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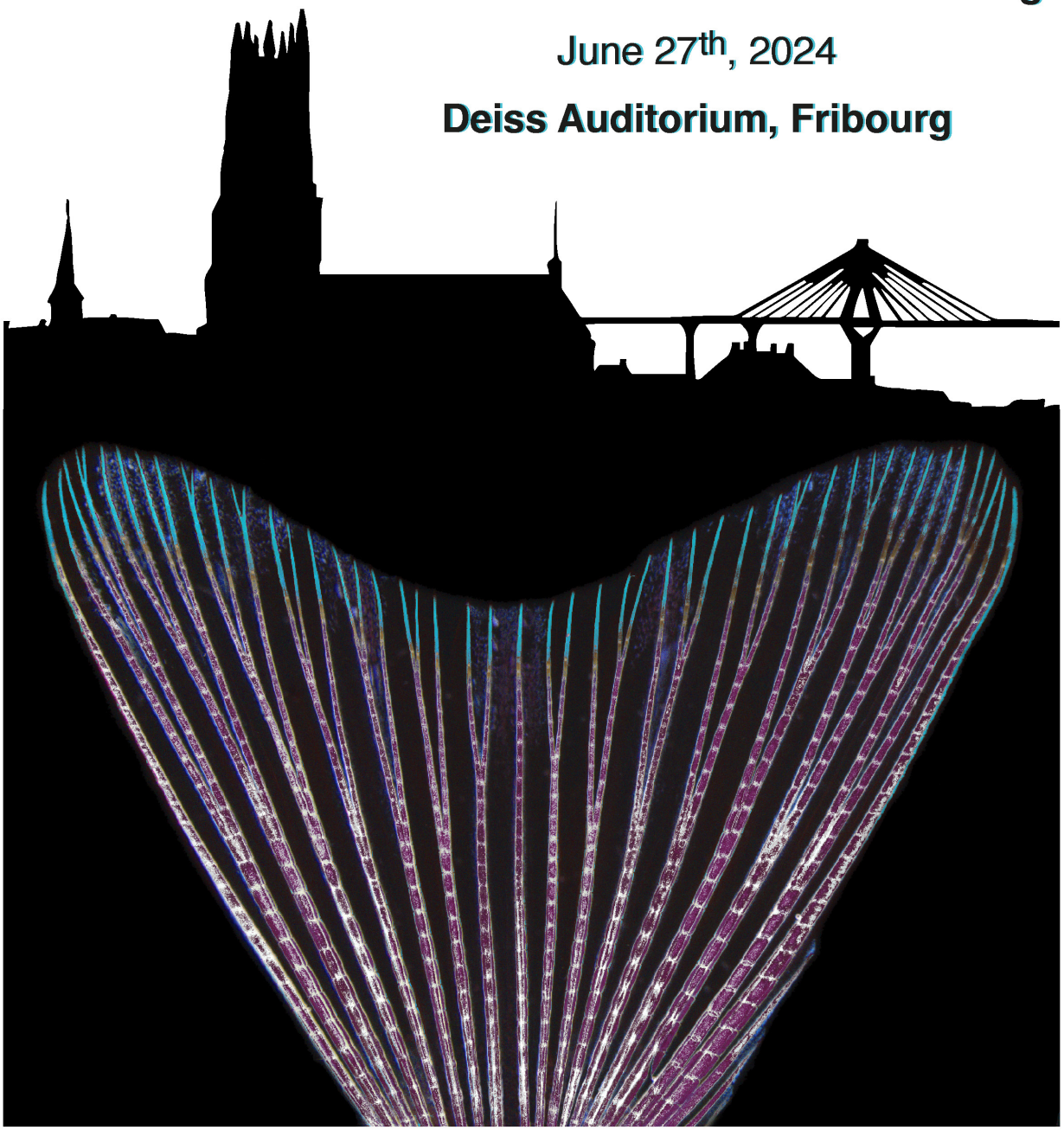
16th Annual Swiss Zebrafish Meeting

June 27th, 2024

Deiss Auditorium, Fribourg



Fri-Zebrafish

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09:20-10:20 Registration – Fixing Posters – Coffee and Croissants – Sponsor Exhibitions

	Session 1 Chair: **Anna Jazwinska**, *University of Fribourg, Switzerland*
10:15-10:25	Welcome
10:25-11:00	Keynote lecture 1 **Christian Mosimann**, *University of Colorado School of Medicine, USA* **Lateral thinking in cardiovascular development and disease**
11:00-11:15	**Nadia Mercader**, *University of Bern, Switzerland* **Spinster homolog 1 (spns1)-dependent endocardial lysosomal function drives valve morphogenesis through the control of Notch1-signaling**
11:15-11:30	**Maria Paraskevi Kotini**, *Biozentrum, University of Basel, Switzerland* **Development and architecture of the midbrain vasculature**
11:30-11:45	**Daria Korotkova**, *EPFL, Switzerland* **Role of palmitoylating enzymes in hair cell formation and function on the zebrafish model**
11:45-12:00	**Sarah Brivio**; *University of Geneva, Switzerland* **Endothelial *mafa* expression controls HSC expansion in the CHT niche**

12:00-13:00 Lunch

13:00-13:30 Poster Session 1 & Sponsor Exhibitions

	Session 2 Chair: **Alessandro de Simone**, *University of Geneva, Switzerland*
13:30-14:10	Keynote lecture 2 **Gilbert Weidinger**, *Ulm University, Germany* **Mechanisms underlying cellular plasticity during regeneration**
14:10-14:25	**Sumeet Pal Singh,** *IRIBHM ULB, Brussels, Belgium* **Dutal-to-hepatocyte transdifferentiation contributes to liver regeneration in the presence of spared hepatocytes**
14:25-14:40	**Alexa Burger**, *University of Colorado School of Medicine, USA* **Conserved enhancers control notochord expression of vertebrate Brachyury**
14:40-14:55	**Jingjing Zang**, *University of Zürich, Switzerland* **Recoverin in Visual Transduction and Beyond: New Insights in Cone Vision**
14:55-15:00	The Swiss 3R Competence Centre **Andrina Zbinden**, *University of Fribourg, Switzerland* **The Swiss Zebrafish Culture of Care Group to increase knowledge and establishing refinements in zebrafish research**
15:00-15:20	The Swiss Zebrafish Society **Stephan Neuhauss,** *University of Zürich, Switzerland*

15:20-16:15 Coffee break

Poster Session 2 & Sponsor Exhibitions

	Session 3 Chair: Volker Enzmann, *University of Bern, Switzerland*
16:15-16:30	**Shivali Dongre**, *University of Lausanne, Switzerland* **Using zebrafish embryos as a vertebrate in vivo model to study TF clustering**
16:30-16:45	New PI **Johannes Larsch**, *University of Lausanne, Switzerland* **Neurogenetics of Social Affiliation in Zebrafish**
16:45-17:05	Sponsors: 4 x 3 min talks **Bionomous, Bruker Nano, Idexx Bioanalytics, Zeiss**
17:05-17:15	Poster Prizes, Closing Remarks

17:15-18:30 Apéro and Drinks

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13:00-13:30 Poster session 1

1	Shayan Shami Pour **Mechanisms underlying symmetry breaking in embryonic development**
2	Arianna Cuoco **Investigation of genetic modifiers in ciliopathies using zebrafish models**
3	Alexandra Noble **Shared and unique consequences of Joubert Syndrome gene dysfunction on the zebrafish central nervous system**
4	Jacqueline Kientsch **Glucose supply and aerobic glycolysis define a retinal metabolic landscape essential for vision**
5	Enno Bockelmann **The role of radial glia and microglia in neuronal clearance during brain development**
6	Veronica Akle **Effectivity and efficacy of bioactive compounds against medulloblastoma: comparison between in vitro and in vivo**

15:20-16:15 Poster session 2

7	Danin Dharmaperwira **Studying brain circuits of kin imprinting**
8	Giacomo Miserocchi **3D culture transplantation in zebrafish embryos: an innovative approach to study in vivo tumor dynamics**
9	Melina Köhler ***sox1a:eGFP* transgenic line and single-cell transcriptomics reveal the origin of zebrafish intraspinal serotonergic neurons**
10	Konrad Marx **Regulation of cell proliferation in regenerating zebrafish scales**
11	Vincent Hisler **Skeletal muscle regeneration after extensive cryoinjury of caudal myomeres in adult zebrafish**
12	Lana Rees **Zebrafish notochord lacks regenerative capacity after embryonic tail amputation**
13	Rebecca Leech **Investigating heterogeneities in zebrafish skeletal muscle fibers**
14	Minh K. Y. Pham **The platyfish caudal fin as a muscularized limb model in regenerative biology**
15	Nadia Mercader **Cox7a1 controls skeletal muscle physiology and heart regeneration through complex IV dimerization**
16	Mayuko Harada **The role of tRNA modifying enzymes during zebrafish development**
17	Benedetta Coppe **A paternal cardiac injury induces cardiac adaptation in the next generation**
18	Ines Marques **Changes in collagen cross-linking through lysyl oxidase chemical inhibition affects zebrafish heart regeneration**

19	Ahmed Elhelbawi **The role of N6-methyladenosine (m6A) in vertebrate development**
20	Jialin Liu **Single-cell multi-omics and deep learning decode regulatory logic for early specification and differentiation**
21	Berfin Kartalkanat **Investigation of architectural plasticity of intersomitic blood vessels in zebrafish embryos**
22	Ludovico Maggi **Dynamic Regulation of Actomyosin Tension within Junction-based Lamellipodia (JBL) Drives Endothelial Cell Rearrangements during Blood Vessel Morphogenesis**
23	Andrina Zbinden **The Swiss Zebrafish Culture of Care Group to increase knowledge and establishing refinements in zebrafish research**

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ABSTRACTS

Keynote lecture 1

Christian Mosimann, *University of Colorado School of Medicine, USA*

Lateral thinking in cardiovascular development and disease

Congenital heart anomalies present frequently as part of more complex, syndromic birth defects with seemingly pleiotropic comorbidities. A deeper developmental and mechanistic understanding of how heart and cardiovascular development is integrated in the patterning of our body plan has tremendous potential to advance our predictive diagnostic and potential therapeutic abilities. The heart principally forms from the lateral plate mesoderm (LPM), a deeply conserved multi-lineage progenitor field that also forms cell lineages for endothelium, blood, kidneys, limb connective tissue, mesothelia, and more. This developmental plasticity provides a mechanistic framework to investigate the co-development of seemingly disparate organs, origins of complex syndromic birth anomalies, and key milestones in vertebrate evolution.
Using zebrafish as principal model, we have started to untangle universal concepts of LPM formation and patterning at the level of gene regulation, cell fates, and tissue dynamics. At the gene regulation level, I will outline our advances in decoding earliest gene-regulatory elements involved in early LPM emergence driven by the transcription factors Eomes, FoxH1, and Mixl1. At the cell fate level, I will introduce how LPM-derived Hand2-expressing mesothelial lineages co-develop with associated organs (including the heart) and how cross-species analyses can reveal previously unrecognized cell lineages. On tissue dynamics, I will present how patient mutations in the TBX4 gene inform our LPM-focused congenital disease modeling in zebrafish. Throughout, I will incorporate our latest, community-accessible advances in zebrafish transgenesis and their application for enhancer discovery, variant testing, and more.

Selected talk

Nadia Mercader, *University of Bern, Switzerland*

Spinster homolog 1 (spns1)-dependent endocardial lysosomal function drives valve morphogenesis through the control of Notch1-signaling

Myra N. Chávez[1]*, Prateek Arora[1], Alexander Ernst[1], Marco Meer[1], Rodrigo A. Morales[2,3], Nadia Mercader[1,4,5]*
1 Department of Developmental Biology and Regeneration, Institute of Anatomy, University of Bern, Bern 3012, Switzerland. 2 Division of Immunology and Allergy, Department of Medicine Solna, Karolinska Institutet and University Hospital, Stockholm, Sweden. 3 Center of Molecular Medicine, Karolinska Institutet, Stockholm, Sweden. 4 Department for Biomedical Research, University of Bern, Bern 3008, Switzerland. 5 Centro Nacional de Investigaciones Cardiovasculares, Madrid 28029, Spain.

Autophagy-lysosomal degradation is an evolutionarily conserved process key to cellular homeostasis, differentiation, and stress survival, which is particularly important to the pathophysiology of the cardiovascular system. What is more, both experimental and clinical observations indicate that it affects correct cardiac morphogenesis, including valve development. However, it is unclear which cells upregulate autophagy-lysosomal degradation and for which specific cellular processes it is required for. Here, we introduce novel zebrafish transgenic models to visualize autophagosomes and lysosomes in vivo and to follow their temporal and cellular localization in the larval heart. We determined the kinetics of autophagosome and lysosome vesicle formation and observed a significant accumulation of lysosomal vesicles in the developing atrioventricular and bulboventricular heart regions and their respective valves. Next, we addressed the functional role of lysosomal degradation in cardiovascular development using a spns1 mutant model of lysosomal impairment. spns1 mutants displayed morphological and functional abnormalities of heart development, including abnormal endocardial organization, impaired cardiac valve formation and high incidence of retrograde blood flow. Single-nuclear transcriptome analysis revealed endocardial-specific differences in the expression of lysosome-related genes and alterations of notch1 signaling in the mutant larval heart. Endocardial-specific overexpression of spns1 and notch1 rescued features of valve formation and function as well as overall cardiac morphogenesis. Altogether, our study reveals a cell-autonomous role of lysosomal processing during cardiac valve formation upstream of Notch1 signaling.

Selected Talk

Maria Paraskevi Kotini, *Biozentrum, University of Basel, Switzerland*

Development and architecture of the midbrain vasculature

Maria Kotini, Marina Signer, Daniel Heutschi, Henry Belting, Markus Affolter
Biozentrum, University of Basel, Switzerland

Blood vessel development is an early and crucial phenomenon associated with the growth and survival of a vertebrate embryo. Despite extensive efforts in blood vessel morphogenesis, most previous work focused on early vascular development and little is known about the cellular organisation underlying the developing organ-specific vasculature. In this study, we investigate how junctional remodelling contributes to the observed variation in blood vessel architectures in the developing brain. To characterize the observed variation, we applied in vivo long-term time-lapse Lightsheet imaging on midbrains of transgenic zebrafish lines and combined it with bioimage analysis. Our observations allowed us to describe in detail the process of blood vessel formation in the midbrain. We discovered that more than 50% of the midbrain vasculature consists of unicellular architectures that appear during the maturation of the blood-brain barrier. This novel finding is in contrast to evidence coming from other vascular beds, where the tissue organization is multicellular (ie. the trunk vasculature, hindbrain, etc). Additionally, we revealed unique cell movements governing the midbrain's expansion and the emergence of unicellular architectures, diverging from conventional angiogenic processes. Furthermore, we discovered that in contrast to the rest of the brain, the midbrain architectures are populated by fewer pericytes, opening up the question of how the blood-brain barrier is established in the midbrain. In conclusion, our study presents a comprehensive model of how brain vascular architectures form and explain the variation of organ-specific vasculature.

Selected Talk

Daria Korotkova, *EPFL, Switzerland*

Role of palmitoylating enzymes in hair cell formation and function on the zebrafish model

Korotkova DD, Valentin G, Coudray A, Abrami L, van der Goot
EPFL, Switzerland

Palmitoylation is a reversible lipid post-translational modification, regulated by a family of 23 zDHHC enzymes. According to the literature, diseases resulting from mutations in zDHHCs, affect a wide spectrum of organs and often resemble ciliopathy symptoms. This led us to hypothesize that mutations in palmitoylating enzymes and the consequent dysregulation of ciliopathy-related proteins may contribute to the development of ciliopathies.
To test our hypothesis, we have chosen the zebrafish model due to its suitability for in-vivo manipulations and observations of primary cilia. Recent single-cell sequencing efforts have revealed the cellular expression patterns of the zDHHC gene family in zebrafish larvae's primary cilia. We have selected a subset of zDHHCs expressed in primary cilia and performed in-situ hybridization to examine their spatial expression. Our findings have demonstrated the presence of multiple zDHHCs (zDHHC2,4,5a,6,16b,18b,23b) in the zebrafish ear, an organ enriched in primary cilia, which was consistent with single-cell data. Subsequently, we down-regulated this subset of zDHHCs and observed cilia-related phenotypes across several zDHHCs (zDHHC4,23b,6).
In order to identify the potential zDHHCs targets among ciliopathy-related proteins, we performed co-expression analysis of ciliopathy-related proteins that have previously been shown to undergo palmitoylation and zDHHC4 using scRNA-seq data. To further validate the potential zDHHC4 targets we plan to use human retinal pigment epithelium cell line, notable for having primary cilia.

Selected Talk

Sarah Brivio; *University of Geneva, Switzerland*

Endothelial *mafa* expression controls HSC expansion in the CHT niche

Sarah Brivio[1], Chris Mahony[2], Meghna Shankar[3], Sabine Costagliola[3], Rui Monteiro[2], Julien Y. Bertrand[1]

1 Department of pathology and immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland; 2 Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, United Kingdom; 3 IRIBHM Université Libre de Bruxelles, Brussels, Belgium

Hematopoietic stem cells (HSCs) arise as a rare population from the hemogenic endothelium of the aortic floor and subsequently colonise the zebrafish caudal hematopoietic tissue (CHT), where they expand extensively. We previously showed that the transcription factor tfec, expressed by the CHT vascular niche, is crucial for HSC expansion by regulating the expression of many non-cell autonomous factors. ATAC-seq performed on zebrafish embryos identified a potential enhancer – in tfec intron 2 –is active only in endothelial cells, and could be bound by transcription factors from the MAF family. Maf genes are highly conserved throughout species. In vertebrates, they are classified into large MAFs and small MAFs. The zebrafish nomenclature follows the same structure, but accounts for teleost genome duplication. We mainly focused on mafa (c-Maf in mammals) which is specifically expressed in the vascular niche, according to our single cell transcriptome analysis. We show that mafa directly regulates tfec expression, thus regulating HSC numbers in the CHT. Indeed, mafa overexpression augmented tfec expression, and HSC numbers, while mafa mutants lose their HSC population, following loss of tfec. Furthermore, we demonstrated that tissue specific loss of mafa in the endothelium (kdrl:GAL4xUAS:DN-mafa) is sufficient to produce the phenotype. Therefore, mafa appears to be an important regulator of HSC expansion in the CHT. We are now directly targeting putative Mafa binding sites in tfec intron 2 by CRISPR/Cas editing and performing transcriptomic analyses to understand the full genetic network controlled by mafa, to discover new pathways involved in the CHT niche.

Keynote lecture 2

Gilbert Weidinger, *Ulm University, Germany*

Mechanisms underlying cellular plasticity during regeneration

One hallmark of aging is a decline in tissue regeneration, which can be caused by DNA replication stress. Whether highly regenerative species like zebrafish are immune from such hindrances to replication is unknown. In contrast to most mammals, adult zebrafish achieve complete heart regeneration via cell cycle entry and proliferation of mature cardiomyocytes. We found that cycling cardiomyocytes experience replication stress, which is induced by the demands of regeneration, but does not occur during physiological heart growth. Since zebrafish cardiomyocyte regeneration is remarkably efficient, heart regeneration appears to depend on elevated capabilities to overcome replication stress. Indeed, pharmacological inhibition of ATM and ATR kinases revealed that DNA damage response signaling is essential for heart regeneration. Using inducible overexpression of ligands and inhibitors of the Bone Morphogenetic Protein (BMP)-Smad pathway, combined with analysis of genetic mutants, we found that BMP signaling alleviates cardiomyocyte replication stress. In the absence of BMP signaling, cardiomyocytes become arrested in the S-phase of the cell cycle, which prevents progression to mitosis and results in heart regeneration failure. Interestingly, BMP signaling can also rescue neonatal mouse cardiomyocytes and human fibroblasts from hydroxyurea-induced replication stress. DNA fiber spreading assays in human cancer cells and human hematopoietic stem and progenitor cells (HSPCs) indicate that BMP signaling acts directly on replication dynamics by accelerating DNA replication fork progression and by facilitating their re-start after replication stress-induced stalling. Our results identify the ability to overcome replication stress as key factor for the elevated heart regeneration capacity in zebrafish. Notably, the conserved capability of BMP signaling to promote stress-free DNA replication might unlock new avenues towards anti-aging and pro-regenerative applications in humans.

Selected talk

Sumeet Pal Singh, *IRIBHM ULB, Brussels, Belgium*

Dutal-to-hepatocyte transdifferentiation contributes to liver regeneration in the presence of spared hepatocytes

Sema Elif Eski 1, Rita Manco 2, Macarena Pozo-Morales 1, Camille Perazzolo 1, Inés Garteizgogeascoa Suñer 1, Anne Lefort 1, Frédérick Libert 1, Jiarui Mi 3, Olov Andersson 3, Sumeet Pal Singh 1
Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM), Brussels, Belgium

Restoration of damaged organs occurs via stem-cell differentiation, self-renewal, or transdifferentiation. Among these, transdifferentiation has a facultative role in regeneration, contributing to organ recovery when the other two sources are not available. This is the case for adult zebrafish and adult mouse liver. Here, the current model posits that upon mild injury, hepatocytes are recovered from self-renewal of spared hepatocytes. Only complete ablation of hepatocytes, or inhibition of cell-cycle in hepatocytes, leads to ductal-to-hepatocyte transdifferentiation, which represents a facultative, injury-specific lineage. However, how liver recovers from partial damage is not well explored. Here, we develop a new transgenic tool that allows partial ablation along with segregation of spared and de novo lineages. With this, we observe that the liver in late larval zebrafish recovers from partial ablation or partial hepatectomy by not conforming to the current model. Here, ductal-to-hepatocyte transdifferentiation drives recovery in the presence of spared hepatocytes, challenging the current model of liver regeneration in vertebrates. We further show that ductal contribution to liver regeneration occurs upon partial hepatectomy of 25 % of the liver. Our results challenge the current dogma in the field of liver regeneration and show that lineage plasticity, even within one organ, can follow different courses with age. Next, we show that recovery from starvation-induced liver atrophy does not lead to ductal transdifferentiation, thereby suggesting that nutrition, potentially via mTORC1 pathway, regulates ductal cell plasticity. Our model provides a platform to understand cellular plasticity during regeneration, an important consideration in regenerative therapy.

Selected talk

Alexa Burger, *University of Colorado School of Medicine, USA*

Conserved enhancers control notochord expression of vertebrate Brachyury

Cassie L. Kemmler 1, Jana Smolikova 2, Hannah R. Moran 1, Brandon J. Mannion 3, Dunja Knapp 4, Fabian Lim 5, Anna Czarkwiani 4, Viviana Hermosilla Aguayo 6, Vincent Rapp 7, Olivia E. Fitch 8, Seraina Bötschi 9, Licia Selleri 6, Emma Farley 5, Ingo Braasch 8, Maximina Yun 4, Axel Visel 3, Marco Osterwalder 7, Christian Mosimann 1, Zbynek Kozmik 2, Alexa Burger 1

1 Department of Pediatrics, Section of Developmental Biology, University of Colorado Anschutz Medical Campus, 2 Institute of Molecular Genetics of the ASCR, 3 Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, 4 CRTD Technische Universität Dresden, 5 Department of Medicine, University of California San Diego, 6 Department of Orofacial Sciences, University of California San Francisco, 7 Department for BioMedical Research (DBMR), University of Bern, 8 Department of Integrative Biology and Ecology, Michigan State University, 9 University of Zürich

Brachyury/T/TBXT genes encode T-box transcription factors that are critical regulators of notochord development, the defining feature of the chordate body plan. The notochord stabilizes the embryonic axis, secretes signaling molecules to guide neural tube patterning, and forms the template for spine development. Although anomalies in notochord patterning and spine formation are major contributors to disabilities worldwide, how Brachyury expression is controlled in normal and pathological conditions is unknown. The gene-regulatory enhancer elements that drive Brachyury/T/TBXT gene expression in the mammalian notochord have long remained elusive. Here, we combine genomic data from human chordoma tumors, evolutionary conservation analysis, cross-species transgenic reporter assays, and mouse knockouts to define three notochord-specific enhancer elements in the human Brachyury/T/TBXTB locus. Using transgenic assays in zebrafish, axolotl, and mouse, we establish the shared versus species-divergent activities of the three

Brachyury-controlling notochord enhancers T3, C, and I in the human, mouse, and marsupial genomes. Acting as Brachyury-responsive, auto-regulatory shadow enhancers, deletion of all three elements in mouse abolishes Brachyury/T/TBXTB expression selectively in the notochord. We document enhancer dose-dependent trunk and neural tube defects downstream of Brachyury/T activity without impact on gastrulation or tailbud formation. Surprisingly, the three Brachyury/T/TBXTB-driving notochord enhancers are conserved beyond mammals in the brachyury/tbxtb loci of fishes, dating their origin to the last common jawed vertebrate ancestor. Our data define an evolutionary ancient, essential enhancer combination dedicated to notochord control of Brachyury/T/TBXTB that advances our understanding of how this central transcriptional regulator is controlled in development and disease.

Selected talk

Jingjing Zang, *University of Zürich, Switzerland*

Recoverin in Visual Transduction and Beyond: New Insights in Cone Vision

Jingjing Zang, Matthias Gesemann & Stephan C.F. Neuhauss
Department of Molecular Life Science, University of Zurich

Recoverin (Rcv) is a neuronal calcium sensor with a low molecular weight, predominantly found in the photoreceptors of the vertebrate retina. In darkness, Ca2+-bound Rcv is suggested to inhibit G-protein-coupled receptor kinase (GRK). Upon exposure to light, Ca2+-free Rcv releases GRK, initiating the phosphorylation of visual pigment, and consequently quenching the visual transduction cascade. While mammals and sauropsids have in general only one recoverin gene (albeit an evolutionary distinct one), amphibians have retained both the sauropsid and the mammalian ortholog, and fish possess up to four recoverin genes. Therefore, it seems likely that efficient phototransduction can be achieved with a single recoverin gene and the increased number of recoverins in teleost species hints towards additional functions for recoverins such as an intracellular Ca2+ buffer or as Ca2+ dependent regulators of other kinases in and outside the visual system. To address this, we utilized a cone-dominant zebrafish retina to explore the function of a cone-specific Rcv through the generation of a CRISPR-Cas9 Knockout (KO) line. Electroretinogram Recording (ERG) results revealed that Rcv did not modulate cone photosensitivity. Interestingly, Rcv KO fish exhibited accelerated response kinetics under medium to low light conditions, with no effects observed under extremely bright light. Additionally, Rcv KO fish demonstrated robust ERG off response across a broad range of light intensities. Collectively, our findings show that Rcv play a role in regulating the visual transduction cascade. Intriguingly, our data suggest an unexpected role beyond the visual transduction cascade.

The Swiss 3R Competence Centre

Andrina Zbinden, *University of Fribourg, Switzerland*

The Swiss Zebrafish Culture of Care Group to increase knowledge and establishing refinements in zebrafish research

Zebrafish models used for studying diseases and basic research are well described in the specialist literature. In contrast, there is a lack of comprehensive knowledge about the needs of zebrafish themselves, in the areas of husbandry, pain management, handling, etc. Interest in these topics has gained momentum in recent years, as evidenced in publications such as a review on the use of anaesthetics in 20191, the FELASA recommendations on zebrafish housing in 20202, the FELASA-AALAS recommendations for monitoring of laboratory fish diseases and health in 20223 and a FELASA working group report on pain management in zebrafish in 20234. At the same time, the concept of culture of care (CoC) is becoming increasingly important in laboratory animal science, which is concerned with improving animal welfare, scientific quality, care of the staff and transparency for the stakeholders. The Swiss CoC Group, which was initiated by the Swiss 3RCC, has established a working group specifically on zebrafish. This Zebrafish CoC Group has set itself the goal of exchanging information and increasing knowledge, to improve the welfare of laboratory zebrafish in Switzerland and help to implement a culture of care. This poster introduces the Swiss Zebrafish Culture of Care group and gives an overview of the planned work objectives.

The Swiss Zebrafish Society

Swiss Zebrafish Society

Stephan Neuhauss, *University of Zürich, Switzerland*

Communications from the European Zebrafish Society
Discussion

Selected talk

Shivali Dongre, *University of Lausanne, Switzerland*

Using zebrafish embryos as a vertebrate in vivo model to study TF clustering

Shivali Dongre1, Nadine L. Vastenhouw1
Center for Integrative Genomics, University of Lausanne, Switzerland

Transcriptional machinery is clustered in the nucleus. We and others have previously shown that this is important in the context of transcription regulation (Kuznetsova et al. 2023; Ugolini et al., 2024; Boija et al., 2018). It is unclear, however, what regulates the clustering of gene specific transcription factors (TFs). Most TFs have a DNA binding domain (DBD), and one or more intrinsically disordered regions (IDRs). Here, we use the zebrafish embryo to study the contribution of DBD and IDRs to TF clustering. Early zebrafish embryos can be injected with various mRNA constructs, which, after translation, can be imaged over several cell cycles. Thus, they provide a good in vivo model to study the nuclear organization of TFs. We focused on the pioneer factors Nanog, Sox19b, and Pou5f3, important for zygotic transcription and early development. We performed live imaging of nuclear clusters, combining different mutant backgrounds, ploidy manipulated embryos, and expression of different wild-type, mutant, and chimeric constructs to understand the molecular mechanism of TF clustering in the nucleus. Our data revealed that DNA binding governs the specificity of TF clustering, while IDRs contribute in a sequence-non-specific manner. These data add to the existing model on clustering of the transcriptional machinery, and shed light on the underlying mechanisms.

New PI

Johannes Larsch, *University of Lausanne, Switzerland*

Neurogenetics of Social Affiliation in Zebrafish

Many species live in groups and affiliate with conspecifics upon sensory detection and processing of social information. However, investigating sensory processing during social behavior is inherently difficult because in most cases, the mutual interactions between individuals and the resulting sensory experience are beyond experimental control. We investigate affiliation pathways in juvenile zebrafish in the context of shoaling, the innate and perpetual drive to swim in groups with continuously moving conspecifics. Using virtual reality psychophysics, we recently identified self-like biological motion as one visual trigger of shoaling. We traced biological motion into the brain and discovered a specifically tuned tecto-thalamic visual pathway that detects this social signal and drives shoaling. We now use the tools available in zebrafish for whole-brain activity mapping and cell type discovery to generate a more complete picture of the neuronal implementation of shoaling. Using candidate screening and artificial selection for extreme social behavior, we have identified a set of socially diverging zebrafish lines to investigate how genetic polymorphisms alter the neuronal processing of social cues. Thus, we can now investigate how individuals coordinate social affiliation at the interface of behavioral algorithms, neuronal circuits, and genetic factors.

ABSTRACTS OF POSTERS

Poster 1

Shayan Shami Pour, *University of Zürich, Switzerland*

Mechanisms underlying symmetry breaking in embryonic development

Shayan Shami Pour, Florian Curvaia, Chiara Rebagliati, Daniel Hannuschke
Department of Molecular Life Sciences, University of Zurich

During development, initially un-patterned cells obtain distinct identities to collectively give rise to an organism. To accomplish this, cells must integrate multimodal signals from their extracellular environment with their internal cellular state. Yet, how signalling and cellular state features function together to ensure robust and orchestrated cell fate specification during embryonic development remains largely unknown. To answer this question, we aim to determine the mechanisms underlying cellular decision-making at the onset of zebrafish gastrulation upon germ layer segregation. To that end, using novel methods of multiplexed immunofluorescence imaging and in situ hybridisation, we have simultaneously mapped, at single-cell resolution across the whole embryo, the activity of signalling pathways that play a role in cell fate specifications, the physicochemical properties of the cells and their surroundings, and the transcript/protein abundances of lineage-specific markers, thereby generating signalling, cell state and cell fate landscapes. Furthermore, by employing machine learning-based approaches and information theory to measure the mutual information between those landscapes, we demonstrate how mechano-chemical signalling and cellular properties regulate cell fate. We find that each signalling pathway provides partially non-redundant but limited information for cellular decisions and that only by considering the entire signalling landscape and the heterogeneities in cellular state properties the spatiotemporal emergence of the various cell types observed within the embryo can be predicted.

Poster 2

Arianna Cuoco, *University of Zürich, Switzerland*

Investigation of genetic modifiers in ciliopathies using zebrafish models

Arianna Cuoco, Alexandra Züger, Claudia Hofmann, Ruxandra Bachmann-Gagescu
Department of Molecular Life Sciences, University of Zurich

Ciliopathies are a group of recessive Mendelian disorders caused by dysfunction of primary cilia. These ubiquitous organelles protrude from the cell body and are involved in signal transduction. Cilia are microtubule-based structures organized in subcompartments: a modified centriole, the basal body, anchors the cilium in the cell while just apical to it, the transition zone (TZ) controls the protein and lipid composition of the ciliary membrane. Several multi-protein complexes of ciliopathy-associated proteins have been described at the TZ. Ciliopathies are characterized by genetic heterogeneity and phenotypic variability, in particular for the progressive retinal and renal symptoms. This variability is also observed between patients sharing the same causal mutations, suggesting a role for genetic modifiers. To investigate putative modifiers and to determine whether loss-of-function of genes encoding proteins belonging to the same ciliary subcompartment has a stronger or weaker combined effect than when non-interacting proteins are involved, we generated double mutants for ciliopathy genes. Interestingly, we found a phenotypic enhancement, indicating a genetic interaction, between some but not all TZ-protein encoding genes: while single cep290 and rpgrip1l mutants display no retinal morphological anomalies, cep290;rpgrip1l mutants have severe retinal dystrophy with early onset photoreceptor loss. Moreover, the cc2d2a retinal phenotype was enhanced by mutations in rpgrip1l. In contrast, cc2d2a;cep290 double mutants appeared indistinguishable from cc2d2a mutants. Taken together our current findings suggest that only specific combinations of transition zone proteins can phenotypically affect each other, to which we will follow up by investigating if this is correlates to a physical interaction.

Poster 3

Alexandra Noble, *University of Zürich, Switzerland*

Shared and unique consequences of Joubert Syndrome gene dysfunction on the zebrafish central nervous system

Alexandra Noble1,5*, Markus Masek1*, Claudia Hofmann1 , Arianna Cuoco1 , Tamara Rusterholz1, Hayriye Özkoc1, Nadja Greter1, Nikita Vladimirov2,3,5, Sepp Kollmorgen5, Esther Stoeckli1,5, Ruxandra Bachmann-Gagescu1,4,5

1 Department of Molecular Life Sciences, University of Zurich, 2 Zurich Neuroscience Center (ZNZ), 3 Institute of Neuroinformatics, University of Zurich and ETHZ, 4 Institute of Medical Genetics, University of Zurich, 5 URPP Adaptive Brain Circuits in Development and Learning (AdaBD), University of Zurich

Joubert Syndrome (JBTS) is a neurodevelopmental ciliopathy characterised by a midbrain-hindbrain malformation often associated with additional neurological phenotypes. Ciliopathies arise from dysfunctional primary cilia, small sensory organelles present on most vertebrate cells. Causative mutations in over 40 primary cilia genes have been identified for JBTS, and these genes encode proteins with variable localisations and functions throughout the primary cilium. However, we still do not understand how the dysfunction of such diverse proteins results in the neurological phenotypes seen in JBTS. To investigate the consequences of JBTS gene dysfunction on the CNS, we use zebrafish models for the JBTS-associated genes cc2d2aw38, cep290fh297, inpp5ezh506, talpid3i264 and togaram1zh510. We use whole-mount imaging to show that JBTS mutant larvae have abnormal primary cilia throughout the brain, including on Purkinje and eurydendroid cells in the cerebellum, however the morphology of the cerebellum and other brain regions is unaffected. Nonetheless, transcriptomic analysis indicates an enrichment of gene sets related to CNS function in these mutants. Furthermore, automatic movement tracking with the Zebrabox shows that cc2d2aw38 mutant larvae have reduced and abnormal locomotion. Taken together, these transcriptional and behavioural analyses suggest that zebrafish JBTS mutants have underlying abnormalities in neural circuit function. We propose that these zebrafish JBTS models therefore offer a unique opportunity to study the role of primary cilia in neural circuit function.

Poster 4

Jacqueline Kientsch, *University of Zürich, Switzerland*

Glucose supply and aerobic glycolysis define a retinal metabolic landscape essential for vision

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Glucose is the main carbon source for cell energy metabolism, critical for ATP production and biosynthesis. In the retina, a modified Astrocyte-Neuron Lactate Shuttle enhances metabolic output, supporting proper function. Photoreceptors are central to this metabolic landscape, where glycolysis supports surrounding cells' energy needs via lactate. This feature likely contributes to photoreceptor susceptibility to genetic alterations or degeneration. Thus, energy metabolism and a collaborative metabolic environment are crucial for retinal function and vision, though the precise in vivo dynamics are not well understood. We focused on retinal glucose transporters (Gluts) and lactate dehydrogenase subunits (Ldhs) to study glucose uptake and lactate production in a cone-dominant retina. Glut1a/1b were identified as glucose transport mediators in the outer retina, where Ldh levels were highest, suggesting reliance on aerobic glycolysis is localized to the outer retina. We used Seahorse® for assessing the metabolic profiles of larval eyes to probe their bioenergetics. Together with additional analysis of Glut and Ldh mutant retinas, we observed that preventing outer retina glucose entry or lactate production resulted in a significant decrease of retinal glycolysis, leading to morphological alterations culminating in vision loss. These findings highlight the importance of aerobic glycolysis in cone photoreceptors for retinal integrity. Even partial disruption of glucose entry or lactate production reduced

glycolysis, causing degeneration and vision loss that could not be compensated by mitochondrial metabolism. Together, this study emphasizes the irreplaceability of glucose and lactate in the retinal metabolic landscape and paves the way to uncover metabolic mechanisms in retinal diseases.

Poster 5

Enno Bockelmann, *University of Zürich, Switzerland*

The role of radial glia and microglia in neuronal clearance during brain development

Enno Bockelmann and Francesca Peri

Department of Molecular Life Sciences, University of Zurich

During brain development, many neurons that fail to integrate into functional circuits undergo programmed cell death. Microglia, the specialized phagocytes in the brain, play a central role in clearing these dying cells to prevent necrosis and the release of potentially harmful molecules. Although microglia play this crucial protective role, mice and zebrafish lacking specialized brain phagocytes are viable until adulthood. This raises the question of how the neuronal corpses are dealt with under these circumstances. The involvement of other cell types in neuronal clearance remains largely unexplored. Here, I utilize advanced long-term in vivo imaging combined with cellular reporters to visualize radial glia and gain insights into their behavior and their interplay with microglia during brain development in zebrafish larvae. Coupled with a live reporter for apoptosis, I visualize the radial glia's interaction with dead neurons and assess their phagocytic behavior in the presence and the absence of microglia. By integrating perturbations and newly developed real-time reporting tools into this workflow, I aim to uncover the intrinsic molecular and cellular mechanisms governing phagocytic behavior in radial glia and their role in neuronal clearance.

Poster 6

Veronica Akle, *University of Zürich, Switzerland*

Effectivity and efficacy of bioactive compounds against medulloblastoma: comparison between in vitro and in vivo

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Medulloblastoma accounts for nearly 10% of all brain tumors in children. Despite advances in treatment, there is a need for effective therapeutics that target the biological mechanisms that promote tumor progression and limit metastatic invasion. The aim of this work is to contribute to the discovery of such therapeutics by investigating toxicity and validating anti-tumor efficacy of repurposed and de-novo designed compounds, early in the discovery process. Three-dimensional in vitro invasion and viability assays were used to identify efficacious compounds that suppress medulloblastoma cells invasion and viability. In vivo toxicity of shortlisted compounds was evaluated by morphometric screening using a VAST BioImager system combined with computational image analysis. Effect of the compounds in tumor size and cell dissemination was assessed in vivo using fluorescent protein-labeled tumor cells xenografted into zebrafish larvae. Morphometric analysis revealed slight differences in toxicity between molecules. Similarly, the best de novo designed compounds were chosen based on low targeted toxicity in vivo that also caused significant suppression of tumor cell invasion in vitro. Medulloblastoma tumor cells from lines HDMB03, D425 and ONS76 were confirmed in 95% of injected larvae, and tumor cells were still visible up to 5 dpf in about 80% of xenotransplanted larvae. The tumor-suppressive effect of selected compounds is currently being systematically determined in vivo. The zebrafish larval model is well suited for up-front assessment of toxicity of new compounds in vivo using image-based morphometric analysis.

Poster 7

Danin Dharmaperwira, *University of Lausanne, Switzerland*

Studying brain circuits of kin imprinting

Danin Dharmaperwira and Johannes Larsch
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The basis of human cooperation is often a relationship of trust, acquired through repeated instances of interactions between individuals. Likewise, cooperation based on familiarity can be found in other animal species, including primates, bats, rodents, birds, and fish, in the form of reciprocal, altruistic, and kin-biased behaviour. Thus, social bonding increases survival by facilitating cooperative tasks such as gathering of food and predator avoidance. A prerequisite to form such a social bond is the ability to differentiate between individuals. Historically, it has been challenging to pin-point the salient stimuli that define the identity of an individual. In mice, for example, olfaction is thought to underly this recognition, while primates are thought to do so mainly through vision. However, where, in the brain this identity information is encoded, and if such circuits are conserved between species remains an open, fundamental question in social neuroscience. Zebrafish emerge as a powerful model of kin recognition and imprinting. Past 8-days of age, zebrafish larvae preferentially choose to swim in water conditioned with odour of kin, rather than non-kin animals, but only if they have been exposed to kin odour during a critical window at 5-6 days of age. Behavioural evidence suggests that zebrafish sequentially acquire visual and olfactory templates, respectively, during this time window. However, the nature of the olfactory kin cue and the neural circuits for its detection are still unknown. During my PhD, I will leverage the zebrafish' optical and genetic accessibility. To this end I will combine quantitative behavioural analysis, cellular resolution whole-brain activity mapping and genetic circuit perturbation. These experiments will reveal brain areas and cell types mediating the neural representations of individual identity in a vertebrate model system and generate a starting point for comparison with other species.

Poster 8

Giacomo Miserocchi, *IRCCS IRST, Meldola, Italy*

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

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Tumor masses induced in animals remain difficult to control and characterize. Zebrafish xenograft is an excellent option except for the lack of reproducibility of injected single cell suspensions, which does not reproduce spatio-temporal distribution and composition of cancer tissues. A strategy to overcome these limitations is the integration of different validated and characterized models. Our proposal is to perform the transplantation of 3D cultures in zebrafish embryos in order to combine biomimetic systems and in vivo models to better understand the cancer diseases biology. We developed spheroids using immortalized cancer cell lines only or in combination with 3T3 fibroblast cells. 3D cultures were stained and transplanted into the yolk sack of 48 hpf 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. Several cell concentrations were used to develop spheroids with a relative size of 1/3 to ½ of the yolk sack dimension. The transplantation process resulted compatible with the larvae viability, which did not display abnormal alterations during embryogenesis. This technique also allows the recovery of cancer cells through the damage of the yolk membrane. 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. This method provides a tool to better understand the pathophysiological tumor processes in order to accelerate information transfer from bench side into clinical practice.

Poster 9

Melina Köhler, *IBCS-BIP, Karlsruhe Institute of Technology, Germany*

sox1a:eGFP transgenic line and single-cell transcriptomics reveal the origin of zebrafish intraspinal serotonergic neurons

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IBCS-BIP, Karlsruhe Institute of Technology, Germany

The vertebrate spinal cord comprises an enormous diversity of functionally distinct cell types which arise from a variety of different progenitor domains. However, the progenitor populations and the mechanisms underlying the differentiation process of specific neurons often remain elusive. Transcription factors of the Sox family play a critical role in vertebrate central nervous system development and are expressed in neural progenitor cells and their derived neurons of the ventral spinal cord in zebrafish. In this study, the transcriptome of sox1a lineage progenitors and neurons from the transgenic zebrafish Tg(sox1a:eGFP) line was obtained, sequenced, and analyzed at four different time points. Using scRNA-seq, we have found that sox1a is also expressed in late-born intraspinal serotonergic neurons (ISNs) in addition to the previously characterized sox1a-positive neurons. Developmental trajectory analysis and ablation of the lateral floor plate (LFP) by morpholino knockdown indicate that the LFP progenitor population is the origin of ISNs. Furthermore, inhibition of Notch signaling revealed the involvement of this pathway in timely ISN differentiation. These results suggest that the zebrafish LFP is not only a source of earlier developing KA'' and V3 interneurons but also of ISNs.

Poster 10

Konrad Marx, *University of Geneva, Switzerland*

Regulation of cell proliferation in regenerating zebrafish scales

Konrad Marx, Simone Cicolini, Guillaume Salbreux, Alessandro De Simone
Department of Genetics and Evolution, University of Geneva

Cell proliferation needs to be precisely controlled for a regenerating body part to grow back to its original size and shape. Difficulties in visualizing cell signals and behaviours in vivo limited our understanding of how signalling pathways orchestrate cell proliferation in adult regenerating tissues. We investigate this question in regenerating zebrafish scales using live imaging and transgenic biosensors. Scales are appendages including a bone disk deposited by a monolayer of osteoblasts. After scale loss, new osteoblasts form by differentiation and then proliferate for about two days. Thereafter, osteoblasts stop proliferating but continue growing in size (hypertrophy) driven by Erk activity waves. We find that Erk activity is also required for osteoblast proliferation. Thus, we characterized its pattern using a live sensor: Erk is initially uniformly active, then it switches off starting from the scale center. Intriguingly, osteoblasts appear divided in two populations: a first population proliferates following Erk activation, a second one grows hypertrophically. Single-cell and bulk transcriptomics show that these two populations are, respectively, less and more mature osteoblasts. We find that mature osteoblasts appear centrally in the scale and extend in the entire tissue, similar to the pattern of Erk deactivation. Remarkably, the hypertrophic region follows the growing edge of the bone, suggesting that osteoblast-secreted bone matrix drives their maturation and Erk switch-off. We are now using new transgenic markers of osteoblast maturation and perturbations to investigate this hypothesis. Overall, this project is revealing how the interplay of signals, bone formation and cell state controls appendage regeneration.

Poster 11

Vincent Hisler, *University of Fribourg, Switzerland*

Skeletal muscle regeneration after extensive cryoinjury of caudal myomeres in adult zebrafish

Hendrik Oudhoff; Vincent Hisler; Florian Baumgartner; Lana Rees; Dogan Grepper; Anna Jaźwińska

Department of Biology, University of Fribourg

Skeletal muscles can regenerate after minor injuries, but severe structural damage often leads to fibrosis in mammals. Whether adult zebrafish possess the capacity to reproduce profoundly destroyed musculature remains unknown. Here, a new cryoinjury model revealed that several myomeres efficiently regenerated within one month after wounding the zebrafish caudal peduncle. Wound clearance involved accumulation of the selective autophagy receptor p62, an immune response and Collagen XII deposition. New muscle formation was associated with proliferation of Pax7 expressing muscle stem cells, which gave rise to MyoD1 positive myogenic precursors, followed by myofiber differentiation. Monitoring of slow and fast muscles revealed their coordinated replacement in the superficial and profound compartments of the myomere. However, the final boundary between the muscular components was imperfectly recapitulated, allowing myofibers of different identities to intermingle. The replacement of connective with sarcomeric tissues required TOR signaling, as rapamycin treatment impaired new muscle formation, leading to persistent fibrosis. The model of zebrafish myomere restoration may provide new medical perspectives for treatment of traumatic injuries.

Poster 12

Lana Rees, *University of Fribourg, Switzerland*

Zebrafish notochord lacks regenerative capacity after embryonic tail amputation

Lana Rees and Anna Jaźwińska
Department of Biology, University of Fribourg

The notochord is an embryonic midline structure that provides cues for development of the segmented axial skeleton in vertebrates. In zebrafish, larval tail amputation results in regeneration of a phenotypically normal fin. In this model, however, the restoration of the notochord remains insufficiently characterized. To determine the restorative capacity of the notochord, we performed resections of the embryonic tail at 4 days post-fertilization and monitored the regenerative process throughout development till adulthood. Consistent with previous studies, fin fold regrew within a few days after amputation, whereas the notochord healed with a callous-like structure, called a notochord bead. Using a regeneration reporter, careg:EGFP, we found that the callous persisted throughout 4 weeks of development, suggesting a failure in the reconstruction of the original notochord tip. Morphometrical analyses of juvenile fish revealed a shorter body and fewer post-abdominal vertebra in the group after tail amputation, compared to uninjured control. This finding indicates that the truncated axial length failed to regrow even after one month. In adult zebrafish, the caudal fin had a normal structure in both groups, nevertheless, the endoskeleton displayed persisting malformations due to embryonic tail amputation. These anatomical abnormalities suggest a disruption of the notochord-associated organizers that pattern this region. Thus, the notochord size and morphogenetic function were not restored after partial amputation. Given that many other zebrafish organs, such as the caudal fin and heart, can be fully restored after injury, this study provides a non-regenerative example, indicating an organ-dependent heterogeneity in the regenerative capacity in this species.

Poster 13

Rebecca Leech, *University of Fribourg, Switzerland*

Investigating heterogeneities of skeletal muscle fibers during homeostasis and regeneration in adult zebrafish

Rebecca Leech, Hendrik Oudhoff, Florian Baumgartner and Anna Jaźwińska

Department of Biology, University of Fribourg

Zebrafish lateral musculature consists of profound fast- and superficial slow-twitch fibers, classified according to their distinctive physiological and structural properties. The subpopulations of each muscle types have not yet been deeply characterized in adult zebrafish. Focusing on the region of the caudal peduncle, we identified that the *careg:EGFP* transgenic reporter is expressed in an inner layer of the slow muscle compartment. This pattern complements the expression of the *slow myosin heavy chain 1 (smyhc1)* reporter line, which is restricted to the outer layers of slow muscles. The non-overlapping expression of both reporters suggests differences within the slow myofiber population. Following myomere cryoinjury, both reporters were upregulating in regenerating myofibers, but at different stages of new fiber formation. This finding suggests that during regeneration, the intermediate differentiation state might transiently involve a slow muscle property, irrespectively of the terminal muscle identity. To investigate heterogeneities in skeletal muscle fibers and the regenerative dynamics of myogenesis, we will perform single myofiber RNA-sequencing. This study will expand knowledge on the diversification of myofibers during homeostasis, as well as during regeneration after inducing profound myomere injury in adult zebrafish.

Poster 14

Minh K. Y. Pham, *University of Fribourg, Switzerland*

The platyfish caudal fin as a muscularized limb model in regenerative biology

Minh K. Y. Pham, Lana Rees and Anna Jaźwińska

Department of Biology, University of Fribourg

The zebrafish caudal fin provides a valuable model of appendage regeneration in vertebrates. However, its translational suitability is limited because bony elements of the fin, called rays, lack the functional association with skeletal muscles, like in a tetrapod limb. Our laboratory has previously discovered that the platyfish (Xiphophorus maculatus, the Poeciliidae family), evolved an innovative bauplan of the caudal endoskeleton. Here, we report that this species possesses another novelty among teleosts, the presence of ray-associated musculature in the basal part of the fin. We found that the muscle fibers of rays and inter-rays are reversely oriented, suggesting a contractile cooperation between bone and soft tissue compartments. This anatomy suggests that the muscularized caudal fin displays some analogy to a tetrapod limb. To determine whether such a limb-like fin can regenerate skeletal muscles, we analyzed tissue regrowth after amputation. We found that wound healing and blastema formation were associated with a partial reshaping of the remaining skeletal muscle. While the progression phase was advancing, muscle regeneration started only at 14 days post-amputation. This delay suggests that the regrowth of the fin size and muscle regeneration were guided by asynchronous mechanisms. The initiation of muscle restoration was associated with differentiation of bony rays, indicating a functional dependence of muscle regeneration on bone morphogenesis. The complexity of the platyfish muscularized fin will provide new insights into the evolutionary concepts of limb restoration in vertebrates.

Poster 15

Nadia Mercader, *University of Bern, Switzerland*

Cox7a1 controls skeletal muscle physiology and heart regeneration through complex IV dimerization

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The oxidative phosphorylation (OXPHOS) system is intricately organized, with respiratory complexes forming super-assembled quaternary structures whose assembly mechanisms and physiological roles remain under investigation. Cox7a2l, also known as Scaf1, facilitates complex III and complex IV (CIII-CIV) super-assembly, enhancing energetic efficiency in various species. We examined the role of Cox7a1, another Cox7a family member, in supercomplex assembly and muscle physiology. Zebrafish lacking Cox7a1 exhibited reduced CIV2 formation, metabolic alterations, and non-pathological muscle performance decline. Additionally, cox7a1-/- hearts displayed a pro-regenerative metabolic profile, impacting cardiac regenerative response. The distinct phenotypic effects of cox7a1-/- and cox7a2l-/- underscore the diverse metabolic and physiological consequences of impaired supercomplex formation, emphasizing the significance of Cox7a1 in muscle maturation within the OXPHOS system.

Poster 16

Benedetta Coppe, *University of Bern, Switzerland*

A paternal cardiac injury induces cardiac adaptation in the next generation

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Organisms are constantly exposed to environmental stimuli that activate genetic and epigenetic responses in the cells. Usually, epigenetic modifications in the gametes are removed upon fertilization. However, if they escape embryonic reprogramming, they can be transferred from one generation to another, resulting in new phenotypic traits. Diet alteration, chemical exposure, and early life trauma experienced by ancestors influence the health of the subsequent generations. In this study, we investigated whether a cardiac injury in zebrafish, which can regenerate its heart, can also influence the immediate progeny. We used multiple omics approaches to assess the effects of a cardiac injury on male gametes and observed differences in the spermatozoa of uninjured and injured fish. Next, we investigated whether the paternal history of cardiac damage induces changes in the offspring. We crossed uninjured and injured males and observed alterations in the cardiac transcriptome of the F1 generation under physiological conditions. We wondered if these changes affect the cardiac regenerative abilities of the offspring. We injured the F1 generation and observed

altered cardiac remodeling abilities in the offspring of injured fathers. Interestingly, these changes were influenced by the time when the father was crossed after the injury, early after the pro-inflammatory peak, or upon regeneration. Overall, our results disclose the intergenerational transmission of cardiac damage "memory" from the father to the immediate offspring. Investigating the effects of cardiac injury in the following generation opens an unexplored field of research and offers a new perspective in the discovery of factors influencing cardiac health.

Poster 17

Ines Marques, *University of Bern, Switzerland*

Changes in collagen cross-linking through lysyl oxidase chemical inhibition affects zebrafish heart regeneration

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Formation of scar tissue, which cannot be replaced by healthy tissue is thought to be one of the main causes of heart failure after myocardial infarction. Scar stiffness has been linked to the maturation of collagen fibres, one of the main components of the extracellular matrix (ECM). The process of collagen maturation occurs through the cross-linking of the more immature collagen fibre, which is mediated by a family of enzymes known as lysyl oxidases. To evaluate if reduced ECM stiffness could improve heart regeneration, we chemically inhibited lysyl oxidases immediately after heart injury, hence impairing collagen cross linking in the early stages of ECM deposition. Our data showed that reduced ECM maturation impaired heart regeneration, by delaying the formation of the compact layer and reducing cardiomyocyte proliferation, indicating that early formation of the ECM, with correct stiffness, plays an essential role in heart regeneration.

Poster 18

Mayuko Harada, *University of Bern, Switzerland*

The role of tRNA modifying enzymes during zebrafish development

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Chemical modifications of transfer RNA (tRNA) are critical for accurate and efficient translation, and their absence can induce codon-specific translation defects, thereby triggering protein aggregation. Wobble uridines (U34) are generally modified in all species. In eukaryotes, the elongator and Urm1 pathways synthesize 5-methoxycarbonylmethyl-2-thiouridine (mcm5s2U). While these pathways were extensively studied in yeast, their role in vertebrate development remains unclear. Furthermore, we know very little about tRNA biology in zebrafish, as there are currently >8600 predicted tRNA genes in Danio rerio compared to approximately 430 in humans. We perform modification-aware tRNA sequencing to thoroughly characterize tRNA gene expression and the regulation of tRNA modifications across tissues and during development. Interestingly, our results show a higher diversity of isodecoders expression in earlier embryonic stages compared to later stages. Furthermore, our data suggest that specific types of isodecoders are differentially regulated during development and differentiation. To better understand the role of U34 during development, we have inactivated U34-modifying enzymes by CRISPR-Cas9. Adult U34 mutants are significantly smaller than wild type and display a lower survival rate. Finally, mutant females are fertile, while mutant males do not mate with female fish. This shows that fish rely on U34 modifications during development and that their absence impairs their reproduction. We are currently investigating whether male sterility is mainly caused by defective sperm function or from behavioral abnormalities. In the future, we will analyze changes in mRNA translational caused by the tRNA modification defects.

Poster 19

Ahmed Elhelbawi, *University of Bern, Switzerland*

The role of N6-methyladenosine (m6A) in vertebrate development

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RNA contains multiple modifications crucial for regulating gene expression and RNA metabolism. N6-methyladenosine (m6A) is the most predominant eukaryotic mRNA modification. It is introduced by a complex containing the catalytic subunit METTL3 along with METTL14 and WTAP. Importantly, m6A can be erased by specific demethylases, suggesting its dynamic use for controlling gene expression. m6A is implicated in diverse biological processes ranging from neurodegenerative diseases to multiple cancers. However, unraveling the intricate mechanistic roles of m6A in these processes in vivo remains a fundamental challenge. To elucidate these mechanisms, we deleted Mettl3 in zebrafish, resulting in mettl3-/- fish mortality within a month of fertilization. Using in-situ hybridization we found that mettl3 is ubiquitously expressed during early embryonic development. Subsequently, the expression is restricted to the brain. Therefore, we combined RNA-seq and single-cell RNA-seq of Mettl3-/- mutant heads to dissect the molecular phenotypes. We found that genes associated with eye diseases are dysregulated and that several eye-specific cell types are underrepresented in the mutants. Histological analysis revealed morphological changes of the mutant retinas, while electroretinography uncovered striking visual defects. Furthermore, mettl3-/- mutants displayed defects in locomotor activity in automated dark-light transition experiments. Notably, Mettl3 orchestrates eye development by regulating the expression of specific eye-related genes. Finally, we found that mutant cells respond to m6A absence by autoregulating the splicing of wtap, the scaffold member of the m6A-writer complex. Our work provides a framework for understanding how m6A functions during vertebrate development and will help to develop treatment strategies for m6A-related diseases.

Poster 20

Jialin Liu, *Biozentrum, University of Basel, Switzerland*

Single-cell multi-omics and deep learning decode regulatory logic for early specification and differentiation

Jialin Liu and Alex Schier
Biozentrum, University of Basel, Switzerland

The interplay between transcription factors and chromatin accessibility regulates cell type diversification during vertebrate embryogenesis. To systematically decipher the gene regulatory networks and principles during this process, we generated a single-cell multi-omics atlas for RNA expression and chromatin accessibility during zebrafish embryogenesis. We developed a deep learning model to predict the chromatin

accessibility of cis-regulatory elements based on DNA sequence, revealing that a small number of transcription factors determines cell type specific chromatin landscapes. Reconstruction of gene regulatory networks uncovered that mesendoderm-associated cis-regulatory elements are primed by maternally deposited pioneer transcription factors. In addition to the classical stepwise specification-differentiation mode of development, we discovered a novel mode: instant differentiation, in which a flat network of multiple transcription factors co-regulates hundreds of differentiation genes to generate early-differentiating cell types such as epidermal seals and lipid-metabolizing tissues. Our study provides a rich resource to dissect embryonic gene regulatory networks and helps reveal the regulatory logic of differentiation.

Poster 21

Berfin Kartalkanat, *Biozentrum, University of Basel, Switzerland*

Investigation of architectural plasticity of intersomitic blood vessels in zebrafish embryos

Berfin Kartalkanat, Ludovico Maggi, Markus Affolter, Heinz-Georg Belting
Biozentrum, University of Basel, Switzerland

The cardiovascular system is the first organ to become functional during vertebrate embryogenesis. We are studying the morphogenetic mechanisms, which underly the formation of intersomitic blood vessels (ISV). ISVs are arranged in a metameric pattern along the body axis and appear phenotypically identical to each other. However, at the cellular level, by labeling cell-cell junctions, two types of vascular architectures, unicellular and multicellular, can be distinguished - and it has been shown that these vessel types are formed by very different morphogenetic processes. In order to find out, whether the initial vascular architectures are fixed or can be converted, we have followed fluorescent junctional reporters over several days of development. Quantification of junctional patterns showed an increase of multicellular ISV at 72 hpf when compared to 48 hpf. Time-lapse analysis of individual ISV over 2 days of development revealed that (1) unicellular tubes transform into multicellular tubes by cell rearrangements; (2) multi-cellular tubes are stable in their architecture; (3) in rare cases endothelial cells deintercalated to form unicellular tubes with autocellular junctions.These observations demonstrate an overall tendency of endothelial cells to form multicellular cellular tubes by cell rearrangement/intercalation. We are now investigating genetic components that may regulate this behavior. To this end we have generated mutants in zebrafish PI3 kinase genes (i.e. pik3c-aa and -ab), a pathway implicated in blood vessel formation and directed cell migration. We are currently investigating the consequences of loss of PI3 kinase function with respect to endothelial cell rearrangement and ISV architecture.

Poster 22

Ludovico Maggi, *Biozentrum, University of Basel, Switzerland*

Dynamic Regulation of Actomyosin Tension within Junction-based Lamellipodia (JBL) Drives Endothelial Cell Rearrangements during Blood Vessel Morphogenesis

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Angiogenesis is the formation of new blood vessels from pre-existing ones. Performing in vivo high-resolution time-lapse imaging and genetic analysis in zebrafish embryos, we have shown that endothelial cells (ECs) rearrange using oriented, oscillating lamellipodia-like protrusions, which emanate and are connected to EC junctions. We call these structures junction-based lamellipodia or JBL. The observations suggest a novel oscillating ratchet-like mechanism, which is used by endothelial cells to move along or over each other and thus provides the physical means for cell rearrangements. Careful analysis of junctional and cytoskeletal reporters reveals that JBL dynamics can be subdivided into distinct steps: 1)“JBL formation” (protrusion), 2)“double junction” (formation of a new (distal) junction) 3)“junction conversion” (merging of distal and proximal junctions) and 4)“junction stabilization”. In order to better understand the regulatory and physical mechanisms underlying the respective steps of JBL function, we have analysed the spatiotemporal

distribution of myosin with respect to endothelial cell junctions (labeled by VE-cadherin and ZO1) and F-actin. We observe wide-spread myosin within JBL throughout the oscillatory cycle. Subsequently, myosin specifically accumulates between distal and proximal junctions during "junction conversion". In line with this observation, inhibition of actomyosin contractility leads to defects in junction conversion thus abolishing junction elongation. Taken together, our findings suggest that actomyosin contractility between proximal and distal junctions represents the driving force of "junction conversion" and that this mechanism provides, together with the protrusion formation, the motive force for endothelial cell elongation/rearrangement. We are currently interrogating candidate signalling pathways in JBL formation and function.

Poster 23

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The Swiss Zebrafish Culture of Care Group to increase knowledge and establishing refinements in zebrafish research

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Zebrafish models used for studying diseases and basic research are well described in the specialist literature. In contrast, there is a lack of comprehensive knowledge about the needs of zebrafish themselves, in the areas of husbandry, pain management, handling, etc. Interest in these topics has gained momentum in recent years, as evidenced in publications such as a review on the use of anaesthetics in 2019[1], the FELASA recommendations on zebrafish housing in 2020[2], the FELASA-AALAS recommendations for monitoring of laboratory fish diseases and health in 2022[3] and a FELASA working group report on pain management in zebrafish in 2023[4]. At the same time, the concept of culture of care (CoC) is becoming increasingly important in laboratory animal science, which is concerned with improving animal welfare, scientific quality, care of the staff and transparency for the stakeholders. The Swiss CoC Group, which was initiated by the Swiss 3RCC, has established a working group specifically on zebrafish. This Zebrafish CoC Group has set itself the goal of exchanging information and increasing knowledge, to improve the welfare of laboratory zebrafish in Switzerland and help to implement a culture of care. This poster introduces the Swiss Zebrafish Culture of Care group and gives an overview of the planned work objectives.

References

1. Martins T, Valentim A, Pereira N, Antunes LM. Anaesthetics and analgesics used in adult fish for research: A review. Lab Anim. 2019 Aug;53(4):325-341. doi: 10.1177/0023677218815199. Epub 2018 Dec 4. PMID: 30514148.
2. Aleström P, D'Angelo L, Midtlyng PJ, Schorderet DF, Schulte-Merker S, Sohm F, Warner S. Zebrafish: Housing and husbandry recommendations. Lab Anim. 2020 Jun;54(3):213-224. doi: 10.1177/0023677219869037. Epub 2019 Sep 11. PMID: 31510859; PMCID: PMC7301644.
3. Mocho JP, Collymore C, Farmer SC, Leguay E, Murray KN, Pereira N. FELASA-AALAS Recommendations for Monitoring and Reporting of Laboratory Fish Diseases and Health Status, with an Emphasis on Zebrafish (Danio Rerio). Comp Med. 2022 Jun 1;72(3):127-148. doi: 10.30802/AALAS-CM-22-000034. Epub 2022 May 5. PMID: 35513000; PMCID: PMC9334007.
4. Sneddon LU, Schroeder P, Roque A, Finger-Baier K, Fleming A, Tinman S, Collet B. Pain management in zebrafish : Report from a FELASA Working Group. Lab Anim. 2023 Dec 5:236772231198733. doi: 10.1177/00236772231198733. Epub ahead of print. PMID: 38051824.

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