and supports the use of NAMs in environmental risk assessment.

From Postdoc to PI Session

Javier Vasquez Marin - COS, University of Heidelberg

Title: Cell autonomy, plasticity and patterning evolution: fish ionocytes as an experimental paradigm

Conserved cell types can vary substantially in size, proportion, and spatial distribution across species. In complex tissues composed of multiple lineages, their precise coordination is essential for homeostasis and post-embryonic growth. This coordination relies on a balance between cell-intrinsic programs and extrinsic environmental cues, yet the relative contribution of these factors and how they evolve across species remains poorly understood.

To address this, I am using the skin ionocytes in fish of the Oryzias genus as a research model. Ionocytes are specialized epithelial cells that regulate osmotic balance by transporting ions in response to external salinity. They are distributed in a characteristic “salt-and-pepper” pattern and exhibit morphological plasticity under changing environmental conditions. Notably, ionocyte density, distribution, and responsiveness to changing environmental conditions vary widely even among Oryzias species, providing a powerful comparative system to investigate cell autonomy.

Using lineage tracing, quantitative morphology, and interspecies chimera approaches, I examined the embryonic origin of ionocytes and their degree of autonomy in defining key traits. My results reveal species-specific strategies: in some cases, ionocyte properties are largely cell-autonomous, while in others they are shaped by the host environment. These findings suggest that even highly conserved cell types can adopt distinct developmental strategies, offering insight into the evolution of cellular coordination mechanisms.

Building on this work, my future research would be intended to explore how different cell lineages coordinate to form and maintain complex tissues, with a particular focus on the role of mechanical cues. During my PhD, I developed expertise in mechanotransduction in early embryogenesis, and I aim to integrate this with my current interest in tissue-level coordination.
As a model, I will use zebrafish or medaka gills, a structured organ composed of branchial arches containing filaments sustained by multiple fate-restricted stem cell populations. Previous work in our lab has shown a hierarchical organization among these lineages, with chondrocytes potentially regulating the proliferation of other cell types. I hypothesize that chondrocytes generate a mechanically stiff microenvironment that promotes proliferation through pathways such as YAP/TAZ signaling. To test this, I plan to combine transplantation approaches with ex vivo gill cultures and the development of filament-like organoids.

David Bierbach - Humboldt Universität zu Berlin

Title: How individuality is linked to large scale collective processes

Phenotypic variation among individuals is thought to be caused by differences in genes and/or environmental conditions. Therefore, if these sources of variation are removed, individuals are predicted to develop similar phenotypes lacking individual variation. In sharp contrast to these predictions, we find substantial individual variation in behavior among genetically identical individuals of the clonal fish, Poecilia formosa, that were isolated directly after birth into highly standardized environments. This variation does not develop over the ontogeny but is present at day 1 of the fish's life. In contrast to the current research paradigm, which focuses on genes and/or environmental drivers, our findings suggest that individuality might be an inevitable and potentially unpredictable outcome of the very early embryonic development. In order to study both causes and consequences of these individual differences, we developed a biomimetic robot - the so-called Robofish - that is able to integrate itself interactively into groups of fish. This tool allows us to decouple behavior from morphology and test hypothesis arising from theoretical considerations on social and collective interactions. I will outline how Robofish helped us to find a link between differences in swimming speed and collective patterns in shoaling fish and how leader-follower interactions depend strongly on the anticipated reaction of the social partner. It remains then largely unexplored if results from those sophisticated and highly controlled laboratory experiments can be used to explain patterns of ecology and evolution in the wild. I will thus showcase my research on free-living Sulphur mollies (Poecilia sulphuraria) in Mexico that indeed use several mechanisms found in laboratory experiments to perform astonishing collective behaviors when faced with real predators in their natural habitats.

Sapna Chhabra - EMBL Heidelberg

Title: Robustness of embryonic development in changing environments

Externally developing embryos experience dynamic thermal environments, including day–night temperature cycles, yet how they maintain robust patterning under such variable environments remains poorly understood. During my postdoc, I investigated this question in the Japanese ricefish (Oryzias latipes, medaka), focusing on axis segmentation: the rhythmic partitioning of the body axis into somites, which give rise to vertebrae and skeletal muscle. Segmentation relies on the interplay between two processes: the segmentation clock, an intracellular oscillator that propagates as a traveling wave to regulate the timing and position of segment formation, and axis elongation, which supplies new tissue to the pre-somitic mesoderm (PSM for segmentation). Using real-time imaging under chronic and cycling temperature regimes, I quantified clock oscillations and axis elongation. I found that both segmentation clock frequency and elongation velocity scale linearly with temperature, but with distinct sensitivities and response times. These differences transiently alter PSM length across temperatures; over time, however, somite length scales with PSM length, revealing a dynamic compensation mechanism that preserves proportional patterning.
This compensation may be governed by the spatial rate of change of the segmentation clock oscillation phase gradient along the axis, which remains invariant across temperatures. To test this idea, I collaborated with physicists to develop a theoretical model in which clock phase dynamics are coupled to elongation velocity, such that somites form once the resulting phase difference between anterior and posterior PSM reaches 2π. The model recapitulates the delayed scaling of somite length to PSM length, suggesting that proportional patterning emerges from the dynamic coordination of distinct thermal responses in the segmentation clock and axis elongation. Together, these findings reveal a previously undescribed mechanism of thermal robustness across developmental modules, which we term chronomorphic compensation.

Joaquin Navajas Acedo - Biozentrum, University of Basel

Title: Spatiotemporal Emergence of Somatosensory Neuron Diversity

How cellular factors combine in space and time to generate the diverse neuron types of a functional circuit in a robust way remains unclear. The fish primary somatosensory system of Rohon-Beard (RB) neurons develops first and is thought to undergo programmed cell death and be then replaced by the DRG. Our live imaging and deep single-cell transcriptomics across development show that contrary to a ~150-year-old assumption, RB neurons do not disappear and are heterogeneous at 3 levels: their transcriptome, their axial distribution, and interindividual pattern. Spatiotemporal analysis of RB subtype emergence reveals that it follows a staggered-layer logic, with a 'pan-RB program' preceding a stochastic 'hybrid neurotrophin receptor' state that resolves into mutually-exclusive subclasses, followed by specialized functional subtypes. Combining in toto cell lineage reconstruction and a custom whole-mount spatial transcriptomics —in the same animal- we show RB neurons possess extremely simple cell lineages and link them to dozens of transcription factors and somatosensory genes in space. We also define the regulatory mechanism by transcription factors underlying the seemingly stochastic, yet robust emergence of the observed neuronal subtypes across time. We also show this mechanism is likely conserved across Anamniotes. Our work demonstrates that zebrafish RB neurons are an excellent model to study the molecular mechanisms underlying the development of neuron diversity at the cellular level.

Thursday September 17th

Time	Session
09:00–09:45	**KEYNOTE : Mathilda Mommersteeg**
09:45–10:52	**Session 3 — Regeneration, Aging & Disease Models (Part I)** Guest Speaker : Dennis de Bakker - Mapping the genetic architecture of brain aging in killifish
11:00–11:30	**Coffee break**
11:30–12:22	**Session 3 — Regeneration, Aging & Disease Models (Part II)**
12:30–14:00	Lunch & meet the keynote speakers
14:00–14:26	**Session 3 — Regeneration, Aging & DiseaseModels (Part III)**
14:30–15:11	**Session 4 — Toxicology & Screening** Guest speaker: Gaëlle Hayot - KIT
15:11–15:39	**Session 5 — Omics & Systems Biology**
15:40–16:10	Coffee break
16:10–17:10	**KEYNOTE : Laure Bally-Cuif**
17:10–19:00	**Poster Session 1**
19:00	**Gala dinner - jil Rooftop Drink&Dine**

Florian Constanty - University Heidelberg

Title: Spatial proteomics uncovers the dynamic composition of the border-zone niche in the regenerating heart

Florian Constanty, Stephanie Roessler, Arica Beisaw

Cardiovascular diseases remain the leading cause of mortality worldwide, with myocardial infarction as the most prevalent and devastating manifestation. In adult mammals, the limited regenerative capacity of cardiomyocytes following ischemic injury leads to myocardial loss and heart failure. Although human and murine hearts have lost regenerative potential, zebrafish exhibit lifelong cardiac regenerative capacity, offering a powerful model to uncover endogenous mechanisms of heart repair.

To determine the spatial composition of the regenerating heart, we applied laser microdissection to three key regions: the remote zone, border zone (BZ), and scar tissue. This novel approach, performed across multiple time points of regeneration, revealed the spatial architecture of the cardiac proteome. Whereas the remote zone remained stable at the proteomic level, the scar tissue showed greater temporal variability, potentially due to multiple cell type infiltration. The BZ exhibited the most pronounced dynamic changes across time. This temporal plasticity defined a unique, time-specific proteomic signature within the BZ that supports regeneration. At 7 days post-cryoinjury (dpci), the BZ was enriched in secretory proteins, identifying the BZ as a secretory hub potentially directing intercellular crosstalk. By 14 dpci, enrichment shifted to proteins involved in cell attachment and spreading, key processes for cardiomyocyte replacement of the scar and, ultimately, functional recovery.

Strikingly, only 17 proteins, representing 3% of total enriched proteins at the BZ, were consistently enriched across all regenerative time points, positioning them as potential master regulators of heart regeneration. These proteins included known pro-regenerative ECM proteins and proteins that are not yet linked to heart regeneration. This underscores the importance of investigating the newly identified candidates for their potential, yet unknown, functions in heart regeneration.

Using spatial proteomics, we mapped the dynamic composition of the BZ niche across time points of heart regeneration. These observations suggest that cardiomyocyte-ECM interactions could direct heart regeneration at the BZ. Currently, we are leveraging genetic models to test how BZ-enriched ECM components functionally regulate heart regeneration.

Tonatiuh Molina-Villa - MPI for Heart and Lung Research

Title: The mechanical properties of the extracellular matrix play critical roles during zebrafish heart regeneration

Tonatiuh Molina-Villa, Miguel Valle-Inclan Cabello, Adarsh Mohabir, Pooja Sagvekar, Julia Kolb, Giulia Mangia, Miloslav Sanda, Daniel Wehner, Didier Y.R. Stainier

The physical and biochemical properties of the extracellular matrix (ECM) control cell communication and maintain cardiac homeostasis. However, how the mechanical properties of the injured tissue control cell behaviour after myocardial infarction remains unclear. A rapid increase in ECM stiffness correlates with the loss of regenerative potential in neonatal mouse hearts. To investigate the interplay between ECM stiffness and collagen crosslinking, we first performed high-resolution imaging to assess collagen organization in neonatal mouse hearts. We observed a significant increase in collagen fiber thickness, but not in density, from postnatal day 1 (P1) to P7 and P14. Notably, collagen deposition and distribution in the neonatal mouse ventricle were comparable to those observed in the adult zebrafish heart. To further explore how ECM stiffness influences cell behavior and regeneration, we tracked collagen dynamics during adult zebrafish heart regeneration. We identified increased crosslinking and remodeling of collagen fibers at the surface of the injured tissue and within the border zone. Using 3D imaging, we followed the migration of cortical cardiomyocytes (cCMs) and found that they migrate toward the injury site along distinct paths. These migrating cCMs did not exhibit increased proliferation compared to non-migrating CMs; however, they displayed elevated nuclear and cytoplasmic Yap levels, suggesting a key role for mechanical signals.

To modulate collagen crosslinking, we generated a loxa full-locus deletion allele. loxa mutant hearts exhibit reduced collagen fiber dynamics, characterized by decreased fiber density and thickness, as well as enhanced coronary revascularization of the injured area.
Unexpectedly, these mutants display increased stiffness of the injured tissue, likely due to reduced elastin crosslinking and compensatory upregulation of the loxl1 gene. In these loxa mutants, cCMs displayed increased Yap levels, reduced dedifferentiation, shorter protrusions, and sarcomere shortening compared with wild-type siblings. Equally, global loxa overexpression resulted in reduced cCM protrusion length and sarcomere shortening following injury. Proteomic analysis and bulk transcriptomic profiling of the injury revealed upregulation of glycolysis at 7 and 14 dpci, and increased expression of genes encoding transcription factors and chromatin regulators in loxa mutant hearts after injury. Ongoing studies aim to elucidate the functional roles of these factors in CMs. Collectively, our findings demonstrate that the mechanical properties of the cardiac ECM critically influence CM behavior during regeneration and that genetic mechanisms tightly regulate these properties.

Tahnee Mackensen - Biozentrum Basel

Title: Neuronal microexons modulate arousal via the cAMP-PKA-CREB pathway in zebrafish

Tahnee Mackensen, Luis Pedro Iniguez, Thomas Soares Mullen, Cristina Rodríguez-Marin, François Kroll, Giulia Zuccarini, Jordi FernandezAlbert, Laia Sancho-Vila, Jon Permanyer, Isaac H. Bianco, Michael Orger, Jason Rihel, Manuel Irimia

Proper regulation of arousal maintains the balance of rest and activity and enables appropriate responses to stimuli; its disruption is a hallmark of many neurodevelopmental disorders. While transcriptional mechanisms of arousal control are well-defined, the contribution of post-transcriptional processes such as alternative splicing remains unclear. Here, we identify a critical role for the microexon splicing regulator srrm3 in maintaining arousal homeostasis in zebrafish. srrm3 mutants exhibit persistent hyperarousal characterized by sleep loss, sensory hypersensitivity, and elevated behavioural and neuronal activity. We identify the cAMP-PKA-CREB signaling axis as a central driver of mutant hyperarousal. Specifically, pharmacological inhibition of cAMP signalling rescues mutant hyperactivity and associated transcriptional changes while wild-type cAMP activation phenocopies the mutant. Downregulation of immediate early genes and reduced CREB phosphorylation further suggest adaptation to sustained neuronal activation. These findings establish srrm3-dependent microexon splicing as a key molecular layer of arousal regulation linking RNA-processing defects to neuromodulatory imbalance.

Roman Franek - University of South Bohemia

Title: A young gonadal environment reverses reproductive aging of male germ cells

Essaikiammal Sodalai Muthu KONAR, Aaron TORRES-MARTINEZ, Tomáš TICHOPAD, Radek SINDELKA, Roman FRANEK

Testicular aging is associated with declining germ cell proliferation, loss of germline-associated programs, and extensive remodeling of the somatic gonadal environment. However, whether reproductive aging is driven primarily by intrinsic deterioration of germ cells or by dysfunction of the aging gonadal niche remains unresolved. Here, we used a germline transplantation approach in zebrafish (Danio rerio) to determine whether germ cells from reproductively aged males retain the capacity to support spermatogenesis when exposed to a young gonadal environment.
Donor spermatogonial cells were isolated from 3-year-old Tg(ddx4) males exhibiting hallmarks of reproductive aging, including failure to spawn, absence of milt upon stripping, reduced gonadosomatic index, decreased germ cell proliferation, and reduced sperm abundance. Spermatogonial cells from 8-month-old Tg(ddx4) males served as young controls. Donor cells were transplanted into sterile recipients generated by dnd knockdown.
Germ cells derived from aged donors successfully migrated to and colonized recipient gonads. Although their initial expansion was slower than that of young donor-derived cells, aged germ cells ultimately established complete spermatogenesis. Chimeras generated from both aged and young donors developed GFP-positive testes and produced motile sperm, confirming functional donor-derived gametogenesis. Importantly, sperm motility did not differ significantly between chimeras derived from aged donors (75%) and young donors (68%). Likewise, fertilization rates were comparable between chimeras generated from aged donors (86.7 ± 14.5%) and young donors (85.0 ± 15.2%).

Despite severe reproductive decline in aged donor males, their germ cells retained the capacity to regenerate complete spermatogenesis and restore fertility in young recipients. These findings demonstrate that reproductive aging is not irreversibly encoded within germ cells and instead highlight a central role of the somatic gonadal niche in determining reproductive function. Together, our results suggest that key aspects of male reproductive aging remain reversible when aged germ cells are exposed to a youthful environment.

Session 3 — Regeneration, Aging & Disease Models (Part II)

Sergey Prykhozhij - CHEO Research Institute, Ottawa, Canada
Title: Zebrafish model of a tRNA modification disease due to a G177A mutation in OSGEP

The OSGEP gene encodes a KEOPS (Kinase, Endopeptidase and Other Proteins of Small size) complex subunit catalyzing N6-threonylcarbamoyladenosine (t6A) modification to A37 of ANN tRNAs. This modification enhances the accuracy and efficiency of protein synthesis, and variants in OSGEP are linked to Galloway-Mowat syndrome, with clinical features, including intellectual disability, developmental delay, seizures, microcephaly, other brain abnormalities and kidney problems. The KEOPS complex may also function in telomere length regulation, genome maintenance, and DNA damage response. OSGEP knockdown in human cells impairs cellular proliferation and translation, increases ER stress and apoptosis, activates the DNA damage response, and disrupts actin regulation. Frameshift zebrafish osgep mutants exhibit microcephaly, increased apoptosis in the brain, and early lethality. Mouse osgep knockouts had significantly shorter cortex lengths, cortex–midbrain midline lengths, and cortex widths. A renal phenotype was not observed in either knockout model, possibly due to early lethality masking renal involvement in older animals. Based on human-genetic data, we generated a zebrafish osgep G177A knock-in mutant. osgep G177A/G177A larvae have reduced eye and head sizes relative to wildtype. Mutants grow poorly and about 70% of them die during the first 4-5 weeks post-fertilization. Elevated apoptosis is a potential etiology for smaller size of larval organs and juvenile animals given that p53 pathway is activated in osgep G177A/G177A embryos.

However, loss of tp53 does not rescue the morphological phenotypes or survival of osgep mutants indicating that p53 activation is not the only mechanism responsible for their phenotypes. RNA-sequencing of single wildtype and osgep G177A/G177A larvae at 6 dpf identified >1000 differentially-expressed genes. Up-regulated genes were significantly associated with protein folding, endoplasmic reticulum, RNA processing/maturation, and ribosome biogenesis. Down-regulated genes were strongly enriched among translation-related terms, developmental terms, immune and hematopoietic terms, inflammatory response, as well as Ribosome, Proteasome, Ferroptosis, Apoptosis and Oxidative Phosphorylation pathways. Maternal-zygotic (MZ) osgep G177A/G177A embryos generated from the surviving zygotic mutants exhibit dramatically exacerbated morphological phenotypes such as overall size and overall developmental progress with increased activations of p53 pathway, interferon signaling as well as Amino Acid and Unfolded Protein Responses targets and effectors. After integrating the transcriptomic data from zygotic and MZ mutant data, we identified that there is common activation of the Amino Acid Response (AAR), known to be active under amino acid starvation or tRNA deficiency condition. AAR may therefore be a suitable mechanism to target for diagnosis and therapeutic development in KEOPS-related diseases. We are now addressing the upstream mechanisms behind the interferon and AAR activation and the phenotypic consequences of manipulating these pathways.

Bailin Wu - Heidelberg University,

Title: Investigating the role of actin stabilization in promoting CM invasion during zebrafish heart regeneration

Bailin Wu , Lara Szilagyi , Greta Muravska , Florian Constanty, Vasiliki Bakali , Arica Beisaw

Unlike adult mammals, zebrafish (Danio rerio) harbor the ability to regenerate their heart, as cardiomyocytes (CMs) undergo dedifferentiation and proliferation, extend F-actin-filled protrusions, and ultimately replenish the fibrotic scar tissue at 60-90 days post cryoinjury (dpci). To understand the underlying molecular and cellular mechanisms regulating replenishment of scar tissue by functional CMs, we compared the transcriptomes of remote CMs and border-zone CMs using single-cell RNA-sequencing.

We discovered new gene candidates that potentially regulate CM invasion during zebrafish heart regeneration. Among the top differentially expressed genes enriched in border zone CMs, we verified Transgelin, a mechanosensing, actin-bundling protein encoded by tagln, as an injury-induced border-zone CM marker. Recent studies have shown that tagln expression is induced at the wound border in several heart injury models. In order to understand the function of tagln in vivo, we generated a CRISPR-based loss-of-function model and transgenic gain-of-function models. Using our gain-of-function model, we find CM-specific overexpression of tagln (CM:taglnOE) leads to stabilization of CM F-actin, premature CM protrusion formation, premature expression of CM dedifferentiation markers, and accelerated scar resolution. Notably, CM:taglnOE likely shapes the regenerative niche at the wound border zone by inducing non-CM autonomous phenotypic changes. We observe that CM:taglnOE results in enhanced Collagen XII deposition, induces vwf/mmp2 expression and increases phospho-Erk presence in surrounding cell types in the wound.

Currently, loss-of-function and additional gain-of-function studies are ongoing to understand the mechanisms underlying the regulation of CM invasion by Tagln and how tagln overexpression in CMs affects the phenotype of surrounding cells at the border zone. Altogether, our data will explore whether Tagln can link cytoskeletal regulation and intercellular crosstalk, and provide molecular and cellular insights into a largely understudied process during cardiac regeneration in zebrafish.

Jana Fuss - COS, University of Heidelberg

Title: Natural genetic variation in spinal cord regeneration of medaka revealed by high-throughput screening of the MIKK panel

Jana Fuss , Gero Hofmann, Javier Palau Alegria, Esther Yoo, Emily Hildebrandt , Florian Kreuder , Sebastian-Alexander Stamatis , Samuel Crossman , Bettina Welz, Philip Watson, Risa Suzuki , Thomas Thumberger, Tomas Fitzgerald , Ewan Birney , Jan Kaslin , Joachim Wittbrodt

The ability to regenerate upon spinal cord injury (SCI) varies greatly among vertebrates. While mammals form scar tissue and lose locomotor function, other animals like zebrafish (Danio rerio) can regenerate the spinal cord (SC) and regain their swimming ability. Yet even another teleost, medaka (Oryzias latipes), has demonstrated lower potential to regenerate not only SC but heart and retina alike. An interspecies comparison however cannot identify the genetic basis of these differences, as high overall genomic variability conceals individual factors in complex traits. With the Medaka-Inbred-Kiyosu-Karlsruhe (MIKK) panel we have established a unique resource to investigate complex traits intraspecifically. The currently 74 nearly-isogenic lines derived from a wild medaka population have been successfully used to dissect the genetic base of temperature sensitivity, drug susceptibility and visual acuity in other projects of our lab. We now apply the same approach to SC regeneration, screening the panel for regenerative potential to subsequently map associated loci by QTL analysis. To assess SC regeneration in the 74 MIKK panel lines, we adapted a manual SCI method for reproducible and high-throughput lesions on medaka hatchlings. We built a temperature-controlled swim assay setup to observe locomotor activity and tapping and white light startle response of 92 hatchlings at once. Uninjured and injured individuals underwent swim assays once a day for 6 days-post-injury.

Lines were classified as high or low 'regenerators' based on the observed locomotor recovery and crosses were set-up to identify the loci associated to each phenotype in a QTL mapping in the F2 generation.
Candidate loci will be functionally validated using precise genome editing to introduce regeneration-associated alleles and evaluate resulting SCI recovery phenotypes. This systematic study demonstrates that natural genomic variability within a species can determine regenerative potential, and is paving the way to uncover the molecular mechanisms governing SC regeneration.

Jérémie Zappia - University of Bristol, UK

Title: Understanding the pro-reparative functions of neutrophils in bone fracture repair and the negative impact of NSAIDs

Bone fractures in the ageing population represent a global health crisis. In Europe, an estimated 33.9 million people suffer from osteoporosis, resulting in 4.5 million fractures annually. In Western countries, fragility fractures surpass diabetes, cardiovascular disease, and cancer in hospital days occupancy. This socioeconomic burden is exacerbated by frailty, which significantly escalates post-fracture mortality. Bone repair in patients associated with frailty is frequently compromised by "inflammaging", the systemic dysregulation of the immune system.

Following fracture, the repair process has 4 main phases: haematoma formation accompanied by inflammation, soft callus formation, hard callus formation (endochondral ossification), and bone remodelling. During the first phase, neutrophils are the first responders and help to prevent infection. While the association of neutrophil-mediated hyperinflammation with poor fracture repair has been well documented, the extent to which neutrophils can play a pro-reparative function in fracture is less well understood. Furthermore, current orthopaedic practices rely on the immediate post-fracture non-steroidal anti-inflammatory drugs (NSAIDs) administration, such as ibuprofen, despite evidence that they may impair bone healing and increase non-union rates, a serious complication.

Our objective is to characterize neutrophil behaviour at the fracture site while relating it to bone repair outcomes and determine how ibuprofen treatment modulates these dynamics by using a zebrafish fin fracture model.

We showed that a heterogenous population of neutrophils is quickly recruited to the site of injury. We observed that a subset of neutrophils extrudes their chromatin to form Neutrophil Extracellular Traps (NETs), a behaviour known as NETosis firstly described as a defense mechanism against pathogens. In an injury context, NETs may serve as an emergency scaffold for stabilization as we showed neutrophils being able to rearrange ECM proteins, such as collagen, fibronectin and laminin, to reorganize the fracture microenvironment.
Our fracture model is particularly relevant for evaluating the impact of NSAIDs. We showed that zebrafish treated with ibuprofen exhibited altered bone repair mechanisms with fractures progressing toward non-union within hours of injury, or delayed bone repair likely due to the suppression of the early inflammatory signals from neutrophils. This mechanism was replicated with the inhibition of neutrophils recruitment by targeting CXCR2, an important receptor involved in their chemotaxis. Our research shows that early immune involvement is a primary determinant of final bone quality at the injury site. Collectively, our research helps to clarify the essential immune mechanisms underpinning fracture healing and suggests that the timing of anti-inflammatory intervention may be a decisive factor in patient outcomes. Moving forward, we aim to investigate these processes within the scope of ageing to elucidate the mechanistic links between neutrophil dysregulation, frailty and poor bone repair outcomes.

Session 3 — Regeneration, Aging & Disease Models (Part III)

Mark Naven - University of Bristol , UK

Title: Live Imaging the Wound Inflammatory Response and the Consequences of Disrupted Circadian Clocks on Angiogenesis and Collagen Deposition

Mark A Naven, Terrence M Trinca, Maria Esther Prada-Sanchez , Lorna Hodgson, Laura Bevan, Kevin Thiessen, Stephen J Cross, Gabriela Marinescu, Zohar Ben-Moshe Livne, Rebecca Richardson1, Yoav Gothilf , Chrissy Hammond1, David Whitmore and Paul Martin

Healthy wound healing is a consequence of many tissues and cell types acting in sequence involving cell migration, proliferation, contraction, matrix synthesis and matrix remodelling. Several of these elements are at some level likely regulated by the circadian clock. Pivotal to the wound healing response is the timely arrival and departure of innate immune cells, which instruct collagen deposition by fibroblasts and regulate angiogenesis during wound healing.

Imaging of zebrafish with fluorescently labelled macrophages, endothelial cells, and basal keratinocyte produced collagen1a2, cellular behaviour, angiogenesis and matrix deposition can be monitored in real time post-wounding. To investigate the role of the circadian clock in wound healing, zebrafish were exposed to “normal” 12-hour light 12-hour dark conditions, or circadian disruption with constant light, constant dark or rapid 4-hour light-dark cycles. Light manipulation impacts all cellular clocks; a complementary genetic approach with neutrophil and macrophage specific circadian clock disruption has revealed specific roles of the circadian clock for these cell types during wound healing.

Zebrafish wounded in light conditions disrupting circadian clocks, both neutrophils and macrophages migrate towards wounds with altered speed and remain within wounds longer compared to controls.
Collagen fibres remain unaligned longer and excessive angiogenesis occurs in wounds made under circadian disrupted conditions. Specific genetic knockdown of neutrophil and macrophage clocks mimics the light driven phenotypes; however, these genetically disrupted neutrophils and macrophages are recruited in fewer numbers and macrophages inappropriately depart before controls. Specific genetic disruption of neutrophil and macrophage circadian clocks also impacts collagen deposition and angiogenesis during wound healing. Zebrafish with neutrophil targeted circadian disruption also spontaneously develop cancers.

Shift work, our lifestyles and modern lighting may disrupt our circadian clocks. Our findings suggest altered inflammatory, angiogenic and collagen deposition responses with circadian disruption. This may suggest circadian disruption could impair wound healing or exacerbate chronic wounds.

MarcoTarasco - MPI for Heart and Lung Research

Title: Investigating the onset of cardiac fibrosis in a novel zebrafish model

Marco Tarasco, Sofia Papadopoulou, Tanishta Bhattacharya, Arnaud Prevet, Jan Detleffsen, Stefan Günther, Mario Looso, Pia R. Lundegaard, Didier Y. R. Stainier

Cardiac fibrosis (CF) is a pathophysiological condition associated with many types of cardiovascular disease. CF is characterized by excessive collagen deposition in the extracellular matrix (ECM), driven by increased differentiation of cardiac fibroblasts into myofibroblasts in response to tissue stress. Despite its clinical importance, the mechanisms underlying the onset and progression of CF remain poorly understood. As existing models to study CF suffer from substantial limitations including ethical considerations, high maintenance costs, and inability to perform live imaging at cellular resolution, we have developed a novel gain-of-function zebrafish model that exhibits increased collagen deposition within the atrial tissue starting by 1-month post-fertilization, followed by its resolution by 6 months. In this model, continuous overexpression of the voltage-gated sodium channel gene scn5lab in atrial cardiomyocytes causes an increased heart rate. These transgenic zebrafish display impaired cardiac function as evidenced by reduced fractional shortening, absent/short P-waves, and disrupted calcium handling. They also exhibit increased blood regurgitation as well as atrial enlargement, possibly a consequence of ECM remodeling, as suggested by the dysregulation of ECM related genes. To investigate the early events leading to CF, we performed scRNAseq at 1 month, which allowed us to capture all cardiac populations present in the atrium. This dataset revealed the increase of different cell types (e.g., macrophages and fibroblasts) during the initiation of CF. Specifically, we identified two cardiac fibroblast subtypes that were increased in the scn5labGOF and may contribute to CF.

Comparative transcriptomics between fibrotic and control conditions identifies many ECM-related genes as well as several transcription factor genes that may play an important role in CF. These findings were further validated by in vitro experiments involving human cardiac fibroblasts. Our findings provide new insights into the mechanisms underlying CF and establish a valuable zebrafish model to study CF during its onset, progression, and resolution.

Session 4 — Toxicology & Screening

Elena K.Nicolay - Helmholtz-Zentrum für Umweltforschung GmbH - UFZ Leipzig

Title: Development of a high-content analysis workflow to screen the effect of endocrine disrupting chemicals on macrophages and enteric neurons in transgenic zebrafish embryos

Elena K. Nicolay, Darya Trofimova, Riccardo Massei, Robert Haase, Fabian Isensee, Ana C. Zenclussen, Tamara Tal

Industrially manufactured chemicals are released into the environment, where their potential adverse effects on living organisms are typically not well understood. One particularly sensitive biological target of chemical pollutants is the endocrine system. Current EU regulations state that Endocrine Disrupting Chemicals (EDCs) must be avoided. However, while there are a range of cell-based testing systems to detect potential EDCs, there are a lack of alternative models that consider the full complexity of the endocrine system. Here, we developed an alternative zebrafish embryo-based assay to detect potential EDC effects on intestinal inflammation and enteric nervous system (ENS) development, both targets of ingested EDCs. Using Tg(mpeg1:mCherry) zebrafish embryos, we developed an imaging and analysis workflow to detect region-specific inflammation based on macrophage count and shape, derived from three dimensional (3D) images. The workflow automates anatomical region detection in transmitted light images using the AI-based nnU-Net model to distinguish structures. We then applied Voronoi-Otsu labelling of cells in regions of interest by combining pre-processing, thresholding, and watershed segmentation algorithms to automatically identify individual macrophages in 3D fluorescence images. To establish the screening assay, multiple reference chemicals and exposure windows were assessed.

Exposure to 0.15% Dextran Sodium Sulfate (DSS) from 3-5 days post fertilization (dpf) increased intestinal macrophages by 10%. Bisphenol A was selected as a prototypical EDC. Exposure to 10 uM BPA from 3-5 dpf resulted in morphological changes in macrophages, including increased number, size and volume, which indicates an enhanced proinflammatory state. Intestinal macrophage infiltration supports normal ENS development. In line with previous work, exposure to 1 µM mycophenolate from 2-3 dpf caused a 35% reduction in ENS cell counts in 5 dpf Tg(phox2bb:GFP) larvae. To adapt this assay for double transgenic Tg(mpeg1:mCherry) X Tg(phox2bb:GFP) embryos, we optimized the exposure conditions to 0.15% DSS and 0.5 µM MPA during the period from 2 to 5 dpf. As part of the HORIZON ENDOMIX project, future work will examine whether exposure to mixtures of co-occurring EDCs identified in human cohorts alters macrophage functionality or ENS development.

Charlotte Philippe - KU Leuven

Title: Nothobranchius furzeri as a model for chronic and transgenerational toxicity assessment: comparing bisphenol A and the bio-based alternative p,p'-BGP

Philippe C. , Cenens N. , Merckx W.

The transition toward a sustainable chemical economy requires the development of safer alternatives to compounds of concern. However, replacing hazardous chemicals with bio-based alternatives does not automatically guarantee reduced biological impact. Thorough assessment of chronic and transgenerational effects is therefore essential to prevent regrettable substitutions. In this context, model organisms capable of rapidly capturing long-term and inherited effects are increasingly important for environmental risk assessment.
The turquoise killifish, Nothobranchius furzeri, is a promising vertebrate model for chronic and transgenerational toxicology. Its exceptionally short lifespan, rapid sexual maturation, high reproductive output, and short generation time allow researchers to investigate life-long exposure scenarios and multigenerational effects within a practical timeframe. Furthermore, as a vertebrate species, N. furzeri provides biologically relevant endpoints related to development, reproduction, aging, and physiology. These characteristics make it uniquely suited to bridge the gap between short-term screening assays and longer, more resource-intensive vertebrate studies.
In this study, we demonstrate the value of N. furzeri for evaluating the safety of emerging bio-based chemicals through a comparative assessment of bisphenol A (BPA) and p,p’-BGP, a bio-based alternative proposed as a replacement for conventional bisphenols. BPA is a well-known endocrine-disrupting chemical associated with adverse reproductive and developmental effects in aquatic organisms and vertebrates.

Although bio-based alternatives are increasingly promoted as sustainable solutions, their potential long-term impacts remain insufficiently characterized.
Fish were chronically exposed to two concentrations of BPA or p,p′-BGP (7.5 and 15 µM) during their whole lifespan, and reproductive performance was monitored over time. In addition, offspring from exposed parents were evaluated to investigate potential transgenerational consequences of exposure. Multiple reproductive endpoints were assessed, including fecundity and fertilisation success, enabling a comprehensive evaluation of reproductive fitness.
Our results reveal a complex toxicological profile for the bio-based alternative. Compared with BPA, p,p′-BGP-exposed fish displayed improved fecundity, indicating that reproductive output was less affected by the alternative compound. However, despite this apparent advantage, fertilisation rates remained severely impaired at equivalent exposure concentrations. These findings demonstrate that increased egg production does not necessarily translate into successful reproduction and highlight the importance of assessing multiple reproductive endpoints when evaluating candidate replacement chemicals. The observed effects also raise concerns regarding the potential for hidden reproductive toxicity that may not be detected through conventional screening approaches focused on a limited set of endpoints.

Session 5 — Omics & Systems Biology

Christabel Bucao - EMBL Heidelberg

Title: Propagation of phenotypic variability in early zebrafish embryogenesis

Christabel F. Bucao, Sophie Kneeshaw , Nick Marschlich , Vikas Trivedi , Michael W. Dorrity

Embryogenesis has evolved to produce highly stereotyped forms despite biomolecular stochasticity and environmental variation. Nonetheless, environmental factors such as thermal stress may challenge the robustness of developmental outcomes. While the influence of temperature on developmental rate and mean phenotype is well-established, its impact on phenotypic variability (i.e., the tendency of a trait to deviate from its mean) remains poorly understood. Crucially, variability measurements are typically studied in isolation, without linking molecular, cellular, and organismal scales; as such, it remains unclear whether and how variability at the molecular level propagates to influence organismal phenotypes.

To investigate the molecular and cellular basis of embryo-to-embryo variability in zebrafish development, we generated an individually-resolved time-series atlas of early zebrafish embryogenesis at different temperatures (473 embryos spanning 8 timepoints, 2–16 hpf, 28°C and 34°C) using single-nucleus combinatorial barcoding and indexing (sci-RNA-seq3). This approach simultaneously captures information on mRNA abundance, cell type abundance, and whole-embryo cell type composition, enabling the quantification of mean and variance at each scale. We leveraged the unique structure of this dataset to develop a hierarchical framework that tracks how phenotypic variability propagates through these layers.

First, we applied a linear mixed model to quantify the relative contributions of different sources of gene expression variance. We show that genes involved in proteostasis are a major axis of global embryo-to-embryo variability, while genes involved in signal transduction drive cell-type-dependent embryo-to-embryo variability. Further, genes involved in post-translational modifications show strong signatures of temporal variation. Treating unexplained residual variance as an estimate of intrinsic gene-level stochasticity, we also identified a class of highly stochastic genes.

Next, we applied this variance model to track how gene-level expression variability within cell types and individual-to-individual variability in cell type proportions shift over development. We compared these estimates to assess whether variability is dampened, maintained, or amplified across scales. Together, we present an embryo-resolved atlas of early zebrafish embryogenesis and a statistical framework integrating multiple layers of phenotypic variability—resources that we are applying to investigate how baseline variability shapes developmental responsiveness under thermal stress.

Friday September 18th

Time	Session
09:15–10:37	**Session 6 — Evolution, Behavior & Physiology** Guest speaker: Swantje Grätsch - MPI Munich Nest building in a shell-dwelling cichlid: From innate motor programs to learned precision
10:40–11:00	**Coffee break**
11:00–11:30	Animal experimentation & discussion
11:30–11:40	Short talk: Archiving of zebrafish lines at the European Zebrafish Resource Center (EZRC) — ***Robert Geisler***
11:40–11:50	Short talk: Managing Rapid Life Cycles: A Scalable Breeding Strategy for Robust Killifish Populations — ***Beate Hoppe***
11:50–12:50	**KEYNOTE :** New genes in old pathways: lymphatics in fish, mice and man **- Stefan Schulte-Merker**
12:50–13:50	Lunch
13:50–15:20	Talk, 'Company of Biologists' postdoc prize, Addgene and Plasmidsaurus talk and GfE poster prizes + Concluding remarks

Session 6 — Evolution, Behavior & Physiology

Ingo Braasch - Michigan State University USA

Title: One Model to Connect them All : Holostean Fishes Inform the Developmental Evolution of Vertebrates and Bridge Fish to Human

Ingo Braasch, Zehua Tony Zhou, Hao Yuan, Jamily Lorena, Chi-Jing Leow, Brett L. Racicot, Allyse Ferrara, Andrew W. Thompson

The macroevolution of vertebrates has been accompanied by gains and losses of genes, regulatory elements, and developmental processes, contributing to major evolutionary transitions such as the emergence of tetrapods from fishes or the unmatched biodiversity of the teleost fishes. Teleosts like zebrafish, medaka, killifish, cavefish, and other biomedical model species are derived from the teleost-specific genome duplication (TGD) that had major impact on their genome and gene function evolution. Together with the earlier two genome duplications before the rise of the jawed vertebrates, this complicates comparative studies across vertebrates due to lineage-specific genome reshuffling and gene losses, obscuring the distinction of orthologs vs. paralogs, and hiding the origins of vertebrate developmental programs.

To overcome these challenges, we are developing holostean fishes – the gars and bowfins used by Darwin to coin the term “living fossils” – as new research organisms for Evo-Devo and comparative developmental genomics. Holosteans serve as the closest ‘unduplicated’ outgroup to the fast-evolving 35,000+ living teleost species as well as outgroup to lobe-finned vertebrates including tetrapods and humans. Holosteans have slowly evolving and thus highly informative genomes, deeply conserved developmental programs, and archaic body plans that provide unique opportunities for wide-ranging biological comparisons among vertebrates.

Using examples from diverse developmental pathways and processes, we demonstrate that comparative genomic analyses and transcriptomic and epigenomic profiling through holostean development are powerful for connecting the disparate sets of genes, gene regulatory elements, and morphologies among distant vertebrate lineages.

The evolutionary inertia of holostean genomes enables the identification of ancestral vertebrate cis-regulatory elements that would otherwise remain hidden for fast-evolving taxa such as teleosts and rodents. Leveraging haplotype-resolved genome assemblies from multiple holostean species – generated in collaboration with the Vertebrate Genomes Project – we use comparative genomics, ATAC-seq epigenomic profiling, and 3D genomics in holosteans to recover hidden gene regulatory landscapes across bony vertebrates. Combining single-cell transcriptomics, our novel computational tool ECHOmap (Evolutionary Cell HOmology map), and Hybridization Chain Reactions, we obtain a complex picture of gene regulatory sub- and neofunctionalization across cell types and teleost species following the TGD.

Developing gars as functional genomic models for comparisons to other biomedical fish species, we developmentally test hypotheses about the evolutionary origins of vertebrate gene functions. The holostean model thus uniquely connects piscine to human biology and provides a powerful "evolutionary bridge" that illuminates vertebrate evolution, development, disease, and regeneration.

Nicholas S. Foulkes - KIT, Eggenstein

Title: A comparative study involving medaka and blind cavefish reveals a "dark" CPDphotolyase function in DNA repair

Nicholas S. Foulkes, Hongxiang Li, Silvia Fuselli, Takeshi Todo, Carsten Weiss, Daniela Vallone, Tilman Lamparter, Cristiano Bertolucci

A complex spectrum of DNA damage is generated upon exposure to combinations of environmental stressors. Therefore, over the course of evolution, DNA repair systems must inevitably adapt to changing environments to ensure the maintenance of genome integrity. Photolyases represent a highly conserved class of enzymes which repair UV-induced covalent crosslinks between adjacent pyrimidine bases (CPD and 6-4 photoproducts) via the visible light-driven process of photoreactivation. In the blind cavefish Phreatichthys andruzzii which has evolved for millions of years completely isolated from UV radiation and visible light, we have documented multiple polymorphisms and loss-of-function mutations affecting both the 6-4phr and DASHphr photolyase genes while strangely, the CPDphr gene remains highly conserved. In order to investigate the possibility of a light-independent function for CPDphr, we have generated loss-of-photolyase-function medaka mutant lines as well as gain-of-photolyase-function mammalian cell lines. By studying and comparing these various in vitro and in vivo models, we reveal that CPDphr (but not 6-4phr or DASHphr), enables the light-independent repair of CPD as well as 8-OHdG, an oxidatively modified form of guanosine, with both types of DNA damage being generated under oxidative stress in the absence of UV radiation. Thereby, using this comparative approach we document selective conservation of light-independent photolyase function in blind cavefish, enabling the repair of DNA damage encountered in an extreme subterranean environment.

Kelly Dooling - University of Münster,

Title: snRNAseq analysis reveals muscular adaptations of Astyanax mexicanus cavefish

Kelly Dooling , Ana Santacruz, Edward S. Ricemeyer , , Wesley Warren , Nicolas Rohner

Caves are challenging environments in which cave-dwelling organisms have evolved mechanisms to survive in conditions of little to no light, hypoxia, and nutrient deprivation. The Mexican cavefish, Astyanax mexicanus, has emerged as a prominent model organism for studying these processes due to their repeated and independent adaptations to cave environments while maintaining reproductive compatibility with their ancestral ecotype, surface fish, which dwells in superficial rivers and lakes. Studies have particularly noted several metabolic adaptations in cavefish, including hyperphagia, insulin resistance, and increased fat storage. One of the most metabolically active areas of the body lies in muscle tissue, making it a promising target for elucidating further metabolic adaptations. Previous research has found that cavefish display markedly reduced muscle mass and contractility yet maintain similar muscular endurance as the surface fish ecotype. To further characterize the changes associated with these phenotypic differences, we performed single nuclei RNA sequencing (snRNAseq) on muscle tissue from cave- and surface fish. We identified more than 10 putative cell types and detected differences in cell type composition between the two ecotypes in clusters related especially to adipocytes, suggesting increased fat storage in cavefish muscle tissue. These findings provide insight into the cellular and molecular muscular adaptations of cavefish, potentially highlighting techniques used to survive extended periods of nutrient deprivation and hypoxia.

Johanna Kowalko - Lehigh University, Bethlehem PA

Title: Uncovering the genetic basis of repeated evolution in cavefish

Marcos da Silva, Aubrey Manning, Stefan Choy, Suzanne McGaugh, Johanna Kowalko

Adaptation to similar environments frequently results in the repeated evolution of similar traits. Cave animals are a striking example of repeated trait evolution, as life within the darkness of subterranean habitats has led to the repeated evolution across taxa, including loss or reductions of eyes and pigmentation, enhancement of non-visual sensory systems, and changes in behavior. The extent to which the same genes and pathways underlie the repeated evolution of cave traits, however, is largely unknown. We investigate this process in Astyanax mexicanus, a species of fish which includes both surface populations as well as multiple cave-dwelling populations. Cave populations of A. mexicanus have evolved many classic cave-associated phenotypes, and some cave populations have evolved independently of one another. Moreover, A. mexicanus is a genetically tractable species, providing a powerful opportunity to investigate the genetic underpinnings of repeated trait evolution. Using a combination of genetic mapping, genomics and functional genetic approaches, we have been investigating the genetic mechanisms underlying repeated evolution across cave A. mexicanus populations. Further, we have recently begun to examine repeated evolution across other cavefish species. Together, this work provides important insight into the repeatability and genetic basis of evolution.

Julia Schwarzer - LIB - Museum Koenig Bonn

Title: From Cells to Structure: Spatial gene expression during plug formation in medaka wild relatives

Alina Schüller, Lazaro Centanin, Javier Vázquez Marín , Daniel Mokodongan , Niki Vontzou, Alexander Suh , Julia Schwarzer

In evolutionary biology, one of the central questions is how novel traits arise that enable species to exploit new ecological niches. One example of such a trait is the transient “plug” structure in pelvic brooding ricefishes from Sulawesi, Indonesia—wild relatives of medaka (Beloniformes; Adrianichthyiedae). The plug forms in the female gonoduct after spawning and anchors egg-attaching filaments, thereby facilitating external egg retention. Recent research on Oryzias eversi suggests that plug formation may involve a modified inflammatory response. To investigate the developmental and evolutionary basis of the plug, we profiled the spatial gene expression landscape of the female reproductive system of O. eversi across four time points. We identified distinct gene expression clusters that correspond to the morphological organization of the reproductive system, including clusters specific to later brooding stages. Notably, key immune-related marker genes (e.g., il1b, tgfb1, SMS, VMP1, and MRC1) are specifically expressed in the developing plug and surrounding tissue. Our results highlight the molecular complexity underlying pelvic brooding and support the hypothesis that inflammatory pathways have been co-opted for the evolution of a novel reproductive structure.

Robert Geisler - KIT Karlsruhe

Title: Archiving of zebrafish lines at the European Zebrafish Resource Center (EZRC)

Laws on animal experimentation aim to reduce the use of animals in European research, stimulating the use of alternative models such as zebrafish embryos. Centralized archiving of genetically modified zebrafish strains can make critical contributions to this aim, since it avoids redundant experiments and improves reproducibility of experimental results. The European Zebrafish Resource Center (EZRC) at the KIT archives 27,000 knock-out mutations from the Sanger Institute (representing nearly half of all protein-coding genes), 2,000 mutations from forward-genetic screens at the MPI for Developmental Biology, and approximately 1,000 mutant and transgenic lines contributed by the community. It also serves a source for standard wildtype lines.Genome editing by CRISPR can easily produce new mutant lines, however each CRISPR experiment requires a permit, molecular biological characterization of the mutations and evaluation of animals for harmful phenotypes. Therefore we offer free archiving of established CRISPR lines as frozen sperm together with the corresponding severity assessments.As a further contribution to standardization in the zebrafish field we provide hands-on training in cryopreservation methods and participate in the International Zebrafish and Medaka Courses (IZMC) held several times per year at the KIT. Finally, European animal health legislation imposes certain requirements on shipping zebrafish embryos or adult fish. Their implementation at EZRC will be discussed.

Beate Hoppe - Leibniz Institute on Aging

Title: Managing Rapid Life Cycles: A Scalable Breeding Strategy for Robust Killifish Populations

Uta Naumann, Clemens Peters , Simone Gruner, Julia Hammerer, Martin Neumann

Efficient colony management is a cornerstone of reproducible research in aquatic model organisms, particularly in systems with rapid life cycles and closed breeding populations. The African turquoise killifish, Nothobranchius furzeri, has emerged as a powerful vertebrate model for aging research due to its naturally short lifespan and pronounced hallmarks of aging. However, these same characteristics pose significant challenges for colony management, including high breeder turnover, rapid generational succession, and increased risk of inbreeding or unintended selection. As genetic input from wild populations or commercial sources is not feasible, maintaining stable laboratory stocks requires carefully controlled and sustainable breeding strategies. Here, we present a colony management framework termed dynamic population breeding (DPB), designed to address these constraints within a fish facility setting. DPB integrates overlapping breeding cohorts, harem-based mating systems, continuous monitoring of reproductive output, and the strategic use of embryonic diapause for flexible stock management.
We evaluated DPB in two widely used wild-type lines with distinct lifespans, GRZ-D and MZCS-08/122. Across both lines, fertilization rates and clutch sizes remained stable throughout the reproductive period. Systematic clutch-level quality control enabled early exclusion of low-performing breeding groups. Notably, DPB reduced inter-cohort variability and improved early-life survival, particularly during the critical post-hatching phase. Overall, DPB provides a robust and scalable strategy for managing killifish colonies in research facilities. By integrating species-specific life history traits with structured breeding and quality control, this approach minimizes inbreeding and unintended selection while enhancing reproducibility and long-term sustainability of aging studies.

Session Chairs & Keynote Chairs

Scientific Sessions

Session	Chair (s)
Development, Patterning and signaling	Nicolas Rohner and Sepand Rastegar
Emerging Technologies and Methods	Arica Beisaw and Lauren Saunders
Regeneration, Aging and Disease Models	Daniela Panakova and Sven Reischauer
Toxicology and Screening	Tamara Tal
Omics and Systems Biology	Laetitia Preau
Evolution, Behavior & Physiology	Nick Foulkes and Rima Siauciunaite
Animal Experimentation and discussion	Thomas Dickmeis

Keynote Talks

Speaker	Chair (s)
Laure Bally-Cuif	Sepand Rastegar
Mathilda Mommersteeg	Arica Beisaw
Ferenc Müller	Lauren Saunders

Postdoc to PI Session

Item	Details
Chair	Joaquin Navajas-Acedo
Best talk selection	Selected by the PhD students

Best talk & Poster Committees

In addition to the Postdoc Session best talk (choosen by PhD Students), the Organisation Committee will select the three best talks overall

Talks Evaluvation	Members
Organisation Committee	Arica Beisaw, Nicolas Rohner, Lauren Saunders, Stefan Schulte-Merker, Joaquin Navajas -Acedo and Sepand Rastegar.

Posters evaluation	Members
Scientific Committee	Nick Foulkes, Tamara Tal, Rima Sciauciunaite, Laetitia Preau, Sven Reischauer, Daniela Panakova