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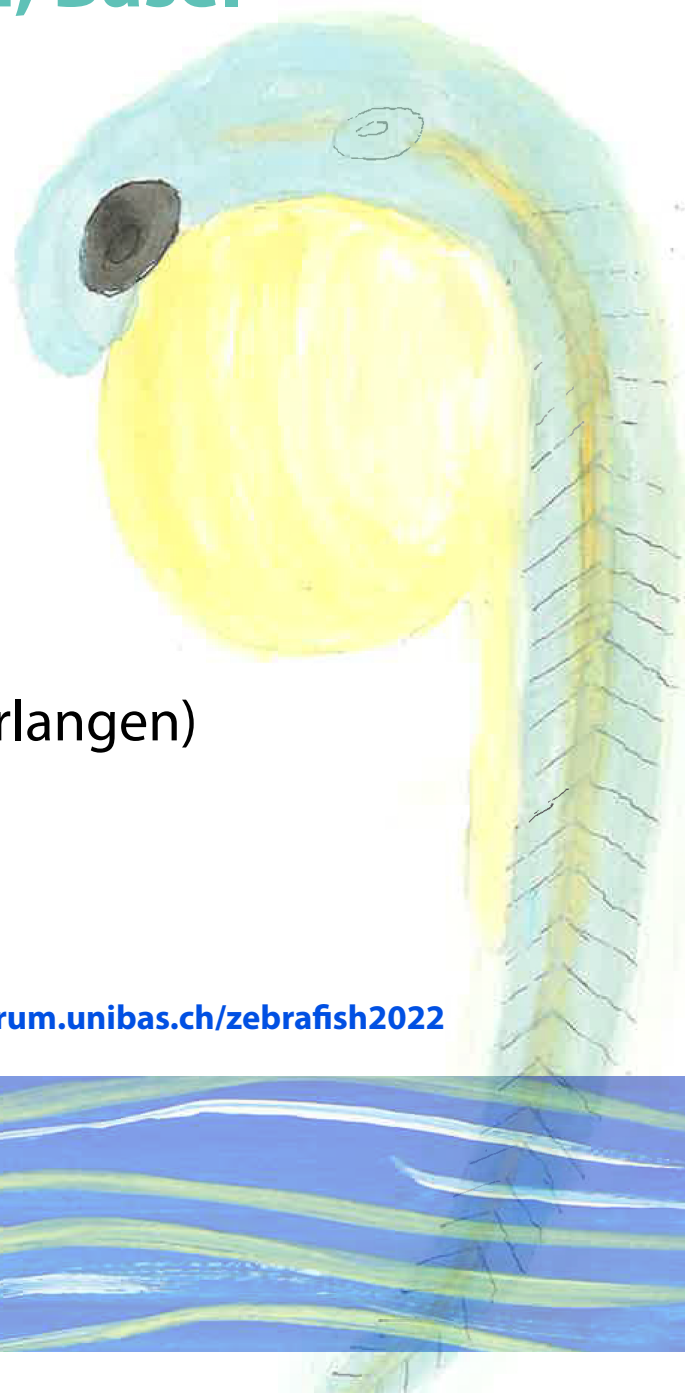
The Center for
Molecular Life Sciences



14th Swiss Zebrafish Meeting

10th June 2022

Biozentrum, Basel



Confirmed Speakers

Herwig Baier

(MPI Neurobiology, Munich)

Wiebke Herzog

(Friedrich-Alexander-Universität, Erlangen)

Alex Schier

(Biozentrum, Basel)

<https://www.biozentrum.unibas.ch/zebrafish2022>

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Swiss Zebrafish Meeting 2022 at the Biozentrum, Basel June 10th 2022

Program:

- 9:00 – 9:45** Registration with coffee and croissants
- 9:45 – 11:20** Opening Remarks and Session 1
- 10:00 – 10:20 *Hybrid cell-lineages and a novel population of plastic δ -cells enable zebrafish to reverse the course of diabetes*
Nikolay Ninov, Center for Regenerative Therapies Dresden, Germany
- 10:20 – 10:40 *Multispectral, live and ultrastructural imaging approaches to study tubular connectivity in the liver*
Sara Caviglia, University of Zurich
- 10:40 – 11:20 *Reconstructing Cellular Biographies*
Alex Schier, Biozentrum, Basel
- 11:20 - 11:50** Poster Session1 and coffee
- 11:50 – 13:15** Session 2
(chair: Stephan Neuhauss)
- 11:50 – 12:10 *Neuronal dynamics and function of the zebrafish forebrain in olfactory perception*
Caudullo Tommaso, Friedrich-Miescher-Institute, Basel
- 12:10 – 12:30 *Spatiotemporal emergence of somatosensory neuron diversity*
Joaquín Navajas Acedo, Biozentrum, University of Basel
- 12:30 – 13:10 *A multimodal digital atlas for mapping genes, circuits and behavior in the larval zebrafish brain*
Herwig Baier, Max-Planck Institute for Biological Intelligence, Munich, Germany
- 13:10 – 14:30** Poster Session 2, lunch break,
guided tour of the Schier lab fish facility
(registered participants only)

14:30 – 16:00	Session 3
14:30 – 14:50	<i>Evolution of pigment patterning in Danio species</i> Uwe Irion , Max-Planck-Institute for Biology, Tuebingen, Germany
14:50 – 15:10	<i>Generating a toolbox for ‘on demand’ manipulation of tissue organization via Rho GTPases in the embryonic zebrafish</i> Robert Bill , University of Zurich
15:10 – 15:30	<i>Mechanics of body axis morphogenesis in zebrafish embryos with robot-assisted microsurgery</i> Ece Ozelci , EPFL, Lausanne
15:30 – 15:50	<i>Cytoneme-mediated transport of active Wnt5b/Ror2 complexes in zebrafish gastrulation</i> Steffen Scholpp , Living Systems Institute, University of Exeter, UK
15:50 – 16:30	Poster Session 3 and coffee
16:30 – 18:00	Session 4
16:30 – 16:50	<i>Zebrafish model to study autophagy and lysosomal processing in zebrafish cardiovascular development</i> Myra Chavez , University of Bern
16:50 – 17:10	<i>In the belly of the microglia: investigating the role of the gastrosome in phagocytosis</i> Joanna Zaręba , University of Zurich
17:10 – 17:50	<i>Development of the Blood Brain Barrier in Zebrafish</i> Wiebke Herzog , Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany
18:00 -	Closing Remarks and Apero, in front of the Lecture Hall

Venue address: The meeting takes place Maurice-E-Müller-Saal (U-1.111) at the Biozentrum, Spitalstrasse 41, 4056 Basel (Switzerland)

Selected Abstracts for Talks

Abstract 1

Hybrid cell-lineages and a novel population of plastic δ -cells enable zebrafish to reverse the course of diabetes

Nikolay Ninov¹

¹Center for Regenerative Therapies, Dresden, Germany

The ability to regenerate β -cells is incomplete in mammals, whereas zebrafish can naturally recover from extreme β -cell destruction and hyperglycemia. However, our understanding of the cellular and molecular processes underlying this regenerative ability is limited. Uncovering the secrets of β -cell regeneration requires new technologies that can track gene expression in multiple cells over time during the process of β -cell regeneration. In this study, we apply a unique blend of in vivo imaging, single-cell genomics and genetics to study de novo β -cell regeneration in adult zebrafish. Using single-cell RNA sequencing to characterize cellular and transcriptional dynamics, we define the gene expression of different pancreatic cells upon β -cell destruction and establish an atlas of β -cell regeneration, spanning the period from β -cell destruction to emergence of new insulin-expressing cells. Among the different cell types, we characterize a previously unrecognized population of plastic δ -cells that undergoes a massive and rapid transdifferentiation, giving rise to insulin-expressing hybrid cells, which share the characteristics of both β - and δ -cells. Using in vivo calcium imaging and cell tracking, we further show that the hybrid cells acquire glucose-responsiveness in the course of regeneration. The overexpression of *dkk3*, a gene enriched in hybrid cells, increases their formation in the absence of β -cell injury. Finally, interspecies comparison at the transcriptomic level shows that the plastic δ -cells are partially related to PP cells in the human pancreas. Our work provides an atlas of β -cell regeneration and indicates that the rapid formation of glucose-responsive hybrid cells contributes to the resolution of diabetes in zebrafish.

Abstract 2

Multispectral, *live* and ultrastructural imaging approaches to study tubular connectivity in the liver

Sara Caviglia^{1,2}, Iris A. Unterweger¹, José María Mateos Melero⁷, Akvilė Gasiūnaitė^{1,3}, Ronja L.S. Heyne¹, Alexandre E. Vanoosthuyse¹, Andres Käch⁷, Francesco Cutrale^{4,5}, Le A. Trinh^{4,6}, Scott E. Fraser^{4,5,6}, Stephan C. F. Neuhaus² and Elke A. Ober¹

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⁵Biomedical Engineering and ⁶Molecular and Computational Biology, University of Southern California, L.A., USA.

⁷University of Zurich, Center for Microscopy and Image Analysis (ZMB), Zürich, Switzerland

During organogenesis, migrating liver progenitors form an organ primordium, where they differentiate into polarized functional units. Hepatocyte apical canaliculi connect to biliary epithelial cells (BECs), giving rise to a joint ductal network. How cell polarity acquisition is spatio-temporally coordinated with tubular connectivity is an open question.

To understand the cellular and molecular mechanisms regulating hepatobiliary network formation, we established *live* imaging at subcellular resolution in the liver and developed FRaeppli (Fish-Raeppli), a genetic next-generation four-colours labeling tool. Cells stochastically express bright membrane- or nuclear-targeted fluorescent protein, compatible with GFP and infrared markers, enabling fast multispectral imaging. High versatility is provided by the Gal4/UAS system together with Cre/lox and/or PhiC31 integrase. Combining FRaeppli with polarity markers revealed previously unknown canalicular topologies between differentiating hepatocytes, reminiscent of the mammalian liver.

Furthermore, similar to mammals, we found that cell polarity acquisition is asynchronous, with BECs forming ramified apical lumina, to which hepatocytes canaliculi fuse significantly later. Interestingly, polarizing hepatocytes show unconventional plasma membrane bleb-like structures, BLiSTRs, directed towards the nascent BEC-network.

To investigate the nature of BLiSTRs and their function, we designed a straightforward method for 3D-correlative light and electron microscopy (CLEM), enabling us to precisely identify liver cell types and their interactions. Thus, we were able to visualize different stages of canalicular formation and apical membrane maturation, including the presence of membrane-rich compartments at the interface between connecting hepatocytes and BECs.

We propose that BLiSTRs may promote hepatocyte polarity orientation and mechanically induce lumen remodeling in neighboring BECs, priming canaliculi connection to ducts.

Abstract 3

Neuronal dynamics and function of the zebrafish forebrain in olfactory perception

Tommaso C. Caudullo^{1,2}, J. Eckhardt, R.W. Friedrich

¹Friedrich Miescher Institute for Biomedical Research (FMI), Basel, Switzerland

²University of Basel

Area Dp of the teleost forebrain is the main synaptic target of the olfactory bulb, and the homolog of olfactory cortex. Its structure and function suggest that it might store odor memories. Consistent with this hypothesis, we found that bilateral lesions of Dp strongly impaired memory recall in an olfactory discrimination task. To explore Dp function in vivo, we measured odor-evoked calcium signals in posterior Dp (pDp) in head-fixed adult fish using a microprism. Results are generally consistent with previous observations in an ex-vivo preparation but contained additional complexity. For example, we found that a strong attenuation of responses after the first application of an odor exhibited a substantially higher stimulus-specificity in vivo than ex vivo. Moreover, odor responses in pDp were temporally structured on a time scale of seconds to tens-of-seconds. This structure contained semi-stable ensembles of active neurons that were separated by sharp transitions. Despite considerable trial-to-trial variability, activity patterns contained consistent and odor-dependent dynamical features. These results support the notion that Dp may mediate multiple functions including novelty detection and memory on different timescales.

Abstract 4

Spatiotemporal emergence of somatosensory neuron diversity

Joaquín Navajas Acedo¹ and Alexander F. Schier^{1,2}

¹Biozentrum, University of Basel, Switzerland

²Allen Discovery Center for Lineage Tracing, Seattle, Washington, USA

The somatosensory system detects a diverse range of sensory stimuli, determined by the molecular and physiological properties of its neurons. How this diversity emerges during development is poorly understood. During zebrafish development, the primary somatosensory system of Rohon-Beard neurons develops first and is thought to disappear, to be then functionally replaced by the secondary somatosensory system, formed by neurons of the Dorsal Root Ganglia. The goal of our project is to discover the molecular and cellular basis of somatosensory neuron diversification. Our preliminary work — combining imaging and single-cell transcriptomics across development— shows that contrary to the 100-year-old paradigm, Rohon-Beard neurons do not disappear during larval stages. Furthermore, our single-cell transcriptomics experiments reveal that the primary and secondary somatosensory systems possess complex neuronal diversity, with shared and exclusive properties. We describe new subclasses of Rohon-Beard neurons and their unexpected heterogeneous axial distribution. Current work aims to use spatial transcriptomics and cell lineage recording to link the observed complex neuron spatiotemporal diversity to cell division and movements during development. This research will help elucidate how neuronal diversity arises and is refined during development using the unique somatosensory system of zebrafish as a model.

Abstract 5

Evolution of pigment patterning in Danio species

Uwe Irion¹

¹Max Planck Institute for Biology, Tübingen, Germany

Pigment patterns are very prominent features of many animals. Their functions range from thermo-regulation and UV protection, to inter-and intra-species communication, e.g., camouflage, aposematism or species recognition. Due to a number of advantages the zebrafish (*Danio rerio*) has become one of the leading vertebrate model organisms to study pigment patterning in recent years. The pattern of the adult fish is highly conspicuous and very reproducible from one fish to another; it is made up from three different types of pigment cells, melanophores, xanthophores and iridophores, that arrange into horizontal light and dark stripes in the skin of the fish. Several mutants are known with disrupted patterns, they allow the identification of genes controlling the process. Other *Danio* species, which are closely related to zebrafish, show an amazing variety of different pigmentation patterns, ranging from horizontal stripes and spots to vertical bars and almost no pattern at all. Based on our knowledge of the patterning process in zebrafish we recently began to investigate the evolutionary changes that lead to this stunning diversity of pigment patterns in the *Danio*-species group. We established breeding colonies of several additional *Danio* species in the lab and started to produce hybrids and gene knock-outs to investigate the genetic changes that underlie the evolutionary diversification of pigment patterns in this group of fish.

Abstract 6

Generating a toolbox for ‘on demand’ manipulation of tissue organization via Rho GTPases in the embryonic zebrafish

Robert Bill¹, Corinna Biermeier, Darren Gilmour and Francesca Peri

¹Department of Molecular Life Science, University of Zurich, Switzerland

Morphogenesis, the process of cells organizing into differentiated tissues, is regulated by signaling pathways and gene regulatory networks that control cell fate decisions. It is becoming clear that the multicellular organization process of developing tissues can feed back to allow contextual control of the differentiation process. However, to investigate the dynamic interplay between tissue organization and differentiation, tools to perturb acutely and on demand the organization of tissues *in vivo* are needed.

In order to change tissue organization at single cell resolution, we are screening potent activators and deactivators of the Rho family GTPases RhoA, Rac1 and Cdc42. Candidate GEFs and GAPs regulators of each GTPase are expressed acutely in the developing zebrafish epidermis, using either chemogenetic (mifepristone) or optogenetic (iLID/sspB system) inducible systems. Their impact on cell morphology and motility is assayed directly by live imaging. Preliminary data indicates that manipulating the three Rho GTPases can alter tissue organisation in a user-defined manner, potentially allowing a more precise investigation into the feedback mechanisms that couple cell differentiation to tissue architecture.

Abstract 7

Mechanics of body axis morphogenesis in zebrafish embryos with robot-assisted microsurgery

Ece Özelçi^{1,2}, Andrew C. Oates² and Mahmut Selman Sakar^{1,2}

¹ Institute of Mechanical Engineering, Ecole polytechnique fédérale de Lausanne, CH-1015 Lausanne, Switzerland

² Institute of Bioengineering, Ecole polytechnique fédérale de Lausanne, CH-1015 Lausanne, Switzerland

Microsurgical techniques that were foundational for experimental embryology in the early 1900s are still in use today. Inspired by these classic techniques we built a user-friendly robotic microsurgery platform which allows precise mechanical manipulation of soft tissues in zebrafish embryos. As a proof of principle, we investigated the contribution of different embryonic tissues to vertebrate anteroposterior axis elongation and segmentation. We targeted specific regions of tail explants, and quantified the response in real-time by following morphogenesis of different tissue parts and segmentation clock dynamics. We found that an extension force is generated through the posterior notochord that is strong enough to buckle the structure. Our data suggest that this force generates a unidirectional notochord extension towards the tailbud because presomitic mesoderm (PSM) around the posterior notochord applies gripping forces and does not let it slide anteriorly. These results complement existing biomechanical models of axis elongation and reveal that tail elongation at the observed developmental stage is driven by coupled interactions of the posterior notochord, the tailbud, and the posterior PSM.

Abstract 8

Cytoneme-mediated transport of active Wnt5b/Ror2 complexes in zebrafish gastrulation

Steffen Scholpp¹

¹Living Systems Institute, University of Exeter, UK

Chemical signaling is the primary means by which cells communicate in the embryo. The underlying principle refers to a group of ligand-producing cells and a group of cells that respond to this signal because they express the appropriate receptors. In the zebrafish embryo, Wnt5b binds to the receptor Ror2 to trigger the Wnt/Planar Cell Polarity (Wnt/PCP) signaling pathway to regulate tissue polarity and cell migration. However, it is still unclear how this lipophilic ligand is transported from the source cells through the aqueous extracellular space to the target tissue. Here we show that Wnt5b, together with Ror2, is loaded on long protrusions. The active Wnt5b/Ror2 complexes are handed over from these cytonemes to the receiving cell to trigger Wnt/PCP signaling, regardless of whether the cell expresses functional receptors. On the tissue level, we show that cytoneme-dependent spreading of active Wnt5b/Ror2 affects convergence and extension in the zebrafish gastrula.

Abstract 9

Zebrafish model to study autophagy and lysosomal processing in zebrafish cardiovascular development

Myra Chavez¹, Alexander Ernst and Nadia Mercader

¹Institute of Anatomy, University of Bern, Switzerland

Autophagy is an evolutionary conserved process key for cellular homeostasis, differentiation and stress survival, which is particularly important to the pathophysiology of the cardiovascular system. Moreover, recent studies suggest that these processes affect correct cardiac organogenesis and tissue regeneration. However, which cells upregulate autophagic processing and what it is required for is unknown. In this work, we generated several zebrafish transgenic tools to visualize autophagosome and lysosome formation in a tissue-specific manner. Using live-microscopy along with image reconstruction algorithms, we could follow the temporal and cellular dynamics of autophagy activation in the developing zebrafish heart from 32 hpf onwards and outlined the kinetics of autophagy activation up to 96 hpf. Interestingly, we distinguished a distinct accumulation of autophagosome and lysosome vesicles in the atrioventricular canal and outflow track and their respective valves. Furthermore, we made use of *spns1*-mutant zebrafish larvae as a genetic model of lysosomal impairment to address the functional role of lysosomal degradation in cardiovascular development. We found severe morphological and functional abnormalities in the developing hearts of *spns1*-mutants, including smaller ventricular size, lower heart rate, retrograde blood flow, impaired valve formation and a higher incidence of heart edema. In addition, we observed that impaired autophagosomal processing in the mutants correlated with an altered expression pattern of genes related to valve development. Our study provides a detailed description of autophagy activation during zebrafish heart development, and proposes an important link between autophagosomal processing and correct cardiovascular development.

Abstract 10

In the belly of the microglia: investigating the role of the gastrosome in phagocytosis

Joanna Zaręba¹, Elena Cattaneo, Francesca Peri

¹Department of Molecular Life Sciences, University of Zurich

Phagocytosis is a central process for immunity and maintenance of tissue homeostasis. In this way, professional phagocytes efficiently remove foreign particles or excess biological material, such as apoptotic neurons, during brain development. While the recognition and engulfment of these apoptotic cells are well understood, subsequent digestion and recycling steps remain mostly unclear, even though they are essential to ensure proper phagocytosis. Recent work in zebrafish microglia and mammalian macrophages led to the discovery of the "gastrosome," a new compartment in the phagocytic pathway. This vesicle fuses with maturing phagosomes and functions as a collection compartment. Increased phagocytosis causes enlargement of the gastrosome and changes in microglial morphology as well as motility. We performed CRISPR and chemical screens to uncover the regulation and role of this newly discovered phagocytic compartment. This led to identifying Npc1 and ABC transporters as important players in phagocytosis and gastrosome biology. The development of new phagocytic markers in zebrafish microglia has allowed real-time mapping of vesicular interactions over time, providing new insights into the spatio-temporal coordination of phagocytosis, the role of the gastrosome, and how microglia react when these transporters are blocked.

POSTER ABSTRACTS

Abstract 11

Autophagy maintains the function of the kidney proximal tubule

Daniela Nieri¹, Zhiyong Chen, Olivier Devuyst & Alessandro Luciani

¹Institute of Physiology, University of Zürich, Zürich, Switzerland

Epithelial cells lining the kidney proximal tubule rely on vesicular membrane trafficking pathways to ensure the reabsorption of essential nutrients. The endosomal-lysosomal system sustains the specialized function of proximal tubule cells capturing and degrading intracellular worn-out constituents through autophagy — an evolutionary conserved process that maintains the cellular homeostasis and functions. Whether autophagy is essential for preserving proximal tubule cells functions and whether it is evolutionarily conserved remain largely unexplored. Taking advantage of model organisms and physiologically relevant cellular systems and combining them with epistasis-driven approaches targeting *Atg7* — an essential gene regulating autophagosome maturation — we disable autophagy in kidney tubular cells. We created a kidney proximal tubule-specific autophagy-deficient mouse model (hereafter *Atg7cKO* mice) and we noted that *Atg7cKO* mice display progressive growth retardation and proximal tubule dysfunction. In *Atg7cKO* derived proximal tubule cells we observed a decreased LC3I-to-LC3II conversion and lower numbers of punctate LC3 structures as well as an accumulation of electron microscopy (EM) structures compatible with autophagy initiation structures/phagophores. To further explore the consequences of autophagy inactivation *in vivo*, we established a whole *atg7* deficient zebrafish model using the CRISPR/Cas9 technology. The homozygous mutant zebrafish display no obvious developmental defects, show a noticeable reduction of life expectancy compared to wild-type littermates and a progressive proximal tubule dysfunction. These changes were reflected by defects in autophagosome biogenesis, mirroring the cellular alterations observed in *Atg7cKO* mice. Taken together, these results indicate that autophagy sustains the homeostasis and transport functions of the kidney proximal tubule, and that this connection is evolutionary conserved.

Abstract 12

Zebrafish platform for high-throughput screens of proximal tubule dysfunction

Zhiyong Chen¹, Alessandro Luciani, Zsuzsa Radvanyi, Stephan C.F. Neuhaus and Olivier Devuyst

¹University of Zurich, Switzerland

The cells lining the kidney proximal tubules reabsorb the ultrafiltered low-molecular-weight (LMW) proteins through the very efficient receptor-mediated endocytosis and endolysosomal system. We have previously developed a transgenic Tg(lfabp:: $\frac{1}{2}$ vdbp-mCherry) zebrafish reporter line for monitoring LMW proteinuria as a consistent and early marker of endolysosomal diseases affecting the kidney proximal tubule. Here we report a newly-generated bioluminescence-based reporter line Tg(lfabp:: $\frac{1}{2}$ vdbp-nanoLuc), in which N-terminal region of vitamin D-binding protein is coupled to nanoLuc luciferase. The $\frac{1}{2}$ vdbp-nanoLuc produced in liver, is secreted into the blood, filtered and reabsorbed in kidney. The detection of $\frac{1}{2}$ vdbp-nanoLuc by luminometry is time- and cost-effective, compared to the mCherry system, more suitable for large-scale screening. Proof-of-concept studies demonstrated that Tg(lfabp:: $\frac{1}{2}$ vdbp-nanoLuc) zebrafish is useful for detecting the LMW proteinuria related to both chemical nephrotoxicity and genetic deletion of endocytic receptor megalin. Moreover, we generated the first zebrafish model of Dent disease by genetic deletion of *clcn5a* and *clcn5b* genes, two orthologues of human *CLCN5*. Urinary analysis revealed abnormal excretion of tracer in 7-day-old *CIC-5* double knockout larvae using both Tg(lfabp:: $\frac{1}{2}$ vdbp-mCherry) and Tg(lfabp:: $\frac{1}{2}$ vdbp-nanoLuc) reporter lines, mimicking the LMW proteinuria observed in patients affected by Dent disease. By developing the Tg(lfabp:: $\frac{1}{2}$ vdbp-mCherry) and the Tg(lfabp:: $\frac{1}{2}$ vdbp-nanoLuc) lines, we have set up a high-throughput zebrafish screening platform relevant for both toxicological testing and phenotypic drug discovery.

Abstract 13

***mettl3* is essential during zebrafish development**

Ahmed Elhelbawi^{1,2}, Antonio Biundo², Theresa Groß-Thebing², Jingjing Zang³, Laura Jahnke⁴, Ursula Jordan⁵, Ngoc Dung Le⁶, Stephen Leib⁶, Erez Raz⁵, Volker Enzmann⁴, Stephan Neuhauss³, Sebastian Leidel^{1,2}

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An exciting finding in recent years was that messenger RNA (mRNA) is modified by a plethora of chemical groups. These modifications play an important role for tuning gene expression and RNA metabolism. N6-methyladenosine (m6A) is the most abundant internal modification of eukaryotic mRNA. It is introduced by a protein complex containing the catalytic subunit Mettl3 and two additional constitutive complex members: Mettl14 and Wtap. Importantly, m6A is reversible and can be removed by the demethylases Fto and Alkbh5 suggesting its use for controlling gene expression. However, how m6A levels are regulated is largely unclear. Furthermore, m6A has been linked to multiple biological processes including hematopoietic stem-cell differentiation, neurogenesis, osteogenesis, and multiple cancers. Understanding the molecular mechanism of how m6A modulates these processes *in vivo*, remains a fundamental challenge. To shed light on these mechanisms, we deleted Mettl3 in zebrafish. Mettl3-knockout fish are born without gross morphological defects. Nevertheless, the mutants die within the first 28 days post fertilization. Using *in-situ* hybridization we found that Mettl3 and other members of the m6A pathway are universally expressed at early stages of embryonic development. Subsequently, their expression is restricted to the brain. Therefore, we combined RNAseq and single-cell RNAseq of Mettl3 mutant heads to identify the source of lethality. We found that genes that are linked to eye diseases are dysregulated in the mutants and that several cell types are underrepresented in the eye. Histological analysis revealed a significant size reduction of the mutant eyes, while electroretinography uncovered striking visual defects in the mutants. Furthermore, analysis of locomotor activity of the mutants in automated dark-light-transition experiments disclosed a drastic behavioral phenotype that worsens over time. Excitingly, we found that mutant cells adapt to the lack of m6A through autoregulation of 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 development treatment strategies for m6A related diseases.

Abstract 14

Circadian regulation of vertebrate cone photoreceptor function

Jingjing Zang¹, Matthias Gesemann¹, Jennifer Keim¹, Marijana Samardzija², Christian Grimm², Stephan C.F. Neuhauss¹

¹University of Zurich, Department of Molecular Life Sciences, Zurich, Switzerland

²Lab for Retinal Cell Biology, Department of Ophthalmology, University Hospital Zurich, University of Zurich, Switzerland

Eukaryotes generally display a circadian rhythm as an adaptation to the reoccurring day/night cycle. This is particularly true for visual physiology that is directly affected by changing light conditions. Here we investigate the influence of the circadian rhythm on the expression and function of visual transduction cascade regulators in diurnal zebrafish and nocturnal mice. We focused on regulators of shut-off kinetics such as recoverins, arrestins, opsin kinases, and GTPase-accelerating protein that have direct effects on temporal vision. Transcript as well as protein levels of most analyzed genes show a robust circadian rhythm dependent regulation, which correlates with changes in photoresponse kinetics. Electroretinography demonstrates that photoresponse recovery in zebrafish is delayed in the evening and accelerated in the morning. This physiological rhythmicity is mirrored in visual behaviors, such as optokinetic and optomotor responses. Functional rhythmicity persists in continuous darkness, it is reversed by an inverted light cycle and disrupted by constant light. This is in line with our finding that orthologous gene transcripts from diurnal zebrafish and nocturnal mice are often expressed in an anti-phasic daily rhythm.

Abstract 15

A novel approach for targeting Diffuse Midline Gliomas using zebrafish patient derived xenografts

Chiara Cianciolo Cosentino^{1,2}, Sandra Laternser¹, Justyna M Przystal¹, Sabine Müller^{1,4}, Stephan Neuhauss⁵, Roger Stephan² and Javad Nazarian^{1,3}

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Diffuse Midline Gliomas (DMG) are rare, highly aggressive pediatric tumors affecting brain's midline structure including brainstem, thalamus, and spinal cord. DMGs are characterized by an extremely poor prognosis and a concerning lack of effective treatments.

Patient derived xenografts (PDXs) murine models are often used for predicting patient-specific drug sensitivity, but their clinical utility is limited by the small size of the biopsied specimens, leading to low initial cell numbers, and the long time (>8 mo) for tumor engraftment.

To overcome these limitations, we established: a) larval zebrafish orthotopic patient-derived DMG xenografts (zPDXs), combining high-throughput capabilities, rapid results and whole organism biology, and b) a zebrafish high throughput drug screening platform to disclose mechanisms of systemic toxicity and neurotoxicity, prioritize safer compounds for mammalian testing, and identify co-therapies.

We implanted patient derived DMG tumor cells in the brain of zebrafish larvae, testing implantation efficacy at different developmental stages and with different cell numbers.

All DMG tumor cells engrafted and propagated in the brain parenchima, with a similar diffuse pattern as observed in human and rodent models. The drug screening platform allowed to test safety and efficacy of several novel compounds, and results were in line with murine toxicity studies.

In conclusion, zPDX can provide rapid in vivo validation of anticancer drugs and drug targets, enhancing the drug discovery process for DMG and other CNS tumors. We anticipate that these pilot studies will provide a framework for establishing an innovative, high-throughput drug screening platform, ideal for testing drug sensitivity directly from patient biopsies in a personalized medicine context.

Abstract 16

Circadian Regulation of Zebrafish photoreceptor function

Laura Köcher¹, Jingjing Zang¹, Stephan C.F. Neuhauss¹

¹Department of Molecular Life Sciences, University of Zurich, Switzerland

Circadian rhythms regulate many aspects of vision. In teleost, the majority of cells are directly light responsive. Therefore, Zebrafish represent a favorable model to study how light regulates circadian clock. The aim of the current project is to investigate the expression and function of genes regulated by circadian rhythm. Different zebrafish single and triple knockout lines of core clock genes have been generated. To investigate the effect of the disrupted core clock genes on gene expression, behaviour and visual ability further analysis will be done.

Abstract 17

Lactate dynamics in the retinal metabolic landscape

Jacqueline G. Kientsch¹, Filipe O. Viegas^{1,2} and Stephan C. F. Neuhaus¹

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²Life Science Zurich Graduate School, Ph.D. Program in Molecular Life Sciences, Zurich, Switzerland

Retinal photoreceptors are among the most energy demanding cells in the body due to tonic synaptic activity and resting potential maintenance. To fulfill their metabolic needs, retinal cells depend on glucose and glycolysis. Despite having oxygen available, aerobic glycolysis is favored in the retina, leading to lactate production. Lactate has been identified as a valuable energy substrate and is proposed to be a major player in retinal energy homeostasis. However, our current knowledge of the metabolic landscape and its dynamics is still incomplete. To investigate the role of lactate in the zebrafish retina, we focused on gene three different genes (*Idha*, *ldhba* and *ldhbb*). These genes encode subunits of the enzyme lactate dehydrogenase (LDH), which converts pyruvate to lactate and vice versa. We used whole-mount in-situ hybridization to characterize the expression patterns of these genes and supplemented this with protein localization via immunohistochemistry. To further examine the role of lactate in retinal metabolism, we created crispant knockouts to investigate LDH contribution to retinal function by using electroretinograms (ERGs) and discover potential compensatory gene expression using RT-qPCR. To uncover specific roles of LDH in retinal cells, we are using a novel CRISPR-based mutagenesis (3C) strategy to create cell-specific mutant lines. With these different approaches, we will shed further light onto the retinal metabolic landscape, especially regarding the role of lactate. This will be important to deepen our understanding of key metabolic pathways that play a role in several prominent retinal diseases, such as age-related macular degeneration or retinitis pigmentosa.

Abstract 18

Investigating the role of cilia in scoliosis and development of the locomotor apparatus using zebrafish mutants in ciliopathy genes

Jacqueline Oberlin^{1,2}, Markus Masek^{1,2}, Ruxandra Bachmann-Gagescu^{1,2}

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Ciliopathies are a group of human disorders caused by the dysfunction non-motile and/or motile cilia. Non-motile cilia are ubiquitous organelles found on the surface of most vertebrate cells where they transduce a variety of external signals to the cell (including sensory, chemical and mechanical signals and signalling pathways during development). Motile cilia on the other hand contribute in a more physically active way as their function is to move extra-cellular fluid. For example, the flow of the cerebrospinal fluid in the brain ventricles is cilia driven. The existing flow of the CSF is especially important for the intact formation of the Reissner fiber. Experiments with zebrafish have shown that the impaired assembly of the Reissner fiber results in a defect axial straightening with a downward curled tail phenotype and adults develop scoliosis. The Bachmann group works with various zebrafish mutants in ciliopathy genes that demonstrate scoliosis but with an upward curled phenotype. In this project we want to further investigate the influence of dysfunctional cilia on the development of scoliosis. We will study the integrity of the Reissner fiber in cilia mutants by using various techniques. Such as, immuno-histochemistry, live imaging in transgenic lines, light and electron microscopy (confocal, spinning disk, scanning electron microscopy), western blot analysis and in-situ hybridization.

Abstract 19

Innovations in the caudal skeleton of Platyfish

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The dorsoventrally symmetrical caudal fin is a morphological hallmark of teleosts. Despite its external morphology, the principal rays of this appendage articulate to bones situated below the notochord, defining a ventral origin of this organ. Here, we report that this typical hypochordal identity of the caudal fin is not fully conserved in platyfish (*Xiphophorus maculatus*), representing the Poeciliidae family. In this species, 3 to 4 principal rays articulate to bones above the notochord, suggesting an epichordal contribution to the fin. In comparison to zebrafish and medaka, platyfish display a high internal symmetry of the caudal skeleton with a mirrored shape of dorsal and ventral processes of the preural vertebrae. Developmental analysis revealed that the first dorsal ray is formed starting at the 10-ray stage of fin morphogenesis.

Our study suggests that an ancestral ground plan with 10 rays expanded in the platyfish by adding supernumerary principal rays in a symmetrical pattern at the dorsal and ventral sides. The skeleton of platyfish represents an evolutionary advancement towards balancing the 28 external with internal symmetry of the caudal fin.

Abstract 20

Skeletal muscle regeneration after extensive cryoinjury in adult zebrafish

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Like in mammals, the zebrafish skeletal muscle can regenerate through activation of satellite stem cells. Here, we established a cryoinjury method causing profound damage of multiple myomeres in the precaudal part of the adult zebrafish. We characterized the sequential activation of muscle precursors using Pax7 and MyoD1 myogenic regulators, and their differentiation into specific types of myofibers. New muscle started to form at 7 and 10 days post-injury, leading to a nearly complete restoration after 1 month. We identified that the transgenic element *careg*, which contributes to fin and heart regeneration, also participates in muscle restoration. In the intact tissue, this reporter was expressed in a subset of slow myofibers, whereas during regeneration, it was induced across all types of newly formed myofibers. The formation of new muscles was dependent on TOR signaling, as the rapamycin-mediated inhibition of this pathway prevented regeneration, resulting in extensive scarring and Collagen XII deposition. Furthermore, blocking VEGFR-signaling partially impaired the efficiency of muscle replacement, suggesting the requirement of angiogenesis in this process. In conclusion, our study demonstrates the ability of adult zebrafish to nearly perfectly re-establish myomeres after extensive cryoinjury and provides a model for further molecular studies of muscle regeneration.

Abstract 21

Zebrafish model for screening effects of Covid-19 drug candidates on cardiovascular system and mediation of immune response to spike treatment

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We developed a medium-high throughput workflow to study the effects of the MMV CovidBox compounds on zebrafish embryonic development with a focus on the cardiovascular system. The experimental part of the workflow comprises of two parallel assays, in which we assess development of cardiovascular system and swimming behavior. On the other hand, we also created a transgenic fish line expressing human ACE2 in tissue specific manner, where we check immune responses to SARS-CoV-2 spike protein in the presence or absence of the candidate drugs.

The recorded data from drug screening were analyzed in a pipeline combining semiautomatic and automatic analysis steps, allowing screening of various compounds for their impact on morphology, heart function, vascular structure and neurological development. We have identified a wide range of effects on embryonic development upon treatment with the CovidBox drugs. Furthermore, we implemented an automated PubMed search, and found that the most studied compounds in the context of Covid-19 eg. Remdesivir and Hydroxychloroquine had little to no impact on embryonic development at the applied concentration, while Ivermectin, a compound in clinical trials, showed toxicity and detrimental effects on zebrafish development. The selected drug candidates were then screened for mediation of immune response to spike protein in presence or absence of human ACE2 protein.

In conclusion, zebrafish is a useful model for specialized screening, which can reveal a wide range of drug side effects on cardiovascular system development. The candidates can also be easily tested on their role in immune response to spike treatment.

Abstract 22

Can Wt1 control cardiomyocyte fate?

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During development, the heart grows through addition of progenitor cells to the poles of the primordial heart tube. In the zebrafish, wilms tumor 1 transcription factor a (wt1a) and b (wt1b) are expressed in the pericardium, at the venous pole of the heart. From this pericardial layer, the proepicardium emerges. Proepicardial cells are subsequently transferred to the myocardial surface and form the epicardium, covering the myocardium. We found that while wt1a/b expression is maintained in proepicardial cells, it is downregulated in those pericardial cells contributing to cardiomyocytes from the developing heart. Sustained wt1 expression impaired cardiomyocyte maturation by reducing chromatin accessibility of specific genomic loci. Strikingly, a subset of wt1a/b-expressing cardiomyocytes changed their cell adhesion properties, delaminated from the myocardium and upregulated epicardial gene expression. Thus, wt1 acts as a brake for cardiomyocyte differentiation and ectopic wt1 expression in cardiomyocytes can lead to their transdifferentiation into epicardium.

Abstract 23

Fuca2 is necessary for HSC expansion in the caudal hematopoietic tissue

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During embryogenesis, a small number of hematopoietic stem cells (HSCs) are specified from the hemogenic endothelium in the floor of the aorta. In mammals, they colonize the fetal liver where they expand. We use zebrafish to understand the molecular non-cell-autonomous signals delivered by the caudal hematopoietic tissue (CHT), the functional equivalent of the fetal liver. The important role of vasculature in HSC expansion has previously been demonstrated. We have shown that many genes are specifically expressed by the CHT vasculature and their importance for HSC expansion.

To identify new genes associated to HSC expansion, we performed single cell RNA sequencing on endothelial cells (flk1:GFP+, trunk and tails) at early stages (22 hpf), before HSCs specification. Cluster analysis showed endothelial cells already formed a heterogeneous population. Specifically, two clusters expressed genes associated with venous identity and the hematopoietic niche from the CHT. *Fuca2* was highly enriched in these clusters, among other genes. After validating *fuca2* expression in the CHT, we knocked-down *fuca2*, and observed a complete loss of HSC markers in the CHT, while their specification seemed unaffected. *Fuca2* (Alpha-L-Fucosidase 2) is part of a gene family (GH29) involved in selectin-mediated leukocyte extravasation and lymphocyte homing. Based on these previously identified GH29 roles, *fuca2* may be similarly involved in vascular extravasation of HSCs from the circulation to the CHT and its loss may affect HSCs ability to leave the vasculature and colonise the niche. We are now investigating this hypothesis by direct live imaging of HSC homing in the CHT.

Abstract 24

New tools to study the onset of lymphopoiesis in the zebrafish embryo

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The blood tissue is constantly regenerated by a rare, multipotent, and self-renewable cell population known as hematopoietic stem and progenitor cells – or HSPCs – through an evolutionarily conserved process called hematopoiesis. In the zebrafish embryo, HSPCs emerge from the endothelium of the dorsal aorta through an endothelial-to-hematopoietic transition, and then seed different hematopoietic organs, such as the caudal hematopoietic tissue (CHT) where HSCs proliferate, or the thymus where T-cell lymphopoiesis takes place. Recent data from mouse and zebrafish models indicate that the newly generated HSPC population is heterogeneous.

Using transcriptome analysis, we identified si:ch211-214p16.1 and si:ch211-214p16.2 – referred to as p16.1 and p16.2 – as two previously undescribed genes specifically expressed by emerging HSPCs at 30-36 hours post-fertilization (hpf). Both genes are expressed in the hemogenic endothelium, then in the CHT and the thymus. In the adult, the p16.1GFP transgenic line shows expression in all lymphoid compartments (T and B cells). Accordingly, transient knock-down of both genes using splice morpholinos leads to a general decrease of lymphopoiesis. Indeed, the ikarosGFPhi subset disappears in the CHT at 48hpf and we observe delays in thymus seeding at 60hpf, as well as a decrease of rag1 signal in the thymus at 5dpf, thus suggesting that these two genes may play an important role in T-cell lymphopoiesis. In order to fully abolish the function of p16.1 and p16.2, we generated a double mutant line using CRISPR/Cas9 and we are looking forward to characterizing hematopoiesis in this double mutant line. Finally, we have also generated an inducible CRE line under the control of the p16.1 promoter to follow the fate of these HSPCs and confirm that they are lymphoid biased. In summary, we generated a new tool to dissect the heterogeneity of newly generated HSPCs in the zebrafish embryo.

Abstract 25

Investigating the cell-junction dynamics during blood vessel morphogenesis in zebrafish

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During vascular morphogenesis, the process of angiogenesis is driven by cell migration, cell shape changes and cell rearrangements. Here, a dynamic balance between inter-endothelial cell adhesion and plasticity allows angiogenic sprouting while maintaining the endothelial seal. Previous analyses on blood vessel formation and anastomosis in zebrafish have shown that junctional remodeling is central to many aspects of morphogenetic endothelial cell-cell interactions. Cell rearrangement is mediated by a particular structure, called junction-based lamellipodia (JBL), which are thought to provide a tractile force for junction elongation. The goal of this project is to exploit the transparency of the zebrafish model system, and to adopt fluorescent reporters and optogenetic tools in order to: image, perturbate and spatio-temporal resolve the molecular events at the base of cell-junction remodeling during vascular morphogenesis.

Abstract 26

The role of PI3-Kinase α in blood vessel morphogenesis in the zebrafish embryo

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The establishment of a functional vasculature is a key element of vertebrate development to ensure availability of nutrients and oxygen to all organs and tissues. Endothelial cells sprouting from pre-existing vessels engage in new contacts with other sprouts, thereby forming new vascular circuits connected to the bigger arteries and veins. During this early stage of blood vessel development, the endothelial cells are positioned in a unicellular segmental manner next to each other. In the zebrafish trunk vasculature, the cells subsequently elongate and rearrange, thereby remodeling the vessel architecture towards a multicellular state (two or more cells outlining the vessel). These morphological changes entail elongation of the cellular interfaces, the cell junctions, from ring shaped structures to long lines tracing the outline of the vessel.

We have previously shown that chemical inhibition of the lipid kinase PI3-Kinase α (p110 α) impairs endothelial cell rearrangements in the zebrafish embryo, with vessels remaining in their unicellular state. To closer inspect this particular function of the kinase, we have generated zebrafish mutants for both PI3-Kinase α encoding genes *pik3ca-a* and *pik3ca-b*. These mutants phenocopy the chemical inhibition, with vessels in the zebrafish trunk remaining unicellular during vascular development. We now aim to investigate the molecular function of the kinase in endothelial cell rearrangements by observation of different key proteins in the mutant background, e.g. VE-cadherin, cortical actin and myosin light chain II. Precise inspection of the stereotypic events during cell junction elongation will shed light on the role of PI3-Kinase α function during vascular morphogenesis.

Abstract 27

Cortactin controls endothelial cell shape by stabilizing junction-associated actin during vascular morphogenesis

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The morphological changes of endothelial cells are crucial for the proper formation of the vasculature. The control of endothelial cell shape relies on intrinsic cellular mechanisms, cell-cell interactions and hemodynamic forces. In this study, we have explored the roles of Cortactin, a member of nucleation promoting factors 2 (NPFs 2), in the development of the zebrafish vasculature. We find that Cortactin associates at cell-cell junctions together with junction-associated actin filament. In the absence of Cortactin, endothelial cells displayed supernumerary junctions at the intersegmental vessels (ISV) and aberrant cell shape at the dorsal aorta (DA). By high-speed in vivo imaging of F-actin dynamics, we observed higher temporal fluctuations of F-actin at cell junctions with respect to intensity as well as spatial localization. Currently, we are further exploring autonomous and non-autonomous contributions of Cortactin and junction-associated actin to the cell shape. In summary, we hypothesize that Cortactin is critical for the cell shape maintenance by stabilizing junction-associated actin in endothelial cells.

Abstract 28

Novel endothelial junctional architecture in the brain of zebrafish larvae

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Establishing a functional vasculature is crucial for growth and survival in the developing embryo. Nutrient and oxygen exchange takes place between the thinnest part of the vascular system, the microvasculature, and surrounding tissues. Previous studies analysing the microvascular system in the brain of rodents have shown that unicellular segments and autocellular junctions exist. Yet how these vessels are formed is not known. These types of junctional architecture couldn't be observed so far in the vascular beds analysed in zebrafish embryos. Formation of all previously described vessels, ultimately always lead to a multicellular architecture. Using confocal microscopy, we have observed that also in the brain of developing zebrafish larvae there are vessels that show unicellular or autocellular segments. To better understand the dynamics of unicellular tubes, we use long-term imaging over several days with low stress light sheet microscopy. To address, whether this is a phenomenon of the developmental window, we compared endothelial cell divisions in different developmental stages and connect them to the expansion of the surrounding tissue. During the same time the blood brain barrier is established, and it is known that junctions, in particular tight junctions, are organizing permeability. To further examine the molecular components that may contribute to unicellular tube formation, we analyse the architecture of the brain microvasculature in larvae, mutant for tight junctional components such as Esama and Claudins. The development of clearing protocols for zebrafish embryos allows us to observe the development of these vessels deep within in the zebrafish brain.

Abstract 29

Development and architecture of the midbrain vasculature in zebrafish

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Blood vessel development starts early on in a growing embryo. The early cardiovascular development has been studied intensively and major advances have been achieved. Less is known about tissue-specific vascular development, which is essential for organ formation and maturation. The formation of complex microvasculature such as the one in the midbrain of the zebrafish embryo is understudied. To understand the development and the tissue architecture of the midbrain vasculature, we used in vivo time-lapse imaging in combination with transgenic zebrafish lines. Our analysis shows that the process of tissue formation is more stochastic than in the trunk or in the hindbrain, but we also found stereotypical patterns. Also, we characterized the microvascular system of the central arteries and we propose a new nomenclature. The study of the junctional architecture of the tubes showed that 50% of the endothelial cells show unique unicellular tube architectures (seamless or autocellular tube types). This novel finding is opposing to evidence coming from other vascular beds, for example the trunk vasculature, where the tissue organization is multicellular. The observations in the 5-day old larvae indicate that the unicellular tubes are a stable and abundant structure in the zebrafish brain.

Abstract 30

A morphometric framework for the embryo-wide quantification of tissue organisation at single cell resolution

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The three-dimensional organisation of cells is a key feature that determines the form and function of tissues, organs, and organisms. Yet, modern systems-level research relies almost exclusively on omics data that often require that samples are dissociated, resulting in the loss of tissue organisational information.

Here we present a quantitative embryology approach that applies modern omics methods to morphometric datasets. By combining in toto light sheet microscopy of developing zebrafish embryos with segmentation of individual cell nuclei, we characterise cells based on organisational features of their tissue neighbourhood. Subsequent spatial partitioning generates features that encode the local cell arrangement and unsupervised machine learning is used to identify organisational motifs across the whole embryo by clustering similar cells based on their organisation rather than their position or gene expression.

Following this approach, we show that local cell organisation follows motifs that are conserved between samples and map to anatomical features. Moreover, we identify a fundamental organisational bipartitioning into regions with converse organisational feature profiles. Interestingly, most tissue specific gene expression patterns respect this partitioning, indicating that local cell organisation provides an important regulatory component that should be integrated for a systems level understanding of development.

Abstract 31

Triggering Organ Self-Assembly in vivo Via Targeted Manipulation of Cellular Architecture

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During development cells collectively undergo shape and fate changes giving rise to tissues and organs. While there is much known about gene-regulatory networks driving tissue differentiation, and how they change cell morphology, there is still little known about the feedback mechanisms that inform cells that they have been successful in their task. One key question is whether the changes in cell shape and organization observed during morphogenesis are able to feedback on cell fate decision making.

In order to address this question, we use interneuromast “chain” cells, multi-potent organ progenitors of the zebrafish lateral line as an experimentally-tractable model system. Our aim is to drive acto-myosin contraction by targeting RhoGTPases in order to investigate the impact of cell clustering on stem cell differentiation.

By using chemo-inducible tools¹ and optogenetic perturbations via the iLID system^{2,3} we can drive acute cell contraction and “de novo” cluster formation in the lateral line. Ongoing experiments using live reporters and scRNA seq as readouts will allow us to explore whether Rho-driven clustering is sufficient to promote cell differentiation and how this is achieved at the level of cell biological mechanism.

Abstract 32

Integrating zebrafish morphometric and gene expression data using point cloud registration

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Progress in developmental biology research is being driven mainly by two state-of-the-art techniques, 3D microscopy and transcriptomics, which generate complementary data sets that are currently difficult to integrate. Computational approaches that can multiplex analyses of gene expression with cell and tissue scale morphometric phenotypes are emerging as a promising solution to this general bottleneck in understanding.

We are working on a multi-sample registration approach in which gene expression and morphometric information is assigned to individual cells, with the aim of generating an average embryo model. To do so, we acquire volumetric images of whole zebrafish embryos using multi view light sheet microscopy and transform them into a point cloud representation via nuclear segmentation. Cell-individual and neighborhood information, such as gene expression or cell density, are assigned to single points (nuclei, cells), and can be analyzed on equal footing. Moreover, representing the cells of embryos as point clouds enables efficient multi-sample registration and with that multiplexing of theoretically unlimited fluorescence channels. We will describe how we have applied this approach to multiplex the expression domains of twelve members of the cadherin cell adhesion molecule family to compare their spatial regulation.

Abstract 33

“Shining light” on tissue resident macrophages -Towards a quantitative understanding of how they search and capture dying cells

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Macrophages are diligent sentinels that continuously scan the surrounding tissue to identify and capture pathogens and dying cells. Their “search and capture” strategies thereby vary depending on the environment. Thus, there are mesenchymal-like macrophages that rapidly migrate across tissues, but also microglia, brain tissue-resident macrophages, that at steady state are stationary and use dynamic cellular extensions to scan the surrounding brain parenchyma. Recent work showed, that these cellular extensions are made of first order microtubule-dependent pseudopods emanating directly from the cell body, and second-order thin filopodia. While these filopodia are highly motile actin-dependent structures thought to sense the brain parenchyma, pseudopods don't show any noticeable motility and are thought to constitute the cellular backbone. High-spatial and temporal resolution in vivo microscopy of the transparent zebrafish embryo has shown, that microglia use microtubule-dependent pseudopods to phagocytose entire apoptotic neurons. Interestingly, however, photo-switchable microtubule destabilization shows, that microglia can rapidly change behavior and move directly toward dying neurons, adopting a different but equally effective phagocytic strategy that resembles that of peripheral macrophages. The aim of this project is to investigate actin and microtubule dynamics and their contribution to migration and phagocytosis in different tissue-resident macrophage populations in vivo using real-time reporters and optogenetic tools.

Abstract 34

Towards a dynamic understanding of microglial response to stimuli

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Microglia, brain phagocytic cells that respond to injuries, and apoptotic cells detect, interpret, and react to several external stimuli in the complex brain environment. A clear feature of microglia is their highly dynamic cellular extensions that these cells use to scan the brain environment and respond to stimuli such as UTP and ATP thought to come from apoptotic and injured neurons, respectively. Although many of these stimuli have been identified, not much is known about their regulation in space and time and how they induce distinct cellular activities and behaviors in microglia.

Our goal is to understand how different signals coming from apoptotic and injured neurons are interpreted, translated, and integrated into dynamic cell behaviors by microglia and other macrophage populations. Thus, we are developing real-time microglial reporters for Ca²⁺, cAMP and diacylglycerol (DAG) signaling. One clear prediction would be that their activities correlate with distinct microglial behaviors; for example, we expect Ca²⁺ being high in migration and DAG at the site of targeted phagocytosis. The development of methods to induce neuronal apoptotic and necrosis and the delivery of diffusible ligands directly into the brain while imaging will enable direct testing of our predictions while also probing for the interdependency of these activities. Recently, we have also established that defects in neuronal processing in microglia, affect these cells in important ways, by abrogating their ability to phagocytose apoptotic neurons and migrate. This points to feedback mechanisms linking cargo processing and higher-order microglial activities.

Our goal is to exploit these feedbacks to develop methods to control microglia, as this can have significant implications for many pathologies where these cells are too "aggressive" phagocytes.

Abstract 35

ISMARA: Completely automated inference of gene regulatory networks from high-throughput data.

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Understanding the key players and interactions in the regulatory networks that control gene expression and chromatin state across different cell types and tissues in metazoans remains one of the central challenges in systems biology. Our laboratory has pioneered a number of methods for automatically inferring core gene regulatory networks directly from high-throughput data by modeling gene expression (RNA-seq) and chromatin state (ChIP-seq) measurements in terms of genome wide computational predictions of regulatory sites for hundreds of transcription factors and micro-RNAs. These methods have now been completely automated in an integrated webserver, called ISMARA (ismara.unibas.ch) that allows researchers to analyze their own data by simply uploading RNA-seq or ChIP-seq data-sets, and provides results in an integrated web interface as well as in downloadable flat form. For any data-set ISMARA infers the key regulators in the system, their activities across the input samples, the genes and pathways they target, and the core interactions between the regulators. One of the recent developments is the zebrafish support for the ISMARA service. The zebrafish annotation contains 475 regulatory motifs associated with 994 transcription factors.

We believe that, by empowering experimental researchers to apply cutting-edge computational systems biology tools to their data in a completely automated manner, ISMARA can play an important role in developing our understanding of regulatory networks across metazoans.

Abstract 36

Reconstructing connectivity between the olfactory bulb and telencephalon in zebrafish

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Distinguishing between different odors and associating them with valence is important for the survival of many animals. In zebrafish, the olfactory bulb (OB) and the pallial area Dp, the homolog of olfactory cortex, are two brain regions involved in this process. In addition to the feed-forward projections from the OB to Dp, there are abundant feedback projections from Dp to the OB . The function of this feedback pathway in odor processing is, however, unclear.

In this project, we will address this question in three steps. First, fish are trained in an odor-discrimination task. Second, good learners are chosen and the odor-evoked neuronal responses in both regions are recorded by calcium imaging. Third, a stack of EM images is acquired from the OB and telencephalon by serial block-face scanning electron microscopy (SBEM) to reconstruct the connectome between the OB and Dp. By comparing the odor-evoked activity patterns in the OB and the feedback projection pattern from Dp to the OB, we will investigate the principles of circuit organization in these two interconnected brain areas.

So far we have generated samples from fish with good learning scores and calcium-imaging data quality. A pilot low-resolution EM stack from the OB to Dp was acquired as a guide for the future acquisition of a high resolution stack.

Abstract 37

Deciphering the molecular logic of odor classification

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Classification of odorants as ‘good’ or ‘bad’ is imperative for the survival of an animal. Good odors are predictive of a reward, while bad odors can signal danger/punishment.

Initial classification of odors is thought to occur during the transition from sensory neurons to mitral cells, in the olfactory bulb (OB). Within the OB distinct spatial domains respond to different elements of the stimulating odors, such as its chemical properties, or its hedonic value. The OB itself is composed of numerous components and cell types; axons of olfactory sensory neurons, outward projecting mitral cells, tuft/tuft cells, and various subtypes of interneurons, which all interact in highly complex ways. Whether and how the molecular properties of the different neuronal populations within the OB are correlated to initial odor classification is not known.

To understand the molecular heterogeneity of cell types in the OB we conducted single cell sequencing. This uncovered the variably expressed genes within the OB. By examining their expression patterns, we are uncovering the organizational principles within the OB. Furthermore, in order to provide functional context to these structures and cell types we developed a novel olfactory choice assay. Using this assay, we assign specific hedonic values to specific odors. Finally, we will examine the functional response to some of the more hedonically relevant odors, and map those to a spatial molecular map of gene expression. Taken together, we aim to examine the molecular logic of odor encoding and classification in the OB.

Abstract 38

Tissue size is regulated by coordination of cell size and cell number throughout early development

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Development is a remarkably robust process that leads to the generation of reproducible species-specific body sizes. Overall body size is largely determined by the number and size of cells. Previous studies in amphibians, mice, and most recently in zebrafish, have demonstrated that polyploids remain the same size as diploids despite having larger cells (due to greater genome content) by compensating with lower cell numbers. How cell size and cell number are coordinated to regulate body size is still a long-standing mystery. To investigate tissue size regulation in the context of increased cell size, we focused on the hatching gland and the eye in triploid zebrafish. The volume of the hatching gland at 2 dpf and the eye at 24 hpf remained the same between triploids and diploids, albeit with larger but fewer cells in triploids. A time course was performed to compare the size of the tissue domains that specify the hatching gland and eye during early development. For the hatching gland, we analysed the volume of its precursor structures at 50% epiboly (5.3 hpf), sphere (6 hpf) and bud (10 hpf). No difference in tissue volume was seen across the time points between triploids and diploids, with negative correlation observed between cell size and cell number. Furthermore, analysis of the nodal signalling gradient, which specifies the early hatching gland domain, revealed no discrepancy between diploids and triploids at 50% epiboly. Similarly, the early eye field volume at bud (10 hpf) and 4-somite (11.3 hpf) was comparable between diploids and triploids. However, the eye volume was significantly larger in triploids between 8 and 15 somites (13 and 16.5 hpf) compared to diploids with both groups having a similar number of cells. These results indicate that the hatching gland and eye sizes are determined by the allocation of space that takes place during early development and is independent of cell size or number. Future work will focus on whether developmental speed underlies the eye size difference detected during mid somite stages and the size compensation that occurs during late somite stages.

Abstract 39

Uncovering the roles of 5' UTRs in translational control during early zebrafish development

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During early developmental stages, metazoan embryos are transcriptionally silent, and embryogenesis is controlled by maternally deposited factors. Developmental progression requires the synthesis of new mRNAs and proteins in a coordinated fashion. Many posttranscriptional mechanisms regulate the fate of maternal mRNAs, but it is less understood how translational control shapes early embryogenesis. Protein synthesis is primarily regulated at the translation initiation step by elements in the 5' untranslated region (5' UTR) of the mRNA. However, we currently lack a systematic understanding of the regulatory information contained within 5' UTRs and how they functionally impact mRNA translation throughout development. Using zebrafish as a model of vertebrate development, we are developing an *in vivo* massively parallel reporter assay (MPRA) to identify 5' UTR motifs involved in translation regulation. By integrating the translational behaviour of 5' UTR reporters throughout embryogenesis with sequence-based regression models, we anticipate to uncover novel *cis*-regulatory elements in 5' UTRs with developmental roles. The MPRA will lay the foundation for future studies dissecting the molecular mechanisms underlying the function of newly identified 5' UTR motifs.

Abstract 40

The regulation of brain-body communication in zebrafish and *C.elegans*

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The regulation of internal organs has been historically considered to be 'self-contained' and autonomous. However, it is increasingly apparent that brain-body communication modulates not just the internal organs, but also the brain itself. To dissect the regulation of brain-body communication, I am combining the specificity of the *C. elegans* pharyngeal nervous system with study of less-characterized visceral innervation in zebrafish.

In *C. elegans*, communication between the 20 neurons of the pharyngeal nervous system and the 280 neurons of the somatic nervous system is mediated almost entirely via neuropeptide signaling. I have generated transgenic lines to induce neuronal activation and silencing of pharyngeal neurons in combination with a behavioral screen to identify novel behavioral outputs modulated by pharyngeal sensation. In a more general approach, I am also using an intersectional degradation system to eliminate neuropeptide processing specifically from the pharynx to evaluate whether there are consequences on somatically-generated behaviors.

In zebrafish, visceral innervation is compact enough that it can be detailed comprehensively, but in contrast to the well-characterized pharyngeal nervous system of *C. elegans*, the genetic identities, modalities, and functions of visceral neurons (from both the vagus nerve and cranial sensory ganglia) are still largely undescribed. I have used scRNAseq approaches to collect transcriptomic data on both of these neuron populations, and will complement this approach with spatial transcriptomics to resolve the anatomical organization of molecular cell types. The combined study of *C. elegans* and zebrafish will help uncover both general principles and unique aspects of brain-body communication.

Abstract 41

Neurog1 and Olig2 integrate patterning and neurogenesis signals in development of zebrafish dopaminergic and glutamatergic dual transmitter neurons

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Dopaminergic neurons develop in distinct neural domains by integrating local patterning and neurogenesis signals. While the proneural proteins Neurog1 and Olig2 have been previously linked to development of dopaminergic neurons, their dependence on local pre-patterning and specific contributions to dopaminergic neurogenesis are not well understood.

Here, we show that both transcription factors are differentially required for the development of specific dopaminergic glutamatergic subpopulations in the zebrafish posterior tuberculum, which are specified by Otp transcription factors and are homologous to the A11 dopaminergic neurons in mammals. Both Olig2 and Neurog1 are expressed in otpa expressing dopaminergic progenitor cells and appear to act upstream of Otpa during neurogenesis. Our epistasis analysis revealed that Neurog1 acts downstream of Notch signaling in dopaminergic specification, while Olig2 acts downstream of Shh, but upstream and/or in parallel to Notch signaling. Furthermore, we identified Olig2 to be an upstream regulator of neurog1 in dopaminergic neurogenesis. This regulation occurs through Olig2-dependent repression of the proneural repressor and Notch target gene her2. In summary, we show how Neurog1 and Olig2 integrate local patterning signals like Shh with neurogenesis to specify the progenitor population of A11-type dopaminergic neurons and initiate neurogenesis and differentiation.

Abstract 42

***Tyrosine hydroxylases th and th2* genes differentially contribute to modulation of physiology and locomotion in catecholamine-free zebrafish larvae**

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Catecholamines (CA) are chemical messengers with neuromodulatory and endocrine functions. They include dopamine, noradrenaline and adrenaline, which have conserved roles in physiology and behavioral modulation. Given its clinical relevance, studying the CA systems contributes to a better understanding of their role in development and disease. So far, most of our knowledge about CA systems derives from pharmacological approaches or neuronal ablation. While these techniques have provided a wealth of information about CA systems, they are less well suited for studying how CA neurons regulate biological systems, and how these neurons function themselves, including contributions by co-transmission of second neurotransmitters like glutamate and GABA. To overcome these issues, we decided to generate a genetic model completely devoid of CA. This goal was achieved by inducing loss of function mutations in the genes involved in L-DOPA synthesis: the two paralogous tyrosine hydroxylase genes (*th* and *th2*) in neurons, and tyrosinase (*sdly*) in melanocytes. ELISA verification demonstrated that *th*, *th2*, *sdly* triple mutant larvae are completely devoid of dopamine. Contrary to rodent CA-deficient models, which die at birth, CA-deficient zebrafish larvae are viable and form functional dopamine-free DA neurons and projections. However, some dopaminergic clusters develop a reduced number of cells, suggesting that these neurons may still have activity of other transmitter systems. Despite this, locomotor outputs as well as physiologically controlled responses like HGC release and cardiac function are impaired in *th*, but not in *th2* mutant larvae, demonstrating the major contribution of *th* to CA-related modulation during zebrafish early development.

Abstract 43

Comparison of spatio-temporal Cre-ERT2 activation by photocaged compounds for lineage labelling in zebrafish

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The Cre-Lox System is commonly used for clonal analysis and spatiotemporal control of transgene expression in zebrafish. Precise and efficient spatial and temporal control of the Cre recombinase activity is critical. In order to achieve spatial control, a fusion protein of Cre and the estrogen receptor (Cre-ERT2), which enables the translocation of Cre upon tamoxifen treatment, was engineered. This genetic system was combined with small photocaged compounds with tamoxifen activity, aiming for spatial control. Actiflash (Sinha et al., 2010) is a compound activated by UV light, whereas tclnd (Fournier et al., 2013) contains a blue-absorbing photolabile protecting group that masks cyclofen. We aimed to perform clonal analysis to identify neural stem cells that give rise to subpallial dopaminergic neurons in the larval zebrafish brain. Thus, we tested Actiflash and tclnd in several transgenic zebrafish lines and different optical uncaging conditions in the embryonic and larval zebrafish brain. We were able to achieve broad activation of the Cre-ERT2 by illumination of whole embryos for both compounds at embryonic stages (30 hpf). However, regional activation within the brain at embryonic and larval stages caused little Cre-ERT2 activation above background, or at higher light levels massive cell death, and thus was not reliable possible for both tclnd and Actiflash. In contrast, cell-type specific CRE-ERT2 expression in defined cell populations and Tamoxifen cause efficient recombination of the lineage marker. Thus, we will focus our further analysis on refining spatial expression of Cre-ERT2 and using limited tamoxifen doses for lineage tracing experiments.

Abstract 44

Anatomical and functional characterization of subpallial dopaminergic neurons in the zebrafish larva

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In mammals, well-known midbrain dopaminergic (DA) neurons of the mesolimbic and mesostriatal pathway play critical roles in emotion and motor control. Degeneration of these neurons in Parkinson's disease comprises proper function of the striatum and limbic system. Zebrafish is easily accessible for developmental and functional interrogation, and has been established as model for DA modulation. Since zebrafish lack homologous midbrain DA neurons, their use as model for Parkinson's is limited. However, zebrafish evolved subpallial DA neurons making them an interesting model to investigate telencephalic DA modulation. Currently, the function of subpallial DA neurons in zebrafish is unknown. Recent studies suggested that they consist of several populations that line the striatum and might be situated within regions homologous to the mammalian amygdala. Our fluorescent in situ co-expression studies of dissected larval zebrafish brains indicate that subpallial DA neurons form part of the extended medial amygdala, among others, critical for odor-cued behavior. In our pilot calcium imaging experiment, we detected the response of subpallial DA neurons to aversive odorant L-cysteine. In the future we aim to continue the characterization of a potential role of subpallial DA neurons in olfactory circuit modulation.

Abstract 45

Mechanosensory habituation and adaptation are modulated by Dopamine

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Different parameters of habituation are modulated by the catecholaminergic system. Here we focus on habituation of a mechanosensory startle response that is mediated by the lateral line. We observe that habituation of the mechanosensory startle response is mitigated by dopamine. Furthermore, we recorded directly from lateral line afferent neurons during water jet stimulation and observe that dopamine has a sensitizing effect on lateral line responses, as it has previously been shown for hair cells. We conclude that dopamine not only modulates habituation of the mechanosensory startle response, but also directly affects sensory adaptation.

Abstract 46

Dopaminergic modulation of mechanosensory systems

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Dopaminergic neurons in the posterior tuberculum and hypothalamus in zebrafish show projections onto several structures of the lateral line system, while also receiving indirect sensory input from the lateral line. This reciprocal connection seems to play a role in the modulation of waterflow perception, which is however, so far not very well understood. To investigate the function of these diencephalic dopaminergic neurons we perform 2-photon calcium imaging and electrophysiological recordings of different neuronal populations involved lateral line perception. Our hypotheses is that dopamine release at hair cells in neuromasts and at afferent neurons in the lateral line ganglions sensitizes the whole system to be more responsive to mechanosensory stimuli. Such a sensitizing effect could allow zebrafish to be readily responsive while or after prolonged stimulation. We therefore established a habituation protocol using mechanical tapping stimulation to evoke lateral line activity. Preliminary results show different response patterns and habituation profiles in the neuronal populations mentioned above. Since water flow is the adequate stimulus for the lateral line system, we also developed a custom built and low-cost flow chamber that allows in vivo recordings of neuronal activity in zebrafish larvae. With this flow chamber larval zebrafish can be exposed to a variety of different flow stimuli, which makes it possible to investigate the different computational stages in the lateral line system in detail.

Abstract 47

A novel role of BCL2L13 at mitochondria-ER contact sites

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The vital role of inter-organelle communication can be highlighted by the many processes taking place at mitochondria contact sites. These include ion transport, lipid exchange and utilization, mitochondrial bioenergetics, dynamics, and mitochondrial quality control. The identification of new molecular players, which regulate organelle communication and tethering, is essential to understand the role of contact sites in physiological and pathophysiological conditions. BCL2L13, a member of the BCL2 family of proteins, was shown to be implicated in various cellular processes, such as apoptosis, mitochondrial fragmentation, mitophagy and energy metabolism. In chronic endurance exercise, a specific role of BCL2L13 for maintenance of mitochondrial quality was suggested. In HeLa cells overexpressing BCL2L13, we found increased mitochondria-ER contact sites and mitochondrial fragmentation. We identified specific domains, which allow BCL2L13 to interact with mitochondria and the endoplasmic reticulum and showed that BCL2L13 is partially located at mitochondria-associated ER membranes. We characterized *bcl2l13* knock out zebrafish, which are smaller, display decreased locomotion, and have lower maximal oxygen consumption rates compared to wild-type fish. Proteomics analyzes revealed altered intracellular calcium dynamics, NAD⁺ homeostasis, and cytochrome c oxidase activity in knock out animals. We suggest that BCL2L13 plays a central role at mitochondria-ER contact sites to regulate proper inter-organelle communication. Thus, the loss of *bcl2l13* affects multiple cellular and physiological processes in zebrafish.

Abstract 48

Investigating the role of the primary cilium in neural circuit development

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Mechanisms controlling development of neural circuits, which are crucial for communication throughout the nervous system, are not fully understood. Emerging evidence suggests that primary cilia, small microtubule-based organelles quasi-ubiquitous on vertebrate cells, play an important role in transducing signalling pathways required for neural circuit development. Dysfunction of primary cilia underlies a group of human disorders called ciliopathies. The ciliopathy Joubert Syndrome (JBTS) is characterised by a cerebellum and brain stem malformation known as the “Molar Tooth Sign” (MTS), which includes abnormal cerebellar tracts. With the aim of investigating the role of primary cilia during cerebellar neural circuit development, we have characterised primary cilia on cerebellar Purkinje, granule and eurydendroid cells within the developing cerebellum of zebrafish larvae and are using zebrafish mutants for the JBTS gene *cc2d2a* to model the disease. Preliminary data from *cc2d2aw38* mutants, which display retinal and renal ciliopathy phenotypes, suggests that primary cilia on mutant cerebellar neurons have reduced levels of *Arl13b*, a protein important for the signal transduction ability of the primary cilium. Nevertheless, *cc2d2a* mutants have normal Purkinje cell number and Purkinje axon tract length during development. Using whole brain tissue clearing of adult *cc2d2a* mutants imaged with light sheet microscopy, preliminary observations show normal organisation of Purkinje cells. Current work focuses on characterising granule and eurydendroid cells and their tracts in *cc2d2a* mutants and on evaluating the cerebellum of other JBTS-gene zebrafish mutants that entirely lack cilia such as *talpid3*, to determine the role of this organelle in cerebellar circuit development in zebrafish.

Abstract 49

Opioid exposure leads to differential behavioural effects in zebrafish larvae

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Opioids are used to treat acute and chronic pain or are consumed as recreational drugs due to their euphoric effect. Through human excretion of the parent compounds and metabolites, accidental spillage or disposal opioids are released into wastewater and are increasingly detected in the aquatic environment. However, their impact on aquatic species is currently only poorly understood.

In this study, we used the zebrafish model to investigate the impact of fentanyl citrate and tramadol hydrochloride on the behaviour, physiology and morphology of zebrafish larvae. Exposure of zebrafish larvae to fentanyl citrate for 120 hours resulted in reduced locomotor activity and a reduced heart rate. Moreover, the number of animals with an uninflated swim bladder (SB) was increased compared to control conditions, and sizes of the inflated SBs were smaller. When larvae were exposed to fentanyl citrate and tramadol hydrochloride for 24 hours, their SB sizes were expanded compared to control larvae. Moreover, larvae swam mainly close to the surface. SB expansion occurred around 3 hours of exposure to fentanyl citrate, whereas the surfacing behaviour was already induced after about 20 min of exposure. Our results show that different windows of exposure to the opioid fentanyl citrate during zebrafish development lead to differential behavioural, physiological and morphological effects. While developmental exposure leads to sedative effects and SB non-inflation, short exposures lead to uncontrolled surfacing behaviour and SB expansion. These effects can lead to impaired predator avoidance and feeding success and consequently to reduced survival chances of fish early life stages.

Abstract 50

Zebrafish (*Danio rerio*) larva as an *in vivo* vertebrate model to study renal function

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The study of renal function remains a challenge. *In vitro* cell-based assays are approved to study e.g. ABC/SLC mediated drug transport but do not cover other renal functions as glomerular filtration and tubular reabsorption. For this purpose, *in vivo* studies are needed, which are time-consuming, expensive, and often rely on *in vivo* experimentation with higher vertebrates. In view of these limitations, there is a high unmet need for cost-effective and animal reducing *in vivo* test systems that include mechanistic studies at a cellular level and allow a translation to humans.

Since the zebrafish larva (ZFL) pronephric kidney shares high similarity with the anatomy of nephrons in higher vertebrates and does not count as an animal, we explored in the present study whether 3 to 4 days old ZFE have a fully functional pronephron. The aim is to use ZFL as an *in vivo* vertebrate model to study glomerular filtration, ABC/SLC mediated drug transport and folate receptor 1 mediated tubular reabsorption.

We could show that ZFL has a fully functional pronephron at 4 days post fertilization and is, therefore, an attractive translational vertebrate screening model to bridge the gap between cell culture-based test systems and pharmacokinetic experiments in higher vertebrates.

Abstract 51

Zebrafish (*Danio rerio*) larva as an *in vivo* vertebrate model to assess neurotoxicity of solvents

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The blood brain barrier (BBB) is formed by endothelial cells between blood and brain parenchyma. BBB helps in maintaining brain homeostasis and restricts the entry of pathogens and toxins into the brain. Occupational and environmental exposure to organic solvents may lead to the development of neurological disorders like encephalopathy, peripheral neuropathy and motor neuron diseases. Neurotoxicity studies in rodents are very complex, expensive and difficult to apply in a standardized manner. We used zebrafish larva as an in-vivo vertebrate model to assess the neurotoxicity of organic solvents. The structure of the blood-brain barrier (BBB) is conserved between teleost fish and humans, thus enabling zebrafish as a powerful model to study BBB functionality in-vivo. The aim of this study is to investigate the effect of solvent exposure on BBB integrity. We used transgenic zebrafish lines Tg(kdrl:GFP), Tg(kdrl:mcherry) to visualize the brain capillaries in zebrafish embryos. Acute endpoints like LC50 and IC50 were measured by exposing the embryos to solvents. To assess the effect of solvent on BBB integrity, a specified volume of fluorescent tracer is injected into zebrafish through the common caudal vein and exposed to test solvent. The mid brain region of zebrafishes exposed to solvent and control fishes were taken using confocal microscope. Tracer intensity inside the brain and plasma were quantified for each fish.

Abstract 52

Larval zebrafish model for Benzodiazepines-induced neurotoxicity endpoints

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Compelling evidence from preclinical studies and clinical reports has demonstrated developmental neurotoxic effects of general anaesthetics and sedatives, raising considerable concerns about administering anaesthetic agents during pregnancy and in early infancy. In this study, we aim to establish high-throughput behavior-based screening assays for testing developmental neurotoxicity of established and widely used anaesthetic and sedative compounds in a zebrafish model. We tested two Benzodiazepines, the long-acting Diazepam and the short-acting Midazolam, in wild-type zebrafish larvae at 3, 4, and 5 days post-fertilization (dpf). A behavioral paradigm based on the visual motor response (VMR) was used for neurobehavioral assessment. First, as proof of method we carried out in-treatment tests administering Diazepam and Midazolam at five different doses in 5 dpf larvae. Our results confirmed a dose-dependent sedative effect of both drugs. Next, we sought to determine any neurobehavioral alteration following early developmental exposure to the general anesthetics. Diazepam and Midazolam were administered to 3dpf larvae at two different doses and three different durations of exposure, subsequently, post-treatment VMR experiments were conducted at 4 and 5 dpf. Our results revealed an exposure time-dependent decreased activity in treatment groups of higher Diazepam dosage and both Midazolam dosages at 1-day post-treatment. Surprisingly, in certain post-treatment and in-treatment recordings of both drugs we recorded a peculiar hyperactivity, resembling the paradoxical reactions to Benzodiazepines in clinical practice. Based on our study outcomes we propose to use the zebrafish as a feasible alternative animal model in the assessment of neurotoxicity related to general anesthetic exposure.

Abstract 53

Assessing cold-shock domain family proteins (Csps) involvement in the virulence of *Listeria monocytogenes* (Lm) in a zebrafish embryo infection model

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Listeria monocytogenes (Lm) is a foodborne pathogen associated with contaminated dairy products and deli meats. Lm causes a life-threatening disease in humans known as listeriosis and accounts for serious public health and food safety problems.

Cold shock-domain family proteins (Csps) are highly conserved nucleic acid binding proteins regulating the expression of various genes including those involved in metabolism, virulence, and stress tolerance in bacteria. In Lm there are three Csps proteins (CspA, CspB and CspD), that have been shown to promote both cold and osmotic stress adaptation responses. Their phenotypic and regulatory roles, however, are yet to be fully elucidated.

In this study, we examined the impact of Csps in virulence by comparing Lm wildtype (WT) strains of different genetic backgrounds and their corresponding csp deletion mutants.

The consequences of deleting csp genes on Lm virulence was examined using a zebrafish embryo-based infection model. We infected 48 hours post fertilization (hpf) zebrafish larvae with approximately 500 colony-forming unit (CFU) into the blood circulation. Embryos were then observed up to 72 hours post infection (hpi) for signs of disease and eventual death.

We found that zebrafish embryos infected with csp deletion mutants survived better than those infected by the WT strain. Our results indicate that all 3 Csps are functionally relevant for Lm virulence. Overall, our study shows that Csps play important roles in Lm virulence, one of trait central to the public health and food safety impacts of Lm.

Abstract 54

Deciphering the role of endothelial plasticity in metastasis

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About 90% of cancer-related deaths are caused by metastasis 1,2, the process in which circulating tumor cells (CTCs) leave the primary tumor entering the bloodstream to colonize distant organs 3,4. Importantly, extravasation events, the critical step where tumor cells exit the vasculature crossing the endothelium, are rare and technically difficult to capture in vivo. Despite its importance, most of the current knowledge on extravasation derives from in vitro dynamic data, which do not recapitulate the complexity of the environment, and from in vivo mouse models, where dynamic imaging approaches are challenging to implement, limiting the mechanistic understanding of this important process. Notably, the nature and function of ECs signaling occurring during CTC extravasation is completely unknown.

We take advantage of the zebrafish model that allows high-temporal and cellular resolution in a biomechanical relevant context, to dissect the complex interplay between tumor cells' biomechanical properties and endothelial cellular responses, rising a whole new clinical potential.

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