



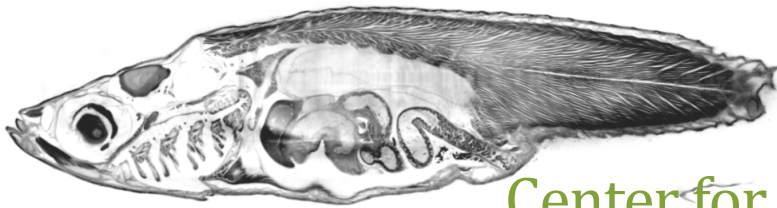
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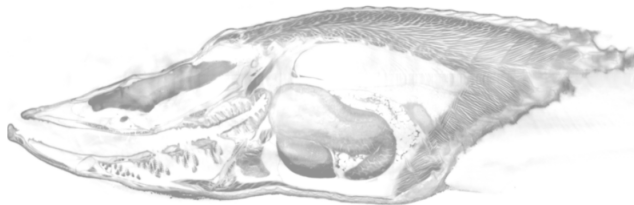
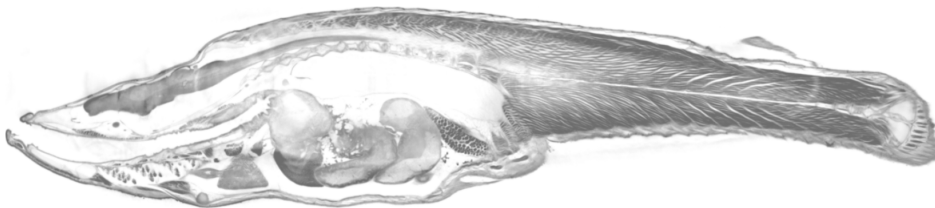
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4th Strategic Meeting for Medaka Research & 3rd Regional Fish Meeting



April 16-18, 2018
Center for Organismal Studies (COS)
Heidelberg University, Germany





Centre for
Organismal
Studies
Heidelberg



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4th Strategic Meeting for Medaka Research & 3rd Regional Fish Meeting

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Heidelberg University, Germany

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SCHEDULE - MONDAY, APRIL, 16th

Location: Alte Aula, Heidelberg University

09:00 - 09:10 Welcome Words

09:10 - 10:50 Plenary Session I

Chair: Lazaro Centanin

- T1 - **Hiroyuki Takeda**, Japan - *The structure and epigenetics of the pluripotent genome in medaka fish*
- T2 - **Kristin Tessmar Raible**, Austria - *More than meets the eye: Vertebrate non-visual photoreceptors*
- T3 - **Alexander Aulehla**, Germany - *The role of signaling oscillations during embryonic mesoderm patterning*
- T4 - **Takashi Yoshimura**, Japan - *Medaka as a model to understand the underlying mechanism of vertebrate seasonal adaptation*

10:50 - 11:20 Coffee Break

11:20 - 13:00 Plenary Session II

Chair: Hiroyuki Takeda

- T5 - **Hideaki Takeuchi**, Japan - *Individual recognition in medaka fish*
- T6 - **Saori Yokoi**, Japan - *Analysis of molecular basis underlying decision making according to social familiarity*
- T7 - **Yukiko Ogino**, Japan - *Diversification of androgen receptor function underlies secondary sex characteristics development of teleost fishes*
- T8 - **Kiyoshi Naruse**, Japan - *Evolution of the sex chromosome and sex-determining genes in Oryzias fish*

13:00 - 14:30 Lunch

SCHEDULE - MONDAY, APRIL, 16th

Location: Alte Aula, Heidelberg University

14:30 - 16:10 Plenary Session III

Chair: Manfred Schartl

T9 - **Minoru Tanaka**, Japan - *Germ Cells - More than Gametogenesis*

T10 - **Christoph Winkler**, Singapore - *Transcriptome profiling of osteoblasts in a medaka osteoporosis model identifies novel mediators of bone repair*

T11 - **Ronald B. Walter**, USA - *The Genetic Response to Fluorescent Light Exposure Within Internal and External Organs Is Conserved Among Vertebrates (*Danio rerio*, *Oryzias latipes*, and *Mus musculus*)*

T12 - **Jochen Wittbrodt**, Germany - *Library of life: the matrix. Medaka to tackle the genetics of individuality*

16:10 - 16:35 Key Note Lecture

T13 - How the Medaka came to Europe

Manfred Schartl

Chair: Jochen Wittbrodt

16:35 - 18:00 Reception - Bel Etage

18:00 Wine Tasting: Knipsers Halbstück, Bissersheim

SCHEDULE - TUESDAY, APRIL, 17th

Location: COS, Im Neuenheimer Feld 230, Room 00.005

08:45 - 10:00 Community Meeting

Chairs: Kiyoshi Naruse & Jochen Wittbrodt

T14 - **Thomas Thumberger**, Germany - *Acute and inducible knockdown of GFP-tagged proteins using a nanobody-destruction box fusion*

T15 - **Tomas Fitzgerald**, UK - *Whole Genome Analysis of the Inbred Medaka Kiyosu Panel*

10:00 - 10:50 Plenary Session IV

Chair: Kiyoshi Naruse

T16 - **Joaquín Letelier**, Chile - *cis-regulatory logic of Shh expression reveals common history of unpaired and paired fins*

T17 - **Satoshi Ansai**, Japan - *The molecular genetic basis of diversified sexually dimorphic traits in Oryzias species endemic to Sulawesi, Indonesia*

10:50 - 11:20 Coffee Break

11:20 - 13:00 Plenary Session V

Chair: Minoru Tanaka

T18 - **Haobin Zhao**, China - *The Proteins Interacting with Prmt5 in Medaka (Oryzias latipes)*

T19 - **Baubak Bajoghli**, Germany - *Long search for the "Holy Grail" in the evolution of adaptive immune system: Identification of lymph node equivalent structures in medaka fish*

T20 - **Soojin Ryu**, Germany - *Characterization of the endocrine and behavioural stress responses of medaka larvae*

T21 - **Narges Aghaallaei**, Germany - *Basal lamina in the base of intestinal furrow is an inductive site for the mucosal adaptive immune response in medaka fish*

13:00 - 14:30 Lunch

SCHEDULE - TUESDAY, APRIL, 17th

Location: COS, Im Neuenheimer Feld 230, Room 00.005

14:30 - 16:35 Plenary Session VI

Chair: Juan Ramon Martinez-Morales

- T22 - **Alexander Froschauer**, Germany - *Suppressed recombination of the homomorphic Y chromosome in the medaka*
- T23 - **Toshiya Nishimura**, Japan - *Germline sex determination by nanos3, a component of germ plasm, in medaka*
- T24 - **Tonomori Deguchi**, Japan - *Drug discovery screening for lymphatic vessel related diseases using medaka*
- T25 - **Tomohiro Ueno**, Japan - *in vivo MR microscopy of disease models in Medaka*
- T26 - **Felix Loosli**, Germany - *A panel of medaka inbred lines: a resource to study the genetics of individuality*

16:35 - 17:10 Coffee Break

17:10 - 19:00 Plenary Session VII

Chair: Jochen Wittbrodt

- T27 - **Yasuhiro Kamei**, Japan - *Biological and Optical improvement of IR laser-mediated gene induction microscope system*
- T28 - **Lazaro Centanin**, Germany - *Fixed Developmental Timing Revealed by Trans-Species Transplantation*
- T29 - **Andrea Pauli**, Austria - *Small proteins with big roles: Bouncer is necessary and sufficient for species-specific fertilization*
- T30 - **Juan Ramon Martinez-Morales**, Spain - *Analysis of YAP/TAZ-dependent transcriptional response during early morphogenesis in teleost embryos*
- T31 - **Didier Stainier**, Germany - *Genetic compensation is triggered by mutant mRNA degradation*

19:00 Poster Session & BBQ

SCHEDULE - WEDNESDAY, APRIL, 18th

Location: COS, Im Neuenheimer Feld 230, Room 00.005

09:00 - 09:30 Key Note Lecture

T32 - Evolutionary emergence of the rac3b/rfng/sgca regulatory cluster refined mechanisms for hindbrain boundaries formation in zebrafish

Juan Ramon Martinez-Morales

Chair: Lazaro Centanin

09:30 - 10:15 Technical Session

Chair: Lazaro Centanin

T33 - **Jochen Gehrig**, Germany - *High content screening by ACQUIFER - Automated microscopy for whole organism screening applications*

T34 - **Omar Hammouda**, Germany - *A Change of Heart: Medaka as a model for Human Cardio-Vascular Diseases & GWAS*

T35 - **Malte Wachsmuth** - *Luxendo Light-sheet Microscopy: Seeing Life from a Different Angle*

10:15 - 10:45 Coffee Break

10:45 - 12:25 Plenary Session VIII

Chair: Lazaro Centanin

T36 - **Clara Becker**, Germany - *Growth control in the retinal stem cell niche of medaka*

T37 - **Anabela Bensimon-Brito**, Germany - *Zebrafish heart valve regeneration: a model for valve recellularization*

T38 - **Michelle Collins**, Germany - *Pitx2c orchestrates embryonic axis extension via mesendodermal cell migration and oriented cell division*

T39 - **Alexander Cook**, Germany - *Developing a zebrafish model to identify novel molecular resilience mechanisms*

T40 - **Chaitanya Dingare**, Germany - *The Hippo Pathway effector Taz is required for the formation of the Micropyle in Zebrafish*

12:25 - 15:00 Lunch and Poster Session

SCHEDULE - WEDNESDAY, APRIL, 18th

Location: COS, Im Neuenheimer Feld 230, Room 00.005

15:00 - 16:20 Plenary Session IX

Chair: Nick Foulkes

- T41 - **Mohamed A. El-Brolosy**, Germany - *The mRNA surveillance machinery control transcriptional adaptation to mutations*
- T42 - **Jakob Gierten**, Germany - *Genome-wide Association Study of Cardiac Phenotypes in Medaka Inbred Strains*
- T43 - **Alexander A. Goloborodko**, Germany - *Molecular Mechanisms of Germ Plasm Localization during Early Zebrafish Embryogenesis*
- T44 - **Christian Helker**, Germany - *In vivo secretome-wide and chemical screening to identify novel regulators of pancreatic β -cell function*

16:20 - 16:50 Coffee Break

16:50 - 18:30 Plenary Session X

Chair: Virginie Lecaudey

- T45 - **Jens Kroll**, Germany - *Loss of erythropoietin aggravates hyperglycemia induced renal damage and alters embryonic renal development via induction of apoptosis in zebrafish*
- T46 - **Ravindra Peravali**, Germany - *From Morphology to Behavior: Phenotyping Medaka Inbred Lines*
- T47 - **Ali Seleit**, Germany - *Neural stem cells induce the formation of their physical niche during organogenesis*
- T48 - **Erika Tsingos**, Germany - *Neural stem cells coordinate post-embryonic morphogenesis in the eye of medaka*
- T49 - **Carina Beatrice Vibe**, Germany - *Visualizing signaling oscillations during embryonic patterning in the Medaka model*

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General Meeting Information

Location: Alte Aula, Heidelberg University



Address: Grabengasse 1, 69117 Heidelberg

Access via Public Transit:

Universitätsplatz is directly in front of the venue of day 1 and is located in the heart of the historic center. **Available Busses: 31/32**

WiFi Access:

WiFi-SSID: UNI-WEBACCESS

User-ID: rl1

Password: auni#2016



Location: COS, Im Neuenheimer Feld 230, 69120 Heidelberg



Access via Public Transit:

Bunsengymnasium is the nearest stop to the venue of day 2/3 and just a 2min walk away. The venue is directly behind the new Mathematikon-building. **Available busses: 31, available trams: 21/24.**

WiFi Access:

WiFi-SSID: UNI-WEBACCESS

User-ID: rt1

Password: FischeIN230

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- Jochen Wittbrodt: +49 152 09340870

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Oral Presentations
4th Strategic Meeting for Medaka Research

T1 - The structure and epigenetics of the pluripotent genome in medaka fish

Hiroyuki Takeda

Department of Biological Sciences
Graduate School of Science, University of Tokyo
Japan

The epigenome of pluripotent cells ensures active transcription of pluripotency-related genes and repression of developmental genes. We generated epigenomic profiles of pluripotent cells which include base-resolution DNA methylomes, histone modification and chromatin accessibility maps using the medaka (Japanese killifish, *Oryzias latipes*), a vertebrate model suitable for genome science. Native pluripotent cells from medaka blastula embryos were used in this study. We first addressed the nucleosome organization and the contribution of DNA sequences to nucleosome positioning in DNA hypomethylated domains (HMDs) using a supervised machine learning algorithm, k-mer SVM. We found that HMDs specifically possess accessible nucleosome organization with longer linkers and identified the strong link between nucleosome positioning and specific DNA sequences at gene promoters in HMDs. Surprisingly, the sequence preference of the nucleosome and linker in HMDs is opposite from that reported previously. We then investigated the dynamics of three-dimensional genome organization during zygotic genome activation (ZGA) using Hi-C in medaka embryos. As reported in *Drosophila* and mouse, we found that higher-order structures such as compartments are established during ZGA in medaka, independent of transcription. The chromatin accessibility heterogeneity emerges almost simultaneously with compartmentalization. These results suggest that the reprogramming of chromatin folding in early embryos is conserved.

T2 - More than meets the eye: Vertebrate non-visual photoreceptors

Bruno M. Fontinha, Miguel Gallach, Florian Reithofer, Alison J. Barker, Maximilian Hofbauer, Ruth M. Fischer, Stephanie Bannister, Arndt von Haeseler, Herwig Baier and *Kristin Tessmar-Raible*

Platform Rhythms of Life
MFPL/ University of Vienna
Austria

The eyes are not the only sites that can perceive light. Light perception by cells in the inner brain of vertebrates, independent of eyes and pineal organs, was already discovered more than 100 years ago. The responsible encephalic photoreceptors have been thought to be specialized cells, similar to the photoreceptors present in the eye and pineal. Consistently, the expression of several opsins has been described at places harboring such deep brain photoreceptors, and hence these opsins were independently claimed to mediate non-visual light responses, such as seasonality. During recent years, an impressive number of non-visual opsins was identified and shown to be in principle able to function as light receptors.

In order to obtain a better understanding of this puzzling complexity of light perception in teleosts, we investigate several 'non-visual' Opsins on a functional level. Our particular focus is on TMT/Encephalopsin group, since these Opsins exhibit a particularly slow sequence evolution and some members are conserved across all vertebrate phyla. We have recently shown that the expression of several members of this group is enriched in medaka and zebrafish inter- and motoneurons. Using molecular and behavioral assays we can show that *tmt1b* in medakafish modulates neurohormone transcript levels and different behaviors in a light-dependent manner. Different types of 'non-visual' Opsins have been proposed to convey the light information controlling light-dependent seasonal breeding response, as it is present in medakafish. Null-alleles of medaka *tmt1b* do not impact on the fish's gonadal status.

T3 - The structure and epigenetics of the pluripotent genome in medaka fish

Alexander Aulehla

Developmental Biology Unit
EMBL Heidelberg
Germany

In our group, we study origin and function of signaling oscillations during embryonic development. In vertebrate embryos, oscillatory signalling activities of the Notch [and in mouse embryos also of the Wnt, and Fgf pathways] have been identified during periodic mesoderm segmentation and are part of the somite segmentation clock. Using the mouse model, we have combined quantitative real-time imaging, novel in vitro assays and perturbation approaches to reveal principles and function of these collective signalling oscillations. I will present our recent findings indicating that the relative timing between Notch and Wnt signalling oscillations are critical for mouse embryo mesoderm patterning.

To establish medaka as a powerful complementary model to quantitatively study signalling dynamics in vivo, we have initiated experiments to generate segmentation clock real-time reporter medaka knock-in lines; goals, strategies and recent results will be discussed.

T4 - Medaka as a model to understand the underlying mechanism of vertebrate seasonal adaptation

Takashi Yoshimura

Institute of Transformative Bio-Molecules
Nagoya University
Japan

The appropriate timing of various seasonal processes, such as reproduction, migration and hibernation, is crucial to the survival of animals living in temperate regions. Although this phenomenon attracts great interest, its underlying mechanisms are not well understood. By using non-model organisms that have highly sophisticated seasonal responses, we have uncovered the universality and diversity in the signal transduction pathway regulating seasonal rhythms in vertebrates. Although humans are not typically considered seasonal animals, some evidence suggests that seasonal variation in physiology and behavior also exists in humans. For example, the wavelength settings for the unique yellow hue are shifted to shorter wavelengths in summer compared with those in winter. Seasonal affective disorder patients, experiencing recurrent winter episodes of depressed mood, overeating and hypersomnia, show electroretinogram changes in winter, with lower sensitivity compared with healthy subjects. These observations highlight the potential importance of the retina in seasonality, but the molecular basis of these seasonal changes remains unknown. We have recently discovered dynamic plasticity in phototransduction regulates seasonal changes in color perception in Japanese medaka fish (*Oryzias latipes*), an excellent model for studying seasonal adaptation. I will discuss how we can better understand human seasonal rhythms using unique animal models.

T5 - Individual recognition in medaka fish

Hideaki Takeuchi

Graduate School of Natural Science and Technology
Okayama University
Japan

Previously, we demonstrated that medaka females recognize familiar males following prior visual exposure, and social familiarity influences female mating receptivity. Medaka females can discriminate familiar males from unfamiliar mates and prefer to mate with the former (Okuyama et al., Science, 2014). Here, we found that medaka use faces for individual recognition. Females can discriminate between two male faces and two objects, but upside-down of the faces made it more difficult to discriminate them. When discriminating between two non-face objects, upside-down did not affect it. Thus faces may be special for fish, just as humans (Wang and Takeuchi, elife, 2017). This is the first study that shows the face inversion effect in animals other than mammals.

T6 - Analysis of molecular basis underlying decision making according to social familiarity

Saori Yokoi, Satoshi Ansai, Yasuhiro Kamei, Masato Kinoshita, Yoshihito Taniguchi, Larry J. Young, Teruhiro Okuyama, Takeo Kubo, Kiyoshi Naruse, Hideaki Takeuchi

Faculty of Pharmaceutical Sciences
Hokkaido University
Japan

Some social animals can recognize socially-familiarized conspecific individuals (social recognition) and familiarity affects their behavior, which may be important for social adaptation. Disorders in human brain function for this system are assumed to cause mental illnesses, such as autism, and great attention has recently been paid to the underlying neural/molecular mechanisms of these disorders. Oxytocin (OT) is considered to be involved in social recognition and social behavior in rodents and humans. For example, social familiarization (repeated encounters) decreases approach behaviors of male wild-type mice toward unfamiliar females, but not OT KO males. Thus, the OT KO males are thought to have defects in social recognition (social amnesia). In this study, we investigated social behaviors of individual medaka fish, which is a model organism commonly used for molecular genetics, toward group-reared fish (defined as familiar fish) in the same tank and toward unfamiliar conspecifics reared in a different tank. Wild-type males exhibit mating behaviors, irrespective of social familiarity. In contrast, we found that social familiarity strongly affects male social behaviors (e.g., courtship, aggressive, and mate-guarding behaviors) in isotocin-related gene mutants. Isotocin (IT) is a fish homologue of OT. This result suggested that IT-related gene mutants lost social motivation toward conspecifics. In some fish, imprinting affects the social preferences of the juvenile fish based on the traits of their parents that care for them during the early development period. Next, to examine whether or not imprinting mediates social recognition of mutants for IT mutants, we investigated whether social familiarization (rearing in the same tank only as adults) could recover the lost of social motivation in the mutants toward unfamiliar fish.

T7 - Diversification of androgen receptor function underlies secondary sex characteristics development of teleost fishes

Yukiko Ogino, Hirotaka Sakamoto, Eiji Watanabe and Taisen Iguchi

Graduate School of Natural Science and Technology
Okayama University
Japan

Gene duplication is a dominant driving force of evolution. Steroid hormone receptor gene family is thought to have arisen from gene duplication. However, the molecular events which produce new protein functions after the genome duplication have not been fully understood. Teleost fishes present a good model to investigate an accurate evolutionary history of protein function after whole genome duplication, because the teleost-specific whole genome duplication (TSGD) 350 million years ago resulted in a variety of duplicated genes that exist in modern fishes. We focused on the androgen receptor (AR) gene, since two different subtype genes, AR α and AR β , were generated in the TSGD. AR β has retained the ancestral function, whereas the AR α evolved as a hyperactive form of AR in the teleost lineage. Results of our combined functional and 3 dimensional analyses of medaka (*Oryzias latipes*) ARs identified the substitutions that led to changes in protein structure and function between medaka AR α and AR β . One substitution located within the helices 10/11 of ligand binding domain is sufficient for generating higher transactivation of AR α . This amino acid change affects the stability of ligand binding by modifying the hydrogen bonds between ligand and AR. To further address the functional differences between AR α and AR β in vivo, we isolated the nonsense mutants of AR α and AR β from the medaka tilling library. AR β have predominant function for external masculine phenotypes, and AR α might participate the development of traits for male-male competition. Our findings would provide an historical explanation for the retention of the duplicated AR copies in euteleost genome.

T8 - Evolution of the sex chromosome and sex-determining genes in *Oryzias* fish

Kiyoshi Naruse, Taijun Myosho and Yusuke Takahana

Lab of Bioresources
National Institute for Basic Biology
Japan

Sex chromosomes harbor a primary sex-determining signal that triggers sexual development of the organism. In mammals, Sry is the dominant male-determining gene located on the Y chromosome, and has evolved from the neural gene Sox3 on the X chromosome probably through a regulatory mutation. Medaka fishes in the genus *Oryzias* have different sex chromosomes with different systems (XY and ZW), providing ideal conditions for investigating the mechanisms that lead to the rapid turnover of sex chromosomes. So far, different sex-determining genes, Dmy and GsdfY, have been isolated from the *Oryzias* species, demonstrating that turnover of sex chromosomes is associated with the substitution of master sex-determining genes. Recently, we identified Sox3 as a novel sex-determining gene on the XY sex chromosomes in the marine medaka *Oryzias dancena/melastigma* by positional cloning. Sex reversed phenotypes in transgenic fish and loss-of-function mutants of the Y chromosomal Sox3 allele all point to its critical role in sex determination, suggesting that the neo-Y chromosome of *O. dancena* arose by co-option of Sox3. Furthermore, we found the Sox3 gene also on the XY sex chromosomes in distantly related *Oryzias* species, *O. marmoratus* and *O. profundicola*. Fine mapping and association analysis identified the Y chromosome-specific 430-bp insertion at the Sox3 locus, which appeared to be involved in its male determination function. The Sox3-dependent sex determination system in *Oryzias* species is polyphyletic, and the Y-specific insertion has not been found in *O. dancena*, suggesting that Sox3 has evolved as the sex-determining gene independently in different lineages of *Oryzias*. These results suggest that Sox3 might have acquired the novel male-determining function repeatedly and independently during vertebrate evolution.

T9 - Germ Cells - More than Gametogenesis

Minoru Tanaka

Graduate School of Science
Nagoya University
Japan

Germ cells are the only cells indispensable for producing the next generation. But it is generally recognized that germ cells are passive cells that can develop into only gametes. Actually the gametogenesis is strictly regulated by the surrounded somatic cells. However, our studies have begun to reveal the positive ability that germ cells possess. The germ cells do not only have intrinsic mechanism that determines their fate cell-autonomously, but also have the ability to change their environment. Our recent results indicate that germ cells have an intrinsic power to feminize the gonad and body, which can be against a power of masculinization by male sex determination gene, *dmrt1bY*. Analysis of controlling the germ cell number also suggests that regulation of germline stem cells is related to the size of gonad. I would like to summarize the potential ability of germ cells that were found during the course of our studies.

T10 - Transcriptome profiling of osteoblasts in a medaka osteoporosis model identifies novel mediators of bone repair

Christoph Winkler

Department of Biological Sciences
National University of Singapore
Singapore

Bone is a highly dynamic organ that is constantly remodeled to maintain its high quality. During remodeling, old bone matrix is degraded by bone-resorbing osteoclasts and instantly replaced by new matrix from bone-forming osteoblasts. To prevent diseases such as osteoporosis, remodeling must be tightly coordinated to balance osteoblast and osteoclast activity. To date, many aspects that control bone homeostasis and osteoblast-osteoclast coupling remain unclear. To address this, we have chosen the Japanese medaka (*Oryzias latipes*) as a powerful animal model to mimic aspects of human bone diseases. Previously, we generated transgenic medaka where excessive bone resorption and osteoporosis-like lesions can be triggered by inducible expression of the osteoclast-inducing factor 'Receptor Activator of Nuclear Factor kappa Ligand' (RANKL). Using live imaging, we could demonstrate that osteoblast progenitor cells get activated and recruited to the osteoporotic lesion sites, where they differentiate and efficiently repair and remineralize damaged bone matrix. To identify factors involved in osteoblast activation during bone repair, we performed transcriptome profiling of osteoblast progenitors and mature osteoblasts that were purified from osteoporotic fish by Fluorescence Activated Cell Sorting (FACS). We characterized a set of transcripts that are up-regulated in osteoblast progenitors immediately after ectopic osteoclasts had started to induce bone lesions. Among these transcripts, we identified a novel calcium sensing receptor CaSR, which is expressed in osteoblasts. CRISPR-mediated genome editing in medaka revealed that deficient CaSR signaling affects osteogenesis, as well as interferes with the recruitment of osteoblast progenitors to osteoporotic lesions sites. In conclusion, our study revealed a set of novel transcripts up-regulated during osteoporotic bone repair and identified essential roles for CaSR in osteogenesis and bone homeostasis.

T11 - The Genetic Response to Fluorescent Light Exposure Within Internal and External Organs Is Conserved Among Vertebrates (*Danio rerio*, *Oryzias latipes*, and *Mus musculus*)

Ronald B. Walter, Mikki Boswell, Yuan Lu, William Boswell, Markita Savage, Kim Hildreth, and Christi A. Walter

Chemistry & Biochemistry
Texas State University
USA

We have previously shown 4,100 K or “cool white” fluorescent light (FL) exposure induces cellular stress, inflammation and immune responses in the skin of several varied biomedical fish models including; platyfish (*Xiphophorus maculatus*), medaka, (*Oryzias latipes*) and zebrafish (*Danio rerio*)¹.

Here we present RNA-seq results that establish similar patterns of modulated gene expression within two internal organs, brain and liver, of zebrafish and medaka exposed to 35 kJ/m² FL. In addition, FL light induced modulated gene expression within skin, brain and liver of both fish models is compared to similarly FL exposed hairless mice (*Mus musculus*).

In zebrafish and medaka, each of the three organs tested showed up-modulation of unique gene sets that are expected to activate the Acute Phase Response (APR) signaling pathway, leading to increased inflammation and innate immune response. Our pathway and gene clustering analyses suggest this response is due to increased cellular oxidative stress that promotes induction of the primary up-stream regulator, tumor necrosis factor (TNF). Exposure of hairless mice to FL also serves to induce both inflammation and an immune response that mimics that observed in the fish models for both skin and brain. However, the FL induced genetic response in liver, although consistent and up-modulated in medaka and zebrafish, exhibits a suppressed response in mouse liver, but in the same pathways.

Overall, the sharing of genetic responses to light among divergent diurnal fishes, and a nocturnal mammal, suggests the genetic response to light is likely hard-wired deep within the vertebrate genome, and may extend through the vertebrate classes, perhaps even to humans.

T12 - Library of life: the matrix. Medaka to tackle the genetics of individuality

Felix Loosli, Kiyoshi Naruse, Ewan Birney, *Joachim Wittbrodt*

Animal Physiology & Development
COS, Heidelberg University
Germany

We are all concerned with what makes us special and what is the basis of our individuality. What is the basis for the difference of individuals of the same species and how do nature (genetics) and nurture (environment) contribute? Individual variation is the key parameter in the interplay of intrinsic and extrinsic factors that contribute to states of health and disease, and must be understood if we aim at translating findings between laboratory models and ultimately to the human context. The central issue is to distinguish between phenotypes that are "determined" by widely shared features of genomes, those influenced by individual variation, and stochastic events. In the past ten years we have systematically explored routes to tackle these questions in a model system. One challenge is based in the fact that individuals only represent a very limited resource for systematic studies. Here we would rather need genetically identical groups representing the individuals that in their entirety represent the variability of a population. Strikingly, complex vertebrate model systems had been bred deliberately to reduce variation as a source of noise and to thus ensure experimental reproducibility. Here medaka with the fully established repertoire of laboratory approaches on the one hand and with its rich natural resources provides a unique opportunity. With its high inter-individual variability on the one hand and its resistance to inbreeding on the other, medaka represents the ideal model system to systematically address the genetics of individuality and to delineate the respective genetic and environmental contributions to individuality. Over the last ten years we have identified an unstructured natural population that we have converted into 111 fully sequenced inbred lines reflecting the phenotypic features of the original population. We currently carry out an in-depth phenotyping of these lines at scales ranging from organismal to molecular phenotypes, using cost efficient approaches that can be highly replicated. The project has put a particular focus onto the heart where we integrate information across the cardiovascular systems of vertebrates, especially the wide range of phenotypes observed in humans. I will present an overview of our attempts to serve as an introduction to the topic that will be further deepened by the presentations of Tom Fitzpatrick and Felix Loosli.

T13 - How the Medaka came to Europe

Key Note Lecture

Manfred Scharf

Physiological Chemistry
Biozentrum Universität Würzburg
Germany

An overview will be given on the history of medaka research in Europe from its very beginning in the eighties of the last century. Initial motivations to select medaka as laboratory model, obstacles and selected examples that mark the triumphal procession of medaka to become the truly complementary and even useful alternative to the zebrafish will be highlighted.

T14 - How to CRISPR: precise and efficient genome editing in Medaka

Thomas Thumberger

Animal Physiology & Development
COS, Heidelberg University
Germany

I will present our running approaches using CRISPR strategies in medaka. The focus will be on their efficiency to produce mutants, targeted insertions, precise knock-ins, and the most accurate way to unambiguously identify founder fish.

T15 - Whole Genome Analysis of the Inbred Medaka Kiyosu Panel

Tomas Fitzgerald

EMBL - EBI
United Kingdom

Over the last six years dedicated work has led to the establishment of the inbred medaka Kiyosu panel. Randomly selected mating pairs originating from an outbred wild population in Kiyosu Japan were used to start the inbreeding scheme. After 9 rounds of single brother-sister mating, 111 inbred lines remained from 83 different original wild founding breeding pairs. Thus 28 lines are "sib lines" to another line, a feature we aim to exploit in our statistical analysis. We have completed whole genome sequencing across the entire panel and called homozygous and heterozygous SNPs, INDEL's and CNV's in each line against the improved PacBio derived medaka reference. Amazingly over 75% of the lines are greater than 80% homozygous providing a truly unique model organism resource. We present initial analysis of Kiyosu panel genomes focussing on global sequence characteristics in and around detected variation. For QTL mapping, whole organism phenotypes are unlikely to provide specific loci from panel phenotyping alone, here we aim to boost statistical power using carefully designed F2 cross strategies. We have developed an extensive simulation and statistical power assessment of the genetic and environmental effects we might encounter, this simulation scheme handles polygenic phenotypes, multi-trait phenotypes and can simulate the results of phenotyping the Kiyosu panel and expected phenotyping of crosses. We will present simulation results showing a predicted improvement in loci mapping when following specific F2 cross experiment design strategies.

T16 - cis-regulatory logic of Shh expression reveals common history of unpaired and paired fins

Joaquín Letelier, Elisa de la Calle-Mustienes, Joyce Pieretti, Silvia Naranjo, Ignacio Maeso, Tetsuya Nakamura, Juan Pascual-Anaya, Neil Shubin, Igor Schneider, Juan Ramón Martínez Morales and José Luis Gómez-Skarmeta

Gene regulation and morphogenesis
Centro Andaluz de Biología del Desarrollo (CSIC/UPO)
Spain
&
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Despite their evolutionary, developmental, and functional importance the origin of vertebrate paired appendages remains a mystery. Classical ideas center on three hypotheses: that paired fins relate to gill arches, are derived from a fin fold, or that median fins evolved first and paired fins arose by co-option of ancient genetic patterning modules. Importantly, the third hypothesis makes specific predictions about the function and phylogenetic history of cis-regulatory elements (CREs) involved in appendage patterning. Here we show that a major enhancer for Sonic hedgehog (Shh, a key diffusible morphogen essential for proper growth and patterning of limbs and paired fins) is deeply shared between paired and unpaired fins in fish and with the limbs of mice. In mice, a single CRE termed ZRS is solely responsible for coordinating Shh expression in limbs. We use transgenic assays in zebrafish and mouse to trace the functional equivalence of the ZRS across gnathostome phylogeny and its likely absence in agnathans. CRISPR/Cas9-mediated deletion of the medaka core ZRS sequence, inspection of *shh* epigenetic landscape and *in vivo* enhancer assays reveal the existence of ZRS shadow enhancers in both teleost and human genomes. Deletion of both ZRS and shadow ZRS abolish *shh* expression and completely truncate pectoral fin formation. Strikingly, deletion of the medaka ZRS, an experiment expected to only affect the paired fins, results in an almost complete ablation of the dorsal fin. This finding indicates that a ZRS-Shh regulatory module is shared by paired and medial fins, and that paired fins likely emerged by the co-option of developmental programs established in the median fins of stem gnathostomes. Later on, this critical ZRS-driven Shh function was reinforced in pectoral fin development with the recruitment of shadow enhancers, conferring additional robustness.

T17 - The molecular genetic basis of diversified sexually dimorphic traits in *Oryzias* species endemic to Sulawesi, Indonesia

Satoshi Ansai, Koji Mochida, Shingo Fujimoto, Daniel F. Mokodongan, Atsushi J. Nagano, Atsushi Toyoda, Kiyoshi Naruse, Kazunori Yamahira, Jun Kitano

Laboratory of Bioresources
National Institute for Basic Biology
Japan

Sexual dimorphism is prevalent, but often differs remarkably between closely related species. However, we know little about which genetic changes can actually contribute to diversification of such sexually dimorphic traits. Sulawesi endemic Adrianichthyidae species (commonly referred to as medaka) serves an excellent model system because their sexual dimorphic body colorations are significantly diversified in closely-related species. As a first step, we generated a de novo long-read genome assembly of *Oryzias celebensis* as a reference assembly for the Sulawesi species. This assembly was anchored to 18 chromosomes by a linkage analysis and was annotated using RNA-seq data from adult and embryonic tissues. Using this genomic information, we have studied the molecular mechanisms underlying red coloration in pectoral fins, a characteristic feature of *O. woworae* males. Quantitative trait loci (QTL) mapping in a F2 intercross between a male of *O. woworae* and a female of *O. celebensis* revealed that an autosomal locus controls the red pigmentation. Subsequent RNA-seq analysis showed that *csf1* gene is a strong candidate responsible for the red fins in 18 differentially-expressed genes in the QTL region. This was supported by loss-of-function analysis of the *csf1* gene by CRISPR/Cas system, which caused a defect in xanthophores. Semi-quantitative allele specific expression analysis indicates cis-regulatory mutations would cause higher expression in pectoral fins of the males. Further genomic analysis are underway to identify the cis-regulatory mutation(s) responsible for responsible for the red fin traits.

T18 - The Proteins Interacting with Prmt5 in Medaka (*Oryzias latipes*)

Xiaosha Zhang, Emile Nibona, Runshuai Zhang, Maomao Guo, Gongyu Xu,
Kongyue Wu, Hao Shen, Xueping Zhong, Qingchun Zhou, Chao Qi,
Haobin Zhao

School of Life Sciences
Central China Normal University
China

Prmt5 plays important roles in regulation of gene expression, RNA processing, cell growth and differentiation, signal transduction and germ cell development, etc in mammal. Prmt5 of medaka had been reported. However, the proteins interacting with Prmt5 in medaka are in dim. In this study, medaka Prmt5 was used as a bait to fish the interacting proteins in the library by yeast two-hybrid technology. Positive colonies were obtained by mating yeast cells of Y187 containing pGBKT7-prmt5 and AH109 containing cDNA library on a plate with the deficient medium of SD/-His-Leu-Trp-Ade and by X- α -gal staining. Eight proteins were confirmed interacting with Prmt5 from 69 preliminary positive colonies after four passages, X- α -gal analysis, backcross test and sequencing. The proteins interacting with Prmt5 are methylosome protein 50 (Mep50), apolipoprotein A-I-like (Apo-AI), PR domain containing protein 1a with zinc fingers (Prdm1a), Prdm1b, Tim-like protein (Tim-I), phosphoribosylaminoimidazole carboxylase and phosphoribosylaminoimidazolesuccinocarboxamide synthase (Paics), NADH dehydrogenase subunit 4 (ND4) and sciellin (Scl). The identification of the proteins interacting with Prmt5 is helpful for study and understanding of Prmt5 function in fish.

T19 - Long search for the "Holy Grail" in the evolution of adaptive immune system: Identification of lymph node equivalent structures in medaka fish

Baubak Bajoghli and Narges Aghaallaei

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Germany

In the past two decades, we have witnessed a renaissance in the study of the evolution of the adaptive immune system in vertebrates. In particular, these studies have expanded our knowledge of the evolution of thymopoietic tissues, where T-lymphocytes develop. Furthermore, dendritic cells (DCs), which primarily serve as a bridge between the innate and adaptive immune systems were identified in teleost fishes. Overall, these studies demonstrate that the cellular components of the adaptive immune system are evolutionarily conserved between vertebrates. However, secondary lymphoid organs (SLOs), which spatially organising the interaction between lymphocytes and antigen-loaded DCs, are not well conserved across vertebrate groups. For example, lower vertebrates lack lymph node, a vital SLO in mammals. The great mystery is therefore where the adaptive immune response takes place in lower vertebrates. To address this fundamental question, we used medaka fish as a model system. In a comprehensive study, we discovered specific sites, where T-lymphocytes and DCs traffic and interact akin to mammalian lymph nodes. We found that these sites are distributed throughout the body facilitating a rapid adaptive immune response to local infections. Overall, our work provides a new evolutionary framework of the adaptive immune response in teleost fishes.

T20 - Characterization of the endocrine and behavioural stress responses of medaka larvae

Soojin Ryu

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University Medical School, Johannes Gutenberg University Mainz
Germany

When confronted with a stressor animals modify their physiology and behavior in order to cope with the threat. The stressors can be of diverse type but they typically culminate in the activation of the Hypothalamic-Pituitary-adrenal (HPA) axis whose final output is the pleiotropic stress hormone glucocorticoids (called cortisol in fish). We are interested in using medaka larvae to study the molecular changes caused by stress exposure during development. As a first step, we have begun to characterise the endocrine and behavioural stress response in larval medaka. Our initial aims are 1) to identify different stressors that elevate cortisol in larval medaka, 2) identify behavioural responses to different stressors, and 3) characterise the ontogeny of the stress response during development.

T21 - Basal lamina in the base of intestinal furrow is an inductive site for the mucosal adaptive immune response in medaka fish

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Several organized lymphoid structures provide a niche that facilitates the initiation of adaptive immune response through stimulation of T-cells by professional antigen-presenting cells (APCs), in particular, dendritic cells (DCs). Gut-associated lymphoid tissues (GALTs) are the most ancient secondary lymphoid tissues in vertebrates, although their organization and cellular composition vary across vertebrate groups. Peyer's patches (PPs) and mesenteric lymph nodes (MLNs) are the main initiation sites of the GALTs in mammals. However these organized structures are missing in teleost fishes. It is not yet known where DCs migrate to interact with T-cells within the teleost gut. Hence, the organization of teleost GALT or an equivalent tissue is still not fully understood. We report here that young naïve T-cells after leaving the thymus migrate predominantly in the basal lamina in the base of the medaka intestinal furrow, where they traffic along the intestine and interact with antigen-loaded DCs both during homeostasis and in response to mucosal inflammation. Additionally we found that the epithelial cells in the medaka intestinal furrow produce Ccl25/Ccr9, a chemokine/chemokine receptor axis that is required for the recruitment of T-cells. Our findings suggest that the intestinal furrow provides an environment for T-lymphocyte and APC interaction during homeostasis and in response to inflammation. This indicate that the onset of adaptive immune response is spatially well-organized and the lack of lymphoid structures in teleost fish is compensated by recruitment of cellular components of the adaptive immune system in the basal lamina of intestinal furrow to perform their functions.

T22 - Suppressed recombination of the homomorphic Y chromosome in the medaka

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Institut für Zoologie
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The suppression of meiotic recombination is a key feature of sex chromosomes. After the formation of a sex chromosome pair the lack of recombination keeps the identity of the chromosome pair (WZ or XY) and paves the way for morphological differentiation, accumulation of sex specific genes or degeneration. The relatively young Y chromosome of the medaka *Oryzias latipes* arose by a chromosomal insertion with the *dmrt1by* gene (aka DMY) driving male sex determination. This Y-specific region has a size of 250 kb and is closely linked to phenotypic markers that are described as early as 1921. The region with suppressed recombination was estimated to be roughly 3.5 Mb in size. When we analyzed the recombination frequency of the 39 Mb LG1 chromosomes in different strains of the medaka, we found a suppression of recombination over at least 16 Mb of the X-chromosome, deduced from phenotypic and PCR-markers mapped in the ensemble database. This recombination block between a transgene (EGFP fluorescence) and the leucophore free locus leads to a genetic distance of only 0.4 cM per 16 Mb and is dependent on the sex of the individual and possibly the origin of the sex chromosomes analyzed. This plasticity reflects the young state of evolution of the chromosomes. Our current analyses compare the physical and genetic recombination frequencies on autosomes and different sex chromosomes to elucidate possible mechanisms of the suppression of recombination. As outlook we discuss the artificial evolution of the medaka sex chromosomes to generate a testable model for evolutionary hypotheses.

T23 - Germline sex determination by nanos3, a component of germ plasm, in medaka

Toshiya Nishimura and Minoru Tanaka

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Japan

Germ cells are the common origin cells of sperm and eggs. In many animals, germ plasm, in which germline determinants are contained, is accumulated in oocytes, and cells which inherit the germ plasm during early embryogenesis develop as primordial germ cells (PGC). Nanos is one of the components of germ plasm and evolutionally conserved from flies to mammals. In medaka, nanos3 mRNA and NANOS3 protein are maternally provided to the embryos and expressed in PGCs during embryonic development. Here, we generated nanos3 loss-of-function mutants by TALEN and examined the function of nanos3 in medaka. nanos3 homozygous (-/-) zygotic mutants derived from nanos3 heterozygous mothers formed germ cells. However, after hatching, the number of germ cells decreased dramatically, leading to infertility in both nanos3-/- males and females. Surprisingly, germ cells in nanos3-/- females reduced the expression level of FOXL3, a gene involved in germline sex determination, and initiate spermatogenesis. Therefore, in addition to the maintenance of germ cells, zygotic nanos3 have a novel role involved in germline sex determination, possibly by regulating foxl3 in medaka.

T24 - Drug discovery screening for lymphatic vessel related diseases using medaka

Tonomori Deguchi

National Institute of Advanced Industrial Science and Technology AIST
Health Research Institute HRI
Japan

Evaluation of drug efficacy at the individual-animal-level is essential for advancing to pipeline compounds in all drug development. However, individual experiments take enormous amounts of time and labor and are a major task of drug discovery research. Therefore, we have developed drug discovery screening technology for lymph vessel related diseases using medaka with the aim of constructing a high-throughput screening system at the ultimate individual level capable of screening for drug discovery. First, in order to analyze the lymphatic vessel morphology optically at high speed, we developed a medaka that expresses green fluorescent protein in lymphatic vessels using recombinant DNA technology. In addition, we also developed a special multi well plate for observing medaka, which can observe medaka embryos or fry aligned in the same direction in all wells. Then, we treated the eggs of the lymphatic vessels visualized medaka in the above-mentioned well plate with inhibitor compounds, the images were acquired with an imaging analyzer, and the form change of the lymphatic vessels was examined to evaluate their efficacy. As a result of evaluating the efficacy of about 500 compounds of the existing inhibitor set, death was observed in 126 compounds and teratogenicity was confirmed in 12 compounds. Blood flow abnormality could also be confirmed with 8 compounds. Drug discovery screening for lymphatic-related diseases using medaka, which we have been addressing for years, has become more and more realistic. In the future, we will aim for completion of high-throughput drug discovery screening technology for lymph vessel related diseases using medaka by advancing automation of medaka egg dispensing and automatic analysis of image data.

T25 - in vivo MR microscopy of disease models in Medaka

Tomohiro Ueno

Graduate School of Medicine
Kyoto University
Japan

Magnetic Resonance Imaging (MRI) is a non-invasive imaging technique, which is suitable for differentiating soft tissues of internal organs. Thus, MRI is an essential diagnostic tool in a clinical situation. To investigate various human disease models in small fish, we accomplished in vivo MR microscopy by developing a 14.1 T ultra-high field MRI and a hypothermic anesthesia procedure. In the in vivo MR microscopy, we can exclude a water pool and achieve high spatial resolution enough for the small body size of the fish. We created a non-alcoholic fatty liver disease model in medaka and followed its individual disease progression. We quantitatively evaluated the steatosis level by calculating the MRI-estimated proton density fat-fraction (MRI-PDFF), which estimated triglyceride in liver tissue. The MRI-PDFF results agreed with a histological analysis. Moreover, we applied the in vivo MR microscopy to investigate early indication of disease in p53 knockout medaka. We will also discuss results of the time series analysis of the p53 knockout medaka.

T26 - A panel of medaka inbred lines: a resource to study the genetics of individuality

Felix Loosli, Joachim Wittbrodt, Ewan Birney, Kiyoshi Naruse

Institute of Toxicology and Genetics
Karlsruhe Institute of Technology
Germany

Individuals of a natural population share the same set of genes. However, the polymorphic nature of the gene pool results in genetic individuality with variation in many key phenotypic traits. Statistical approaches, such as genome wide association studies (GWAS) are applied to unravel these complex genetic traits. This requires large numbers of individuals to achieve meaningful statistics and eventually functional tests to prove causality. It is therefore important to establish suitable model systems. We have used medaka fish (*Oryzias latipes*), an economical, fecund vertebrate with a high tolerance to inbreeding to establish a panel of more than a hundred inbred lines from a wild population. By that we have captured and fixed a large diversity of wild genotypes as near isogenic lines. We will use this panel for GWAS to study the underlying genetics of a select number of complex traits. To this aim we are now establishing assays to screen these lines for phenotypic variation. These assays are designed to measure quantitative differences of a given trait, to allow genome wide association studies with the ultimate aim to identify causative QTLs. I will discuss examples of how we assay specific traits relating to organ development and function, as well as behaviour.

T27 - Biological and Optical improvement of IR laser-mediated gene induction microscope system

Yasuhiro Kamei

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National Institute for Basic Biology
Japan

IR laser-mediated gene induction microscope system: IR-LEGO, is a promising technique for developmental biology and biothermology, because the system enables single-cell level local heating. To date, we applied the system to single-cell/local gene induction in many species including medaka and zebrafish. Recently, we utilized the system for a study of heat in biology, especially temperature distribution and dynamics inside cells (we termed this field as biothermology). Now, we are improving the system to be convenient the operation of IR-LEGO and to be match for biothermological experiment. Although temperature measurement is one of key technology for the IR-LEGO and biothermology, in 2017 we established temperature measurement method in vivo by using fluorescent proteins, gTEMP (Genetically encoded fluorescent thermometer). This FPs realize retimetric temperature measurement in cells without harmful effect. Moreover, we are also improving other points of view. In the meeting, we will explain the outline of improvement of the system in three categories, molecular and microscopic; gTEMP, biological; heat shock factors, and optical; adaptive optics.

T28 - Fixed Developmental Timing Revealed by Trans-Species Transplantation

Jana F. Fuhrmann, *Lazaro Centanin*

Animal Physiology & Development
COS, Heidelberg University
Germany

The temporal requirement to go through embryogenesis is stereotypic within a given specie but largely variable among animals, where the very same organ can display different developmental timing even in related species. Considering teleost fish, a Zebrafish embryo develops faster than a Medaka embryo. Here we use Medaka/Zebrafish chimeras to asses if developmental timing relies mainly on genetic information or if alternatively, is an orchestrated process that responds to instructions from the host. Zebrafish and Medaka blastocysts do not mix to each other when put together in a developing blastula. We have exploited this feature to develop exogenous Zebrafish retinae in medaka host and vice versa, and used it to tackle developmental timing using distinctive events: lens recruitment, onset of pigmentation and neurogenesis. Our results indicate that the genetic timing of retinogenesis is maintained when the organ develops in an exogenous environment, suggesting an autonomous mode of organ formation. I will discuss this findings in the context of community cell effects and self-organising models.

T29 - Small proteins with big roles: Bouncer is necessary and sufficient for species-specific fertilization

Sarah Herberg, Krista Gert, Alexander Schleiffer, *Andrea Pauli*

Research Institute of Molecular Pathology
Vienna Biocenter
Austria

Fertilization is fundamental for sexual reproduction, yet its molecular mechanisms are poorly understood. Here, we identify an oocyte-expressed Ly6/uPAR protein, which we call Bouncer, as a crucial fertilization factor in zebrafish. We show that membrane-bound Bouncer mediates sperm-egg binding and is thus essential for sperm entry into the egg. Remarkably, Bouncer is not only required for sperm-egg interaction, but also sufficient to allow cross-species fertilization between zebrafish and medaka, two fish species that diverged over 150 million years ago. Our study thus identifies Bouncer as a key determinant of species-specific fertilization in fish. Bouncer's closest homolog in tetrapods is restricted to the male gonad in internally fertilizing vertebrates, suggesting that our findings in fish have relevance to human biology.

T30 - Analysis of YAP/TAZ-dependent transcriptional response during early morphogenesis in teleost embryos

Vázquez-Marín Javier, Gutiérrez-Triana José Arturo, Letelier Joaquín, Buono Lorena, Mateo Juan L., Wittbrodt Joachim, **Martínez-Morales Juan Ramón**

Gene regulation and morphogenesis
Centro Andaluz de Biología del Desarrollo (CSIC/UPO)
Spain

The Hippo signaling pathway is a genetic regulatory cascade that controls tissue homeostasis and organ size by coordinating the expression of proliferation, differentiation and apoptotic genes during development. The main constituents of this pathway are two effector proteins: YAP and its paralog TAZ (WWTR1). When YAP or TAZ are active, they get translocated into the cell nucleus and regulate the expression of their target genes interacting physically with TEAD, among other transcription factors. Here we show that Yap family's paralogs composition is variable among vertebrates. Whereas many of the vertebrate branches maintained functional copies of both Yap and Taz (such as in tetrapods, spotted gar or zebrafish), Taz is not present in Acanthomorpha, the largest group of teleost fishes (e.g. cod, tilapia, platyfish, stickleback, or medakafish). In contrast, a second copy of Yap1, here referred as yap1b, appears conserved in their genomes. This closer paralog encodes for a protein with a divergent c-terminal transactivation domain. Interestingly, comparative analysis of zebrafish and medaka mutants shows that the mutation of yap1 in medaka (generated by CRISPR-Cas) results in a strong morphogenetic phenotype early during development, which is similar to the simultaneous mutation of yap1 and taz in zebrafish. We use iDamIDseq to identify the potential targets of these transcriptional regulators during gastrulation in medaka embryos. Our results, reinforced after carrying out a transcriptomic approach, indicate that the binding profile of yap1b is limited to a subset of the yap1 TEAD-dependent binding sites. Furthermore, the abundance of genes involved in cell migration in both datasets as well as the evidence from ISH and functional assays suggest the potential role of YAP as a regulatory driver of morphogenesis at early developmental stages.

T31 - Genetic compensation is triggered by mutant mRNA degradation

Mohamed A. El-Brolosy, Andrea Rossi, Zacharias Kontarakis, Stefan Günther, Nana Fukuda, Carter Takacs, Shih-Lei Lai, Ryuichi Fukuda, Claudia Gerri, Khrievono Kikhi, Antonio J. Giraldez and **Didier Y.R. Stainier**

Developmental Genetics
Max Planck Institute for Heart and Lung Research
Germany

Fertilization is fundamental for sexual reproduction, yet its molecular mechanisms are poorly understood. Here, we identify an oocyte-expressed Ly6/uPAR protein, which we call Bouncer, as a crucial fertilization factor in zebrafish. We show that membrane-bound Bouncer mediates sperm-egg binding and is thus essential for sperm entry into the egg. Remarkably, Bouncer is not only required for sperm-egg interaction, but also sufficient to allow cross-species fertilization between zebrafish and medaka, two fish species that diverged over 150 million years ago. Our study thus identifies Bouncer as a key determinant of species-specific fertilization in fish. Bouncer's closest homolog in tetrapods is restricted to the male gonad in internally fertilizing vertebrates, suggesting that our findings in fish have relevance to human biology.

Oral Presentations
3rd Regional Fish Meeting

T32 - Evolutionary emergence of the *rac3b/rfng/sgca* regulatory cluster refined mechanisms for hindbrain boundaries formation in zebrafish

Key Note Lecture

Juan Ramon Martinez-Morales

Gene regulation and morphogenesis
Centro Andaluz de Biología del Desarrollo (CSIC/UPO)
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Developmental programs often rely on parallel morphogenetic mechanisms that guarantee precise tissue architecture. While redundancy constitutes an obvious selective advantage, little is known on how novel morphogenetic mechanisms emerge during evolution. The vertebrate hindbrain is subdivided along the antero-posterior axis in 7 rhombomeres emerging during development through a finely tuned TFs code. In zebrafish, rhombomeric boundaries behave as an elastic barrier, preventing cell intermingling between adjacent compartments. Here, we identify the role of the small-GTPase Rac3b in actomyosin cable assembly at hindbrain boundaries. We show that the novel *rac3b/rfng/sgca* regulatory cluster, which is specifically expressed at the boundaries, emerged in the Ostariophysi superorder by a chromosomal rearrangement that generated new cis-regulatory interactions. By combining 4C-seq, ATAC-seq, transgenesis, and CRISPR-induced deletions, we characterized this regulatory domain, identifying cis-regulatory elements active specifically at the hindbrain boundaries. Our results suggest that the capacity of boundaries to act as an elastic mesh for segregating rhombomeric cells evolved by co-option of critical genes to a novel regulatory block, refining the mechanisms for hindbrain segmentation.

T33 - High content screening by ACQUIFER - Automated microscopy for whole organism screening applications

Jochen Gehrig

ACQUIFER
DITABIS AG
Germany

ACQUIFER is a division of DITABIS, Digital Biomedical Imaging Systems AG, Freiburger Str. 3, 75179 Pforzheim, Germany. Phenotypic screening is increasingly employed in biomedical and pharmaceutical research to address scientific questions in the context of whole organism model systems, such as the zebrafish embryo. However, the complexity of a fully developed body imposes methodological challenges in high content screening, demanding novel technical solutions that are compatible with large scale automated imaging and scoring of cell- or tissue-specific and overall phenotypes. At ACQUIFER, we have developed the Imaging Machine, a versatile and flexible high content screening platform following a unique optomechanical design, optimized for non-adherent and motion-sensitive specimen. Its sample centred approach, including a static stage in combination with moving optics and integrated environmental control, provides optimal in-vivo imaging conditions and renders it ideal for image-based screening or parallel long-term observation of biological specimen such as small model organisms. Here, we will give an overview of imaging hardware, discuss workflow requirements for zebrafish screening and highlight example projects. Please refer to our website at www.acquifer.de or call us at +49 (6221) 435 2035 for more detailed information on ACQUIFER products.

T34 - A Change of Heart: Medaka as a model for Human Cardio-Vascular Diseases & GWAS

Omar Hammouda, Thomas Thumberger, Jakob Gierten, Jochen Gehrig,
Christian Pylatiuk, Felix Loosli, Jochen Wittbrodt

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Cardiovascular diseases (CVD) cover a wide spectrum of disorders involving the heart or blood vessels such as congenital heart diseases, arrhythmias and many more. Together CVD are the leading cause of death globally. Increasing number of Genome Wide Association Studies (GWAS) are being performed, thousands of single nucleotide polymorphisms (SNPs) are being identified and associated to diseases in humans. However, about 93% of identified SNPs lie within non-coding regions of our genome, highlighting the necessity to explore and understand the role of these regions in disease development and progression. Moreover, genetic and experimental limitations in human GWAS urge us to use animal models to fully exploit the advantages of GWAS. My aim is to break down the complex regulatory pathways involved in the development of CVD using medaka (*Oryzias latipes*) as a model for functional validation of human CVD-associated genes/SNPs. While also introducing it as a robust model for GWAS to identify novel CVD-associated SNPs. Medaka offers many technical advantages such as transparent body and a high tolerance to inbreeding; which will aid in the identification, validation & characterization of novel coding/regulatory mutations leading to CVD-related phenotypes. As a first step, using our newly developed high-throughput heart rate screening protocol, I functionally validate various novel CVD-associated gene hits identified in human GWAS in vivo in medaka embryos. Using CRISPR/Cas9 targeting complementary human SNP-containing gene regions in medaka, I observe significant changes in heart rate, heart morphology or arrhythmias already in the injected generation. The high isogenicity of medaka inbred lines, ease of the experimental setup & analysis in addition to the rapid gain of results, all pave the way for medaka as an exceptional model for high throughput functional gene validations.

T35 - Luxendo Light-sheet Microscopy: Seeing Life from a Different Angle

Malte Wachsmuth

LUXENDO, Fluorescence Microscopy Business Unit
Bruker Nano Surfaces Division
Germany

Light-sheet microscopy has become the state of the art methodology to address a wide variety of biological questions. Key features of this technique are the extremely minimized phototoxicity, the high-speed image acquisition, and the large imaging depth. This allows long-term imaging of delicate samples in a volumetric manner. Fast subcellular processes and interactions can be observed in the comprehensive context of an organoid, organ, or entire organism.

Different samples require different conditions, such that there are also various approaches how to image different samples with light-sheet microscopy. Being a company that is dedicated 100% to light-sheet microscopy, LUXENDO decided to reflect this fact by implementing specialized setups without losing general applicability for each of them.

Here, we will introduce the basic concepts of light-sheet microscopy, followed by different implementations. To highlight the advantages suited for specific samples, we will focus on our two recently introduced products: the multiple-view selective plane illumination microscope (MuVi SPIM) and the inverted view selective plane illumination microscope (InVi SPIM).

The MuVi-SPIM is a horizontal setup, that is designed to image large volumes very fast. The 4-fold geometry with its two-sided illumination combined with the two-sided detection allows optimal signal detection from anywhere in the sample without the need for rotation.

Dedicated to live imaging, the InVi-SPIM is a microscope that is optimized for long-term 3D fluorescence imaging of living specimens. Its maximized photon efficiency, and short illumination times enable long-term imaging under ideal environmental conditions. The optical performance combined with the fast acquisition speed makes the InVi-SPIM perfectly suited for in toto imaging of a large variety of specimens, especially if they are sensitive and need precisely controlled conditions.

T36 - Growth control in the retinal stem cell niche of medaka

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All species with life-long growth are facing similar challenges. Organs have to scale in size, while proliferation of stem and progenitor cells has to be tightly controlled to prevent aberrant growth of tissue. The teleost medaka (*Oryzias latipes*) is one of those species, and after completion of embryogenesis the retina continues to grow. Retinal stem cells (RSCs), located in the niche at the ciliary marginal zone, divide life-long, ultimately adding new neurons to the growing organ. Since the function of the eye depends on its shape, the activity of stem and progenitor cells is tightly coordinated to establish the proper cell type composition and number. My project combines advanced genetics and targeted modulation of signaling pathways in RSCs to address mechanisms of growth control in the RSC niche. I found that the activation of the insulin signaling pathway in RSCs is sufficient to stimulate massive proliferation. Here, the retina not only grows in radial size but also in thickness of the individual layers. The proliferation rate of stem and progenitor cells is a key determinant not only for eye size but also for its physiological properties. Strikingly we could only achieve this by the physiological signal, but not by oncogenic triggers of proliferation so far. Addressing this aspect, we found that RSCs are under surveillance of mononuclear phagocytic cells. The stem cells express a chemokine ligand attracting mononuclear phagocytic cells expressing the corresponding chemokine receptor. These cells form a network, maintained life-long, in close proximity to the RSC niche. Preliminary data point to an active role of immune cells in protecting and honing the RSC niche.

T37 - Zebrafish heart valve regeneration: a model for valve recellularization

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Heart valve diseases pose a major threat to human health worldwide, ultimately leading to heart failure and death. The majority of diseased valves are not repairable, and the only possible therapy relies on surgical replacement using mechanical implants or biological scaffolds. Recently, the clinical application of decellularized valves has shown promising results. However, complete recellularization of the implanted valves is still a challenge, which leads to extracellular matrix destabilization, and ultimately valve leaflet degradation. Therefore, identification of cellular and molecular factors promoting efficient recellularization of the implanted valve is crucial to ensure growth potential, repair and effective response to cardiac function demands. Zebrafish are known for their unusual ability to regenerate multiple organs and tissues. Here we show that, upon atrio-ventricular (AV) valve interstitial cell ablation using the Nitroreductase/Metronidazole (NTR/Mtz) system, adult zebrafish are capable of fully regenerating their cardiac valves. Taking advantage of multiple transgenic lines and detailed confocal imaging, we characterized the key stages of valve regeneration. We focused on the positive role of the inflammatory response, cell cycle re-entry, re-differentiation and reconstruction of lost tissue. Moreover, by combining a high-resolution approach of Laser Capture Microdissection with RNAseq, we established a transcriptomic profile of the regenerating valve and surrounding tissues. This dataset allowed us to identify potential pro-regenerative factors driving valve recellularization. Using overexpression transgenic lines and loss-of-function strategies, we are analyzing the role of chemokines and other secreted factors in promoting cell recruitment during valve regeneration. Overall we expect to bring new insights into the field of cardiac valve regeneration, with a particular focus on the molecular factors promoting valvular recellularization.

T38 - Pitx2c orchestrates embryonic axis extension via mesendodermal cell migration and oriented cell division

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Pitx2c, a homeodomain transcription factor, is classically known for its left-right patterning role. However, an early wave of pitx2 expression occurs at the onset of gastrulation in several species, indicating a possible earlier role that remains relatively unexplored. Here, we show that in zebrafish, maternal-zygotic (MZ) pitx2c mutants exhibit a shortened body axis indicative of convergence and extension (CE) defects. Live imaging reveals that MZpitx2c mutants display less persistent mesendodermal migration and randomly oriented cell divisions, which contribute to ineffective CE movements. Transplant experiments indicate that Pitx2c functions cell non-autonomously to regulate these cell behaviors by modulating cell shape and protrusive activity. Using transcriptomic analyses and candidate gene approaches, we identify transcriptional changes in components of the chemokine-ECM-integrin dependent mesendodermal migration network. Together, our results define pathways downstream of Pitx2 that are required during early embryogenesis, and reveal novel functions for Pitx2 as a regulator of morphogenesis.

T39 - Developing a zebrafish model to identify novel molecular resilience mechanisms

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Resilience is a dynamic and active process that ensures a trajectory of stable mental health during and after a traumatizing event or a prolonged period of stress, or a relatively rapid, successful recovery. Elucidating and understanding the underlying mechanisms behind this phenomenon is important to combat the negative trajectories non-resilient individuals' experience. The zebrafish represents a newly established vertebrate animal model in stress research with a high degree of conservation of the stress response system, rendering it an attractive model system to discover molecules contributing to stress resilience. Both acute and chronic stressor paradigms have been reported for zebrafish. However, as of yet, there is no established resilience model, thus, our aim is to develop the first resilience model in zebrafish. Our first objective is to develop a chronic, ecologically relevant stressor in the form of a predator cue and subsequently detect behavioral alterations via multiple behavioral assays. Here we report the development of a high-throughput stressor delivery system suitable for simultaneous chronic stress exposure of a large number of adult zebrafish. The system consists of small monitors fixed above single-housing tanks displaying a 'looming dot' of defined contrast and angular velocity, resembling an approaching predator. To robustly detect the effect of chronic stress on this behavior, we developed an "open tank" assay, comprising of a large novel environment, offering both a confined deep space and an open exposed space. An individuals' choice between these two compartments is a measure of stress-induced alteration in exploratory behavior. We discuss the details of this behavioral paradigm and present results on how acute and chronic predator stress exposures alter adult zebrafish behavior in this assay.

T40 - The Hippo Pathway effector Taz is required for the formation of the Micropyle in Zebrafish

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In some aquatic species such as teleost fishes, the process of fertilization is external and is mediated by a funnel-shaped opening on the vitelline membrane of the oocyte, called 'Micropyle'. The micropyle constitutes a unique sperm entry point at the animal pole of the oocyte. It is left behind by a highly specialized follicular cell, the micropylar cell (MC) that is thought to block the formation of the vitelline membrane at the point of contact with the oocyte. Hardly anything is known on how only one cell of the follicular cell layer is first selected and then specified to become such a specialized structure. We recently uncovered that the hippo pathway effector Taz (encoded by the *wwtr1* gene) plays a major role in this process. *wwtr1* homozygous mutant females are infertile. We could show that this is due to a failure to specify a micropylar cell and, as a consequence, a micropyle. Although it has been clearly shown that the oocyte polarity plays an important role in the specification of the MC, we demonstrate that oocyte polarity is not affected in *MZwwtr1* females. We further identified Taz as the first bona fide marker of the MC, and could show that Taz is restricted very early during oogenesis to only one cell, which later forms the functional MC. Finally we uncover cell properties of the MC as potential downstream effectors of Taz in the specification process. Altogether our results identify Taz as the first potential Master regulator of the micropylar cell fate, thus making also an important contribution to understanding fertility in teleost fishes.

T41 - The mRNA surveillance machinery controls transcriptional adaptation to mutations

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In response to deleterious mutations, genetic compensation by transcriptional upregulation of related gene(s) (also known as transcriptional adaptation) has been reported in numerous systems; however, how such a response is activated is unknown. We developed and analyzed several models of transcriptional adaptation in zebrafish and mouse and observed a correlation between mutant mRNA decay and transcriptional upregulation of related gene(s). To assess the role of the mutant mRNA in triggering transcriptional adaptation, we generated alleles that fail to transcribe the mutated gene and found that they do not exhibit this response. Moreover, genetic inactivation, silencing or chemical inhibition of the nonsense mediated decay factor Upf1 can also lead to loss of transcriptional adaptation. These results identify a new role for the mRNA surveillance machinery in buffering against mutations by triggering the transcriptional upregulation of related genes. In addition, these results will help design mutant alleles with minimal transcriptional adaptation-derived compensation.

T42 - Genome-wide Association Study of Cardiac Phenotypes in Medaka Inbred Strains

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Objectives: Congenital heart disease (CHD) is one of the most common human birth defects. However, only a minor fraction of CHD cases could be linked to genetic variants/mutations. The aim is to dissect genetic determinants of cardiac traits using medaka inbred strains. For a proof of concept genome-wide association study (GWAS) we focused on heart rate (HR) and its dependence on temperature. **Methods:** A panel of 100 medaka inbred strains with fully sequenced genomes was used as a mapping population (Loosli, Naruse, Birney, Wittbrodt; unpublished). The panel was profiled applying automated microscopy to score HR of medaka embryos in a temperature range of 21-35 °C. To resolve genetic elements controlling HR, we leveraged two medaka inbred strains with extreme phenotypes: Mop with fast HR and HdrR as reference with slow HR, which were subjected to F2 segregation analysis combining phenotyping with whole-genome sequencing (WGS). **Results:** WGS of the inbred panel revealed high levels of homozygosity within strains and millions of segregating SNPs across strains. Phenotyping a subset of the panel revealed substantial differences in HRs (max. 24%) and a high broad-sense heritability ($H^2 = 0.72$). The F2 segregation analysis using Mop (fast HR) and HdrR (slow HR) demonstrated an intermediate phenotype in F1-hybrids and a phenotypic distribution in F2 spanning the parental phenotypic interval. To link phenotypic measurements to variants, WGS is currently performed in the F2 population. **Conclusion:** We present a genome-wide approach to dissect genetic determinants of human disease-relevant phenotypes in a medaka genomics resource. A segregation analysis, focused on HR, demonstrated a mode of complex phenotype inheritance. Strategies to detect and validate novel causative genetic elements are currently being established. We expect that downstream evaluation of candidate variants will indicate novel loci relevant for heart development, function and disease susceptibility.

T43 - Molecular Mechanisms of Germ Plasm Localization during Early Zebrafish Embryogenesis

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The zebrafish germline is specified early during embryogenesis by inherited maternal RNAs and proteins called germ plasm. Only those cells containing germ plasm will become part of the germline, whereas other cells will commit to somatic cell fates. Therefore, proper localization of germ plasm is crucial for germ cell specification. To investigate germ plasm localization, we use the Bucky ball (Buc) gene discovered in our lab as a molecular proxy. Buc is indispensable for germ plasm aggregation in zebrafish. Moreover, Buc is the first protein inducing the formation of primordial germ cells *in vivo*. Fascinatingly, Buc protein mirrors germ plasm localization during all stages of zebrafish embryogenesis and oogenesis. For example, Buc is restricted to the cleavage furrows at the 4-cell stage, which will constitute the entire germline of the embryo. Interestingly, I found that a motif of 47 of 639 amino acids is necessary and sufficient for Buc localization. However, it was recently shown that the localization signal of the Buc homologue in *Xenopus* contains prion domains, which are responsible for protein aggregation (Boke et al., Cell, 2016). Nonetheless, I could show that Buc localization is independent of protein aggregation via prion domains. Furthermore, I found that Buc is recruited to cell-cell junctions, since it co-localizes with the adherens junction protein ZO-1 and hemidesmosomal protein integrin- α 5. Further co-localization experiments will determine the subtype of cell-cell junction, which anchors germ plasm to the cleavage furrow. In addition, I plan to use the localization domain as a bait to isolate the biochemical interaction network controlling Buc positioning in the early embryo. Taken together my experiments suggest that Buc acts as a scaffold recruiting germ plasm proteins to four cell-cell junctions, which then separate the germline from the soma during zebrafish embryogenesis.

T44 - In vivo secretome-wide and chemical screening to identify novel regulators of pancreatic β -cell function

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Diabetes Mellitus is foreseen to be the 7th leading cause of death in 2030, warranting a high demand to identify new therapeutics. Type 2 Diabetes, which affects 90% of diabetes patients, initiates with insulin resistance of the target tissue but only manifests in individuals where the β -cells cannot meet the higher demand for Insulin. Several in vitro drug screens have been carried out to identify signaling pathways inducing β -cell expansion and functionality. However, the drugs identified often fail in the following in vivo studies due to the xenobiotic defense mechanism of the organism. To monitor β -cell functionality in vivo, we established two zebrafish transgenic lines using the firefly luciferase as a sensitive and quantitative readout of pdx1 and insulin promoter activity. To specifically identify conserved and clinically relevant proteins, we screened a library of the human secretome (1700 cDNAs) as well as 7000 chemical compounds including FDA approved drugs. So far, our in vivo strategy has identified 29 human proteins that induce the insulin promoter and 7 small molecules that induce the pdx1 promoter. We further validated several of the small molecules and found that the hit compounds indeed induce β -cell function in primary human tissue samples as well as in a stem cell based human β -cell differentiation protocol. Thus, by using our in vivo high throughput screening strategy, we have been able to identify potential modulators of the activity of the pdx1 and insulin promoters in vivo. We speculate that some of these proteins and compounds will provide a beneficial effect when tested in diabetic patients.

T45 - Loss of erythropoietin aggravates hyperglycemia induced renal damage and alters embryonic renal development via induction of apoptosis in zebrafish

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T46 - From Morphology to Behavior: Phenotyping Medaka Inbred Lines

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Johann Bollmann, Joachim Wittbrodt

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With advances in genotyping and cost-effective sequencing technologies, Genome-wide association (GWA) studies have emerged as approaches to study the genetics of natural variation and gene-environment interaction. GWA studies are particularly useful when inbred lines are available (as once they are genotyped, these lines can be phenotyped multiple times) and also with the availability of automated image acquisition and analysis systems for rapid phenotyping. The teleost fish Medaka (*Oryzias latipes*) is an ideal model organism for these kinds of studies because of the presence of still free living wild populations in Japan and East Asia, the ability to generate highly polymorphic inbred lines and the availability of the complete genome sequence. We present the development of automated and intelligent screening platforms for high-throughput acquisition of morphometric and behavioral data. We characterize gross morphological features of different southern and northern inbred Medaka lines and evaluate the level of phenotypic variation between these lines. Furthermore, we show results of a quantitative assessment of fundamental behavioral traits such as locomotion and feeding in larval Medaka. Mathematical algorithms developed for morphometry and behavioral analysis will be discussed. The aim of this work is to identify a variety of phenotypic traits derived from morphology and behavior in order to assist in the investigation of the genetic basis for polymorphism in Medaka.

T47 - Neural stem cells induce the formation of their physical niche during organogenesis

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Most organs rely on stem cells to maintain homeostasis during post-embryonic life. Typically, stem cells of independent lineages work coordinately within mature organs to ensure proper ratios of cell types. Little is known, however, on how these different stem cells locate to forming organs during development. Here we show that neuromasts of the posterior lateral line in medaka are composed of two independent life-long lineages with different embryonic origins. Clonal analysis and 4D imaging revealed a hierarchical organisation with instructing and responding roles: an inner, neural lineage induces the formation of an outer, border cell lineage (nBC) from the skin epithelium. Our results demonstrate that the neural lineage is necessary and sufficient to generate nBCs highlighting self-organisation principles at the level of the entire embryo. We hypothesise that induction of surrounding tissues plays a major role during the establishment of vertebrate stem cell niches.

T48 - Neural stem cells coordinate post-embryonic morphogenesis in the eye of medaka

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How do anatomically and functionally distinct tissues coordinate to direct growth and shape in complex organs?

Teleost fish grow throughout life, providing an excellent system to address this question. The eye consists of many anatomically distinct tissues; i.e. cells in one tissue do not contribute new cells to other tissues in the organ. Thus, the tissues grow independently, and yet their growth rates must be precisely tuned to maintain the 3D shape crucial for visual function.

In medaka (*Oryzias latipes*), the neural retina (NR) and retinal pigmented epithelium (RPE) contain independent stem cells in a ring-shaped domain. In both tissues, cell size is constant, cell death is negligible, and there is no cell rearrangement. Therefore, the critical parameter that needs to be tuned to adjust tissue growth rate to organ growth rate is the proliferation of the tissue stem cells.

We explore conceptual modes of growth and shape-giving in the NR and RPE in an agent-based computational model. Using experimental lineage tracing in medaka, we validate the model predictions and introduce step-wise increments in complexity to explain the experimental findings. The combination of computational modelling and experiments uncovers that - despite the identical topology of NR and RPE - stem cells in the NR drive growth and control shape of the organ, whereas RPE stem cells follow external instructive signals.

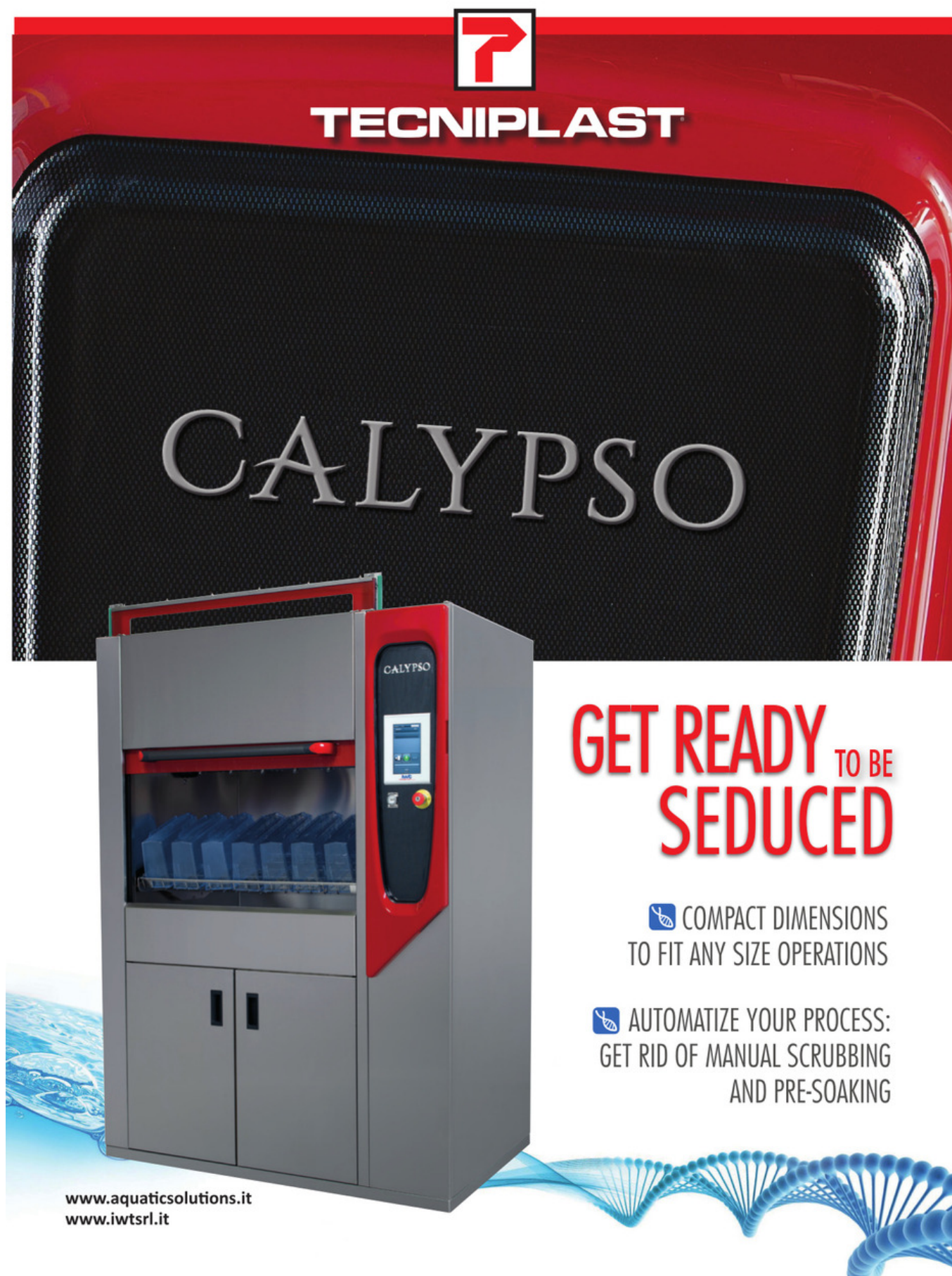
Our work highlights how a minimal target node for evolution – the proliferation of neuroretinal stem cells – can be exploited to adapt complex organ morphogenesis in a vertebrate system.

T49 - Visualizing signaling oscillations during embryonic patterning in the Medaka model

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During development, cells are required to interpret dynamic signals and coordinate a precise response in space and time. Our lab is interested in how embryonic patterning can be encoded in the dynamic properties of such signals. To explore the function of signaling dynamics in development, we study somitogenesis in vertebrate embryos. The timing of somitogenesis is controlled by a “segmentation clock” that periodically activates a system of oscillating genes in the presomitic mesoderm (PSM). It is known that these dynamic signals are important for somite formation, but how cells interpret such signals is an open question. While somitogenesis itself is a conserved process in vertebrates, the oscillatory signaling pathways involved vary between different species. For example, in the Mouse PSM this includes the Notch, Wnt and FGF pathways, while in the Zebrafish PSM there is only evidence for the involvement of the Notch pathway, notably the *her* genes. To complement existing models of vertebrate somitogenesis, we study signaling dynamics in the Japanese Medaka (*Oryzias latipes*). Using CRISPR/Cas9, we aim to knock in reporters in the endogenous locus of molecular players in the Notch, Wnt and FGF pathways, followed by imaging of signaling oscillations in real time. I will present recent progress in the development and imaging of these dynamic reporters. Following this approach, we hope to discover which pathways are involved in the Medaka segmentation clock, and how general the principles of dynamic signal encoding are across vertebrate species. Ultimately, these reporter lines can be used not only to characterize the Medaka segmentation clock, but also to correlate signaling dynamics with spatial patterns through genetic and chemical perturbations.



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Abstracts of Presented Posters

1 - Medaka exposed to 4100 K fluorescent light incite a cellular inflammation and immune response within internal organs

Mikki Boswell, Yuan Lu, William T. Boswell, Markita Savage, Zachary Mazurek and Ronald B. Walter

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We recently reported the transcriptional response to fluorescent light (FL) exposure, in skin, for three commonly used aquatic biomedical research models, *Danio rerio* (zebrafish), *Oryzias latipes* (medaka) and *Xiphophorus maculatus*1 (platyfish). Here, we expand the genetic impact of FL light exposure in medaka to include differential gene expression within two internal organs, brain and liver. Analyses of replicate RNA-Seq data sets showed the transcriptional response to common 4,100 K FL, or "cool white" light, is present in all three organs, and is robust within each organ tested. FL induces transcription of genes associated with the cellular inflammatory and immune responses, consistent with oxidative stress, and likely driven by up-modulation of the TNF α master regulator. Furthermore, comparative RNA-seq analyses of gene expression between the three organs (i.e., skin, brain, liver) suggest the response to light in the internal organs may be via transduction of the acute phase response signaling cascade that modulates cellular stress and increases cellular inflammation. In addition, we exposed adult male medaka to 50 nm wavelength regions between 300-600 nm and were able to determine the 100 nm region between 400-500 nm incites the majority of the genetic response observed for complex FL exposure in all three organs tested. By exposing the animals to 50 nm wavebands, we were able to identify six skin specific pathways could be incited or suppressed by a single waveband and two pathways that could be induced with one waveband exposure while suppressed by another. Likewise, in liver we identified 29 unique waveband specific pathways and two that could be activated or suppressed with specific light sources. Together, these data suggest that not only the type of lighting, but also the specific wavelength composition of the light, play critical roles in animal exposures.

2 - Development of Transcriptional Disease Signature (TDS) to Screen Small Molecules in a Transgenic Medaka Melanoma Model

Yuan Lu, **William Boswell**, Mikki Boswell, Susanne Kneitz, Barbara Klotz, Janine Regneri, Markita Savage, John Postlethwait, Wesley Warren, Manfred Scharl, Ronald B. Walter

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Drug development often fails during late screening stages when candidate drugs are tested in intact animals. Cell culture and target-based compound screening strategies for drug development cannot eliminate compounds that exhibit off target toxicity or biological adaptation in animals. In contrast, phenotypic screening, using intact research animals, has a higher success rate in identifying candidate compounds. However, phenotypic screening is labor intensive, low-throughput, and can be very expensive. To take advantage of target-based and phenotypic screening, we employed medaka as a model to develop a mid-high throughput drug screening regimen involving gene expression profiling to define genetic signatures associated with specific disease (Transcriptional Disease Signature, or TDS). Using this screening strategy and NanoString for direct counting of TDS gene expression, compounds capable of altering TDS gene expression in whole animals may be identified. As a proof of concept, we used transgenic medaka that overexpresses a Xiphophorus mutant egfr gene (xmrk) in melanocytes leading to melanoma development. Using this melanoma model, we have been developing a screening pipeline to identify small molecules capable of reducing melanoma proliferation.

Two anti-melanoma compounds (Cisplatin and Trametinib) were tested and shown to modulate TDS gene expression. Drug effects on TDS profiles were estimated by comparing compound-modulated genes in the TDS NanoString panel using z-score and Kolmogorov-Smirnov statistics. TDS gene probes were designed to target known common signaling pathways including proliferation, development, toxicity, immune function, metabolism and detoxification. Our pilot test showed that both compounds are capable of altering TDS gene expression. In conclusion, we resolved logistics of using intact medaka to screen potential compounds and have developed a data analyses pipeline to establish feasibility of this novel TDS drug-screening strategy.

3 - Towards a fundamental understanding of protein O-mannosylation in vertebrates

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Congenital disorders of glycosylation are diseases of glycosylation that are mostly caused by hypomorphic mutations. These disorders may be very severe such as dystroglycanopathies, which lead to muscular dystrophy and severe brain defects in patients due to loss of O-mannosylation of target proteins. Since our understanding of glycosylation is still very rudimentary, the basis of these human diseases remains obscure.

Hypomorphic mutants for the two key enzymes in the O-mannosylation pathway in the model organism, medaka (*Oryzias latipes*) are already available. The observed phenotypes are clearly reminiscent of defective signalling pathways, with very specific defects. Accordingly, these mutants provide an excellent resource to understand both, O-mannosylation fundamentally and patient phenotypes with O-mannosylation defects. In order to identify critical O-mannosylation sites on signalling pathway proteins and understand their underlying structure-function relationship a combination of the following will be realised: glycoproteomics on established mutants; targeted mass spectrometry on selected candidates and modulating identified O-mannosylation sites in these candidates. Ultimately, understanding the molecular nature of defective pathways may provide access to therapeutic approaches in dystroglycanopathies.

4 - Organogenesis in the lateral line of medaka

Karen Groß, Ali Seleit, Isabel Krämer, Lazaro Centanin

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Fish grow throughout their entire life and their organs handle this challenge of life-long growth by increasing either in size or number of functional units. Using well established 4D imaging tools, the lateral line of medaka is used as a model to assess the mechanisms of how organs are formed both embryonically and post-embryonically to adapt to an ever-growing body. The lateral line is a sensory organ of fish and aquatic amphibians that detects water flow. It is used for orientation, schooling or prey and predator detection. Its functional units are the neuromasts, which are distributed across the entire fish body forming the anterior lateral line on the head and the posterior lateral line on the trunk and caudal fin.

The caudal neuromast cluster (CNC) on the caudal fin of medaka has previously been used as a model to examine post-embryonic organogenesis. Previous work of the lab using lineage tracing and ablation experiments has shown that all neuromasts in the cluster are derived from a single founder neuromast that is deposited on the fin during embryonic development. We observed that new organs are formed by neuromast stem cells that escape the founder neuromast in a stereotypic manner, reminiscent of epithelial to mesenchymal transition: epithelialized cells need to alter their polarization within the tissue, migrate away from their original position and eventually become epithelialized again to give rise to a new organ.

Furthermore, the embryonic development of the anterior lateral line in medaka is described for the first time. Strikingly, primary neuromasts in the anterior lateral line seem to be formed by two different mechanisms: differentiation within sensory ridges and deposition by a migrating primordium. Moreover, the formation of the anterior lateral line pattern is highly stereotypic, hinting towards a tightly controlled development.

5 - The role of transcription factors *zic1* and *zic4* in dorsal somite patterning

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In development, the early specification of axes is a well-studied process, far less is known about patterning events taking place later during embryonic development after the three germ layers are set up. A spontaneous mutant of Medaka (*Oryzias latipes*), the Double anal fin (Da), provides the unique opportunity to study dorsal-ventral patterning during somitogenesis. The Da is an enhancer mutant of the transcription factors *Zic1* and *Zic4* and exhibits a “ventralized” trunk region. This mirror-imaged phenotype is caused by lack of *zic1/zic4* expression in the somites. The Da phenotype cannot be explained with the prevalent model of dorsal-ventral patterning of the somites. There, BMP signaling induces canonical Wnt signaling (*wnt1/wnt3a*) in the dorsal part of the neural tube, which in turn induce the expression of *wnt11* (non-canonical Wnt signaling) in the dorsal medial lip. *Wnt11r* plays an important role in the correct orientation of myocyte elongation. So far, it is unknown how canonical Wnt signaling induces non-canonical Wnt signaling in the dermomyotome. In Da the myotome fails to cover the neural tube, this strongly hints to a role of *Zic1/Zic4* in dermomyotome formation. I want to examine whether *Zic1/Zic4* are mediators between canonical and non-canonical Wnt signaling, since this link is not reported yet. Using ChIP-sequencing we could identify *wnt11r* as a downstream target of *zic1*. Additionally, I could show that inhibition of canonical Wnt signaling led to a decrease of *zic1* expression in the dorsal somites. To validate *zic1/zic4* as downstream targets of canonical Wnt signaling I will upregulate the canonical Wnt signaling pathway using Azakenpaullone, furthermore, to show interactions between players of the canonical Wnt signaling and *zic1/zic4* a ChIP will be performed. Here, I am proposing an additional link in a well-established model, which leads to a more detailed understanding of late dorsal patterning in the teleost trunk.

6 - Role of Toll-like receptor signaling in zebrafish cardiac regeneration

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The innate immune response, one of the earliest responses after cardiac damage, is required for cardiac regeneration in regenerative animals such as the neonatal mouse and zebrafish. However, it is also involved in the pathogenesis of myocardial infarction (MI) in the non-regenerative adult mammalian heart. Despite these important roles, the differences between the immune responses leading to successful and unsuccessful tissue remodeling are unknown. In order to elucidate the molecular mechanisms of the innate immune response promoting cardiac regeneration, we are focusing on Toll-like receptor (TLR) signaling which plays critical roles in innate immunity by recognizing endogenous and exogenous ligands and inducing pro-inflammatory/anti-viral responses. Since TLR signaling depends on the adaptor molecules Myd88 or TRIF, we analyzed the role of Myd88-dependent TLR signaling during zebrafish cardiac regeneration using *myd88* mutants. In these mutants, neutrophil recruitment, a main inflammatory response, was diminished in cryoinjured hearts. Additionally, we found that cardiomyocyte proliferation was significantly decreased in the mutants at 7 dpci (day post cryoinjury). However, these mutants eventually regenerated their hearts by 60 dpci, displaying regressed or no scars. We also generated *trif* mutants using the CRISPR/Cas9 technology and are using them to investigate the roles of TRIF-dependent TLR signaling during cardiac regeneration. Our study will provide new fundamental knowledge about the exact roles and mechanisms of the innate immune response during heart regeneration.

7 - Analysis of foxl3-downstream genes, focusing on intrinsic mechanisms of the germline sex determination in medaka

Mariko Kikuchi, Daisuke Saito, Miyo Tsuda, Mikita Suyama, Shuji Shigenobu,
Toshiya Nishimura, Minoru Tanaka

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Sex-determination of germ cells is a critical issue for sexual reproductive organisms. Foxl3 (Forkhead domain-containing transcription factor 3) is the germline sex determinant identified in medaka (Nishimura et al, Science, 2015). In the foxl3-mutant ovaies, germ cells change their fate to spermatogenesis, indicating that foxl3 suppresses spermatogenesis in female germ cells.

To examine the foxl3-downstream pathways, we conducted RNA-seq using wild-type and foxl3-mutant germ cells, and identified 1,480 differentially expressed genes (DEGs). Interestingly, the genes involved in ubiquitin pathway and regulation of microtubules showed sex-specific expression patterns in pre-meiotic germ cells. Mutant analyses of these DEGs suggested the importance of chromosomal dynamics and/or cyst expansion during mitosis. Sex differences in microtubule alignment was also seen in mitotic germ cells. Collectively, these results unveil the novel views that pre-meiotic cell phase is critical period for commitment to either eggs or sperms. To examine whether these DEGs are FOXL3-direct targets or not, we conducted iDamIDseq (Gutierrez-Triana et al., Development, 2016) to determine the FOXL3-binding element. As a result, we successfully identified the FOXL3-binding element, and it was quite similar to general consensus element of Forkhead-family. In the future, we will confirm the interactions between the DEGs and FOXL3 by ChIP-qPCR using anti-FOXL3 monoclonal antibody we generated.

8 - Identifying the best fluorophores and anesthetics for fish

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Due to recent advances in microscopy in vivo-Imaging is becoming more widespread. Furthermore, the CRISPR/Cas9-system enables to integrate fluorophores at any given locus into the genome, creating the ability to create endogenous fusion proteins. These revolutionizing techniques raise several challenges: 1. Which fluorescent protein should be used? 2. Which anesthetic is most suitable for deeply sedating the embryo with the least impact on development? 3. How can the light-obstructing pigments be abolished?

Here we present a systematic review of fluorophores and anesthetics in Medaka (*Oryzias latipes*). Both have been tested in vivo in embryos using the Acquirer imaging machine and automatic extraction of quantitative measurements. The highest ranked fluorescent proteins and the highest ranked anesthetic were subsequently validated via light-sheet microscopy. The fluorescent protein data was cross-referenced with a recent publication by Balleza et al., which determined the predictive in vitro parameters for fluorescence of proteins in *E. coli*. The anesthetics were scored for their deepness of sedation, animal welfare aspects and developmental impact (mainly on the cardiovascular system).

The obstructing pigments were abolished by using the CRISPR/Cas9 system. We performed a knock-out of *Oca2* and *Pnp4a*, which inhibits pigment formation in the retinal pigment epithelium (RPE) and the iridophores, leading to transparent RPE and peritoneum allowing microscopy of the eye and inner organs. This knock-out exists as a stable line, but can also be used in transient for microscopy of already existing reporter lines.

This study shows the first systematic comparison of fluorescent proteins in vivo in a vertebrate. Furthermore, it is the first systematic comparison of anesthesia in Medaka. The abolishment of pigment for imaging is the final addition to use Medaka as a toolset for in vivo-Imaging.

9 - Notch signalling in lineage restriction during retinal postembryonic growth in medaka

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The vertebrate retina consists of six neuronal and one glial cell type. The presence of all cell types and their correct arrangement are necessary for a proper function of the organ. Its structure and architecture is very well conserved across vertebrates. However, in contrast to mammals, the fish retina grows life-long. The stem cells residing in the ciliary marginal zone in the postembryonic retina support this constant retinal growth throughout life. These multipotent stem cells give rise to progenitors that in turn get restricted to, in the end, produce all cell types in the neural retina. A precise regulation along this differentiation path is crucial for the correct composition of the retina. The factors and pathways maintaining the stem cells and regulating progenitor fate restriction are still under investigation. The Notch pathway is well known to play a role in neural development regulating the balance between stemness and differentiation. Using a transgenic fish line, which reports Notch signaling activation, we could observe that Notch signalling is constantly active in a subset of retinal progenitors in the teleost fish medaka. What is the role of Notch signalling in retinal growth? Is it regulating lineage restriction and/or proliferation of retinal progenitors? In order to address these questions, on the one hand, we determined the potential of the Notch-positive progenitor cells. On the other hand, making use of a genetically targeted overactivation of the pathway as well as its chemical inhibition we could conclude that Notch signalling plays a crucial role in the generation of the correct proportions of retinal cell types.

10 - Clonal Analysis of the Medaka Gill Reveals Fate Restriction during Post Embryonic Growth

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Post embryonic organogenesis occurs in all organisms by either increasing the size of an organ or by adding new functional units to it. Unlike any other vertebrates, fish grow during their whole life. But how do organs adapt to a constant growth and what are the mechanisms behind this homeostatic growth? Long term cell tracking and lineage tracing in vivo is a powerful tool to reveal stem cell fate and understand their contribution to postembryonic growth.

The Medaka gill, a new model for post embryonic growth, offers several advantages such as structural simplicity, compartmentalization and easy access for imaging and experimental manipulation. In this study we determine post embryonic growth in the medaka gill and identify stem cells and their niches which constitute to the formation of new functional units within the organ. Furthermore, we characterize fate restriction of stem cells during homeostatic growth.

The experimental strategy consisted of IdU incorporation experiments, inducible labeling and subsequent lineage tracing. By combining these approaches with confocal microscopy, we demonstrate that the organ increases in size by addition of new filament stacks at the extremes, and in turn, those filaments increase in a proximal-distal manner by adding new lamellae. We also prove that stem cells generating new filament tissue are located at the tip of each filament.

Furthermore, we characterize four fate restricted stem cell populations residing in the gill, each giving rise to distinct patterns of cell types. These patterns occur at different rates, indicating differences in stem cell numbers.

Ultimately, these findings will contribute to understanding post embryonic growth, stem cell behaviour and tissue homeostasis in vivo.

11 - Subfunctionalization of rx genes in Medaka

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In teleost fish, retinal neurogenesis continues postembryonically, thus providing an ideal system to study embryonic and postembryonic stem cells in their physiological environment.

The retinal stem cell domain, the ciliary marginal zone (CMZ), is located at the periphery of the retina and represents a bipartite stem cell niche. It contains rx2⁺ stem cells giving rise to either the retinal pigmented epithelium (RPE) or the neuroretina (NR), which contains one glial and six neuronal cell types.

We hypothesize that the bifunctionality of the niche is controlled by the interplay of two transcription factors Rx1 and Rx2, which favor formation of RPE and NR respectively. These genes belong to the conserved family of retina-specific homeobox transcription factors (Rx). They are expressed early on in the presumptive eye field and play a crucial role in early eye development in vertebrates.

We identified Rx2 as a transcriptional hub balancing stemness of NR and RPE cells in the adult medaka retina (Reinhardt and Centanin et al., 2015, EMBOJ, 34(11):1572-88). rx2-mutant cells display a striking phenotype when challenged with wild-type cells within the same niche. In a mosaic retina, loss of Rx2 activity favors the formation of RPE and consequently prevents wild-type stem cells to contribute to RPE. Thus, Rx2 activity is required for the balance between RPE and NR stem cells. rx1 is a close paralog of rx2. Its expression is temporally and spatially overlapping with that of rx2, its function however is unknown.

To overcome the potential functional redundancy of the co-expressed rx1 and rx2 genes, we established homozygous double mutants.

I will present data on these mutants addressing the function of rx1 and rx2 in the retinal stem cell niche.

12 - The role of IL6 family of cytokine signalling in zebrafish heart/fin regeneration

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Ventricular cardiac hypertrophy is a way for the body to compensate for increased cardiac afterload, but it can lead to cardiac insufficiency and failure. In contrast, moderate physical exercise has been shown to be able to reverse the effects of severe ventricular hypertrophy. Why physical exercise, being a pro-hypertrophic factor itself, can reverse the severity of pathological right heart hypertrophy is poorly understood. The aim of this study is to isolate and dissect cardioprotective elements of exercise and regeneration using zebrafish as a model system. A comparative microarray expression analysis of exercised and regenerating hearts reveals that the JAK-STAT pathway orchestrated by the IL6 family of cytokines is activated in both cases.

Various transcriptomic datasets during heart/fin regeneration in zebrafish show a higher upregulation of il11 transcripts compared to the other IL6 family ligand genes in response to injury. To investigate the effects of the IL6 family of cytokines, we obtained a loss-of-function allele of the common receptor complex component gene il6st/gp130 and generated a loss-of-function allele for the specific Interleukin-11 receptor alpha gene (il11ra). Interestingly, we found that both il6st and il11ra homozygous mutants exhibit severe regenerative defects at various developmental stages. Specifically, il6st mutants fail to regenerate their caudal fins and fin fold at adult and larval stages, respectively. Similarly, il11ra adult mutants retain a fibrous cardiac scar even at 72 days post cryoinjury and also lack the ability to regenerate their caudal fins. Furthermore, il11ra mutant larvae exhibit a pronounced reduction in macrophage infiltration to unamputated control levels after 12 hours post amputation (pa) and a delay in neutrophil infiltration at 90 minutes pa. These observations lead us to hypothesize that IL11 signaling is fundamentally involved in a specific aspect of regeneration that is shared between the heart and fin.

13 - TGF- β regulation of outflow tract development and function in zebrafish

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TGF β (Transforming Growth Factor Beta) signaling plays crucial roles in many biological processes, including embryonic development, tissue homeostasis, cell survival and proliferation. Mutations in TGF β genes lead to defects in the cardiovascular system and multiple other tissues. Due to the multi-functional and context-dependent role of this pathway, as well as to the early lethality of mouse embryos deficient for TGF β members, the role of TGF β signaling during heart and blood vessel development is still poorly understood. Since zebrafish can survive several days without a functional cardiovascular system, it is a promising model to study the role of this pathway in the developing cardiovascular system.

By generating a transgenic reporter for TGF β receptor type 1 (Tgfb β 1), we detected its expression in the zebrafish larval heart and outflow tract. A CRISPR/Cas9 induced genomic lesion in the zebrafish tgfb β 1 locus leads to defects in the development and function of the heart outflow tract and its connecting vessels. These defects bear a striking similarity to thoracic aortic aneurysm and dissection (TAAD) in humans, a genetic disease specifically affecting the ascending artery. We are currently investigating the cell-type specific requirement for Tgfb β 1 as well as the cellular and molecular defects leading to the phenotype.

With this new in vivo model, we aim to understand the role of Tgfb β 1 in the outflow tract and to provide novel insights into the pathologies of TAAD, a disease with a still debated involvement of the TGF β pathway.

14 - Tie-1 signaling in zebrafish cardiovascular development

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Tie-1 is an orphan tyrosine kinase receptor involved in vertebrate cardiovascular development. It is considered to be a context-dependent modulator of Angiopoietin/Tie2 signaling. Knockout mice for Tie-1 die embryonically due to cardiovascular defects, but the exact role of this protein remains unclear. We aim to investigate the role of Tie-1 in cardiovascular development using zebrafish.

We generated tie-1 mutant fish using CRISPR/Cas9 technology and recovered a 7 base pair deletion allele (tie-1 Δ 7) resulting in a premature stop codon. It is predicted to encode a truncated protein lacking the intracellular tyrosine kinase domain. qRT-PCR analysis shows a dramatic reduction of tie-1 transcript level in tie-1 Δ 7/ Δ 7 at 24 hours post-fertilization (hpf), suggesting that tie-1 Δ 7 is a potential null allele. At 48 hpf, homozygous mutant embryos display a reduced width of the common cardinal vein (CCV) and a significant reduction in the diameter of the dorsal aorta and parachordal hind-brain channel. Furthermore, mutants also exhibit reduced blood flow and a detachment between the endocardial and myocardial heart layers followed by pericardial edema at 72 hpf. Using a nuclear vascular line, we observed a significant reduction in the number of endothelial cells in tie-1 Δ 7/ Δ 7 both in the CCV and endocardium. This phenotype may be due to enhanced apoptosis, reduced proliferation and/or defect in endothelial cell migration. To distinguish among these possibilities we are now using live imaging and proliferation assays at 32 and 48 hpf to elucidate the primary phenotype in tie-1 Δ 7/ Δ 7 animals and explore the physiological mechanisms underlying the compromised cardiac function especially.

15 - The role of retinoic acid signaling during heart regeneration in zebrafish

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The adult zebrafish heart is able to fully regenerate after severe injury, enabling the study of molecular mechanisms governing cardiac regeneration. One of the earliest responses that take place after cardiac injury is the epicardial and endocardial upregulation, at both the transcriptional and translational levels, of the enzyme retinaldehyde dehydrogenase (Aldh1a2), which synthesizes retinoic acid (RA). Previous studies have reported that interfering with RA levels and action results in reduced cardiomyocyte proliferation. However, the mechanisms by which RA signaling positively regulates cardiac regeneration remain elusive.

First, we investigated the spatial and temporal activation of RA signaling during heart regeneration. By qPCR analysis, we found that the expression levels of different downstream targets of RA signaling were dynamically changed during regeneration. By analyzing the expression pattern of these genes, and using different RA reporter transgenic lines we hope to determine in which cell types RA signaling is activated. Furthermore, we want to understand the tissue-specific role of retinoic acid signaling during heart regeneration; for this purpose, we are developing different reagents that will allow us to block RA signaling in a spatio-temporal manner. With these tools we will be able to test the role RA signaling in various tissues during heart regeneration. Overall, these studies will aid in understanding the mechanisms by which RA signaling modulates cardiac regeneration in zebrafish, with potential therapeutic implications for human heart regeneration following infarction.

16 - Acute and inducible knockdown of GFP-tagged proteins using a nanobody-destruction box fusion

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Tissue specific and inducible loss of function on the level of the protein provides the ultimate means for acute intervention with protein function. It is not only a useful tool to overcome embryonic lethality but eventually allows addressing the function of the protein of interest. Here we present an experimental setup that allows the analysis of protein function in an in vivo context and combines effective knock down with the tagging of the respective cells facilitating functional in vivo studies. We have successfully adapted an acute knockdown approach for GFP-tagged proteins in the Japanese rice fish medaka (*Oryzias latipes*). This is achieved by the conditional expression of a GFP-targeting nanobody (camelid single-domain antibody) fused to medaka SPOP, an adaptor protein of the Cullin-RING E3 ubiquitin ligase complex. We have established medaka-specific SPOP-anti-GFP nanobodies that can be expressed in any cell type (of the retina) in a temporo-spatially controlled manner. As proof of concept, the endogenous GFP-tagged Retinal homeobox protein 2 (Rx2) was acutely targeted to degradation in a cell-specific and inducible manner. Using a stream-lined CRISPR-Cas9 mediated homology directed repair strategy (from off-target prediction via CCTop to advanced Golden Gate cloning for specific donor plasmid creation) to fuse the GFP sequence with endogenous open reading frames offers an easy and reliable way for acute protein knock-down. The approach works in transient as well as in stable transgenic lines. Successful knockdown of GFP in affected cells is visualized by the co-expression of nuclear RFP with the nanobody construct. The approach presented allows an efficient and acute knock down of in vivo tagged proteins and the analysis of the immediate consequences of that acute loss of function.

17 - Understanding the molecular mechanisms of interstitial cell development during zebrafish heart valve development

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Heart valve interstitial cells (VICs) are fibroblast-like cells important for maintaining the structural integrity and homeostasis of mature valves. During development, cells in the endocardial cushions in the atrioventricular canal (AVC) and the outflow tract (OFT) undergo endothelial-to-mesenchymal transition (EndoMT), invade the interstitial space and differentiate into fibroblast-like cells. While it is known that many heart valve diseases are characterized by VIC activation, little is known about their role during valvulogenesis or even their origin. As a model system, the zebrafish heart presents the possibility of live imaging with single-cell resolution, allowing a detailed investigation of these questions.

We propose to investigate the cellular and molecular mechanisms underlying zebrafish heart valve morphogenesis, focusing on EndoMT/EMT transcriptional regulators. Studies in mice have shown that *Twist1* and *Snail1* are enriched in the AVC in early stages of development. However, since mice deficient for these genes die at embryonic stages, it is not possible to analyze the later stages of valve development, during which VICs are recruited. Our results show that these genes are also enriched in the zebrafish AVC, making them promising candidate regulators of valve development. To better understand the role of these transcription factors, we plan to analyze VIC invasion dynamics using loss-of-function (generation of mutants using CRISPR/Cas9 technology and dominant negative transgenes) and gain-of-function (tissue-specific overexpression) models.

Overall, this study aims to deepen our knowledge of the role of VICs in maintaining the homeostasis and structural integrity of the heart valve, as well as the molecular mechanisms that govern their development.

18 - Tight coordination between cardiomyocyte polarity and junctional rearrangements regulates cardiac trabeculation in zebrafish

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Trabeculation is a highly dynamic and regulated process whereby clusters of ventricular cardiomyocytes (CMs) delaminate and expand into the cardiac jelly to form sheet-like projections in the developing heart. Factors like Nrg/ErbB2 signaling, and blood flow and contractility, are required for trabecular formation. Still many developmental and mechanistic aspects of cardiac trabeculation remain unknown. Recently we were able to visualize in vivo changes in apicobasal polarity in CMs during trabeculation. Based on these observations we proposed that cardiac trabeculation is an EMT-like process.

Similarly, during EMT, epithelial apicobasal polarity is regulated by the coordination of polarity complexes and cellular rearrangements, where the Crumbs complex plays a key role. To better understand how changes in CM polarity are regulated during delamination, we set out to study Crumbs. We found that *crb2a* is the highest Crumbs isoform expressed in the embryonic zebrafish heart before the onset of trabeculation. Next, we analyzed Crb2a localization at different stages in embryonic heart. We found that before the initiation of trabeculation (50 hpf) Crb2a is highly enriched in CM junctions and once trabeculation starts (79 hpf), it relocalizes to the apical side of these CMs. Blocking Nrg/ErbB2 signaling or blood flow/contractility led to an increase in junctional Crb2a between CMs at 79 hpf. To test whether lack of Crb2a affects cardiac trabeculation, we are analyzing *crb2a* mutants. Interestingly, trabeculation is severely affected in these mutants as they display a multilayered compact wall that fails to form clear trabeculae. Moreover, tight and adherens junctions are affected in these mutants. Overall, our data suggest a role for Crb2a in the regulation of CM polarity during trabeculation. These findings will contribute to a better understanding of the mechanisms underlying ventricular morphogenesis and maturation.

19 - Repressing to enhance: In vivo chemical screen identifies novel regulators of pancreatic alpha-cell fate and beta-cell regeneration

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Glucose homeostasis is tightly regulated by insulin production from β -cells and glucagon production from α -cells. Changes in the balance of these hormones lead to Diabetes Mellitus (DM), which is foreseen to be the 7th leading cause of death by 2030, warranting a high demand to identify new therapeutics. DM is characterized by a reduction in β -cell mass and reduced insulin production from β -cells. α -cell development and fate mainly depend on the activity of the homeodomain-containing transcription factor Aristaless related homeobox (Arx). Conditional loss-of-function of Arx in α -cells leads to their conversion into functional insulin-producing β -cells and thus an expansion of β -cell mass. Therefore, inhibition of Arx is an interesting target for the expansion of β -cells. The zebrafish model provides a fast, cost-effective and reliable translational platform for drug discovery in an in vivo setting. Here, we screened ~6217 small molecules on a transgenic zebrafish line (TgBAC(arxa:Luc2)) in which the arx promoter drives the expression of the luciferase gene which allows a sensitive and quantitative readout of promoter activity. Small molecule screening allowed us to identify 36 candidate repressors of arxa promoter activity. Furthermore, we started to validate these candidates in other assays. Preliminary results showed that DMAT, a potent CK2 inhibitor increases functional β -cell regeneration. By lineage tracing α -cells during β -cell regeneration, we could show that DMAT promotes α - to β -cell transdifferentiation. We intend to test further the other target molecules identified in the screen.

20 - Upstream modulators of npas4l expression and early angioblast specification

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Endothelial cells, which constitute the luminal layer of all blood vessels, derive from angioblasts, progenitor cells that arise from the mesoderm. Sequential transcriptional waves direct the differentiation of endothelial cells starting from pluripotent mesodermal cells. At the top of this specification cascade lies *cloche*. Zebrafish embryos mutant for *cloche* show a striking phenotype: the lack of most endothelial and blood cells. Recently, our lab discovered that the *cloche* gene encodes a PAS-domain-containing bHLH transcription factor called Npas4l. We studied the spatiotemporal pattern of npas4l expression during gastrulation using qPCR and ISH, detecting npas4l mRNA as early as the late blastula stage. We used gain-of-function approaches and chemical screening to identify signaling pathways and morphogens responsible for npas4l induction and for angioblast specification, in vivo. To identify factors modulating npas4l expression, we combined transcriptome analysis after drug treatment and in silico prediction of putative binding sites in the cis-regulatory elements of npas4l. Among the candidates, the T-box transcription factors *Eomesa* represents the most promising putative upstream regulator of npas4l expression. Importantly, in vivo overexpression experiments show that *eomesa* has the potential to significantly induce npas4l expression. Loss-of-function experiments in mammalian cells indicate the conserved function of EOMES upstream of endothelial cell differentiation, modulating the expression of angioblast markers such as *Etv2* and *Tal1*. Using in vivo and in vitro murine models, we aim to find a mammalian PAS-bHLH transcription factor that promotes *Etv2* expression. The identification of the upstream regulators as well as the functional ortholog of npas4l would refine our understanding of the differentiation of primitive mesoderm into angioblasts, and may result in a significant improvement for the generation of endothelial cells for clinical purposes.

21 - Tol2in1 – A more efficient approach for the generation of transgenic zebrafish

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The generation and effective use of transgenic lines is a cornerstone of zebrafish and medaka research. Although Tol2-mediated transgenesis is established and efficient, the workflow for generating stable lines is laborious and time-consuming. Characteristics such as brightness, mosaic expression and complicated sorting of non-fluorescent transgenes often create bottlenecks that significantly limit the usability of the method. The Tol2in1 approach aims to refine the generation of transgenic zebrafish lines. Based on interweaved pairs of incompatible recombination sites, Tol2in1 makes it possible to generate multiple stable transgenic lines from a single founder. This approach allows, for instance, founder screening and validation by a fluorescent marker and places a genetic tool such as a Cre or Gal4 expression cassette at the same genomic locus. It also facilitates pre-screening for positional effects and expression patterns. Similar setups using common transgenesis markers, e.g. eye-specific fluorescent protein expression cassettes, allow the systematic generation of homozygous transgenic lines based on phenotypic sorting – a straightforward approach to improve the signal-to-noise ratio, brightness and hence image quality. It could also be useful in experimental setups that require a high number of transgenic fish such as drug screenings. Therefore, this system would not only improve the efficiency of current state of the art approaches but also diminish the requirements for animals and space.

22 - Cell junction associated protein complexes and control of proliferation in the zebrafish posterior lateral line primordium

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The lateral line system of zebrafish is a mechanosensory organ necessary for responding to motion of water (currents). It comprises of a set of sensory organs, so called neuromasts, which are deposited along the side of the embryo by a group of collectively migrating cells, the posterior lateral line primordium.

Amotl2a, a junction-associated protein of the Motin family was shown to limit proliferation in the posterior lateral line primordium. We have identified Merlin, a known tumour suppressor as an interacting partner of Amotl2a in zebrafish. Interestingly, Merlin regulates coherent migration and proliferation of epithelial cells in different contexts. The aim of the project is to determine the role of the Amotl2a-Merlin interaction *in vivo* in controlling proliferation and migration in the posterior lateral line primordium.

23 - Molecular mechanisms in the hypothalamus underlying impaired negative feedback by the stress hormone glucocorticoid

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Hypothalamus-pituitary-adrenal (HPA) axis is the main regulator of the stress response whose dysregulation is implicated in stress-induced disorders including depression. In fact one of the hallmarks of depression is elevated level of the corticotropin releasing hormone (Crh) produced by the hypothalamus. This is thought to be due to impaired negative feedback by glucocorticoids (GC), which are produced by the adrenal gland and feedback negatively to control the levels of hypothalamus and pituitary hormones. Despite its importance, the precise molecular alterations underlying impaired negative feedback are currently poorly understood. In this work, we used an optogenetic approach in zebrafish to first elevate the GC level during development and afterwards analyzed the molecular consequences in hypothalamic cells. Specifically, we employed a transgenic fish expressing *Beggiatoa* photoactivatable adenylyl cyclase (bPAC) in adrenal gland using the Steroidogenic acute regulatory protein promoter. When reared under ambient white light conditions sufficient to activate bPAC, 6 and 12 dpf transgenic larvae exhibited elevated basal cortisol level and a completely compromised stress response. Also, the 6 dpf transgenic larvae showed increased crh expression indicating an impaired negative feedback control of hypothalamic nuclei. Next, in order to identify the molecular alteration in the hypothalamus caused by elevated GC, another transgenic fish was used in which hypothalamic stress controlling (neurosecretory preoptic area) region is labelled with GFP using an enhancer element of *orthopedia a*. Using FACS, these hypothalamic cells were isolated from the bPAC-positive and -negative larvae at 6 dpf and transcriptomic analysis is being carried out. The elucidation of molecular targets by this approach will enable the mechanistic understanding of cellular processes implicated in depression.

24 - An Automated High Content Screening Platform for Identification of Cystic Kidney Disease-Modifying Substances in Zebrafish

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This project aims at the establishment of high content screening pipeline for identification of cyst-modifying substances in zebrafish model for human cystic kidney disease (CKD) by implementing automated high-resolution imaging and compound-induced phenotypic change classifying algorithms.

Morpholino-based (MO) knockdown of intraflagellar transport (ift) 172 gene induces glomerular cysts in GFP-expressing pronephrons of transgenic Tg(wt1b::EGFP) zebrafish. Embryos are imaged after exposure to compounds until 72 hours post fertilization. A Smart Imaging module automatically detects the pronephric kidney, zooms in to capture details at higher magnification. Cystic areas are quantified by running through a series of scripted modules written in ImageJ and Python.

The developed pipeline was implemented for optimizing dose response curve of MO, time lapse experiments for cyst formation characterization and model system validation using rapamycin. The platform has already scrutinized 530 out of 1280 compounds from the Prestwick drug library. Of these, 35 compounds showed cyst-suppressing activity. While one third of these compounds also caused severe general toxicity, 13 showed medium and 5 strong cyst-inhibitory activity with no or mild off-target effects. In conclusion, a unique pipeline for automated cyst-suppressing compound categorization in in vivo large scale screening is developed which might be considered for treatment of CKD.

25 - Pak2 function in cardiac development

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The zebrafish system has been extensively used to study cardiovascular development due to the small size, external and rapid development, and optical transparency of the embryos.

The p21 Activated Kinases (PAKs) are serine threonine kinases involved in various biological processes including inflammation, pro-apoptotic events, cancer and cardiovascular development. Particularly, PAK2 has been implicated in endothelial cell proliferation, migration, and vascular integrity. However, since global and endothelial specific knockout of Pak2 results in early embryonic lethality in mice, most of this knowledge is based on in vitro studies.

Unlike mammals, zebrafish embryos can survive without a functional cardiovascular system for the first days of development, thus allowing the comprehensive analysis of severe cardiac mutants. The zebrafish genome contains two pak2 paralogs: pak2a and pak2b. While pak2a loss-of-function leads to cerebral haemorrhage, pak2b morpholino injected animals do not show any obvious phenotypes. Using the CRISPR/Cas9 system, we generated pak2a; pak2b double mutants (pak2 mutants). We found that loss of pak2 function results in reduced heart size, contractility defects, and absence of circulation. We are currently studying these cardiac defects in more details by investigating sarcomere assembly, cardiomyocyte junction stabilization and calcium mobilization using live imaging and immunofluorescence stainings. We are also trying to distinguish between the autonomous and non-autonomous effects of pak2 loss-of-function by expressing pak2a in a tissue specific manner. One goal of our study is to understand the cellular and molecular steps that lead to such cardiac defects. Thereby, our study reveals for the first time the possible involvement of Pak2 in cardiac function which might ultimately be explored as a potential therapeutic treatment in heart failure.

26 - The immune response modulates zebrafish cardiac valve regeneration

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Cardiac valve diseases present a significant threat to human cardiac health, and can potentially lead to cardiac failure. valve replacement therapies, using artificial scaffolds or bio-prosthetic valves, are the most common therapies to replace diseased valves. However, frequent exacerbated host immune response to the graft leads to deleterious effects thereby causing severe complications in postoperative care. Recent work from our lab has shown that zebrafish are capable of regenerating their heart valves. Using the Nitroreductase/Metranidazole (NTR/Mtz) system we perform a targeted genetic ablation of the valve cells, avoiding the potential effects of a surgical invasive approach. Taking advantage of this model, we propose to study the role of the immune response during the regenerative process. We started by determining the profile of recruitment of the immune cells during valve regeneration, focusing on macrophages and neutrophils. In parallel, we used Clodronate Liposomes, to perform a chemically mediated depletion of phagocytic cells during regeneration. Our results suggest that impairment of the immune response leads to a reduction in cell recruitment during valve regeneration.

Overall, we expect to bring new insights about the potential role of immune cells in valve cell recruitment and remodelling of the valve extracellular matrix (ECM) during cardiac valve recellularization.

27 - The E3-ubiquitin ligase component Rbx1 regulates cardiac trabeculation in zebrafish

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Cardiac trabeculae are ridge-like structures within the ventricular wall that in zebrafish form mostly through the delamination of cardiomyocytes from the compact wall, and are crucial for cardiac function. Defects in proteosomal degradation have been associated with decreased cardiac function in a number of disease models, however their effect during cardiac development has not been extensively analyzed. Here we report a role during cardiac trabeculation for the E3-ubiquitin ligase component Rbx1, which is known to regulate the degradation of key signaling molecules including cell cycle regulators (e.g., p21, p27), transcription factors (e.g., Foxo) and signal transducers (e.g., Notch 1 and 4). Although development is largely unperturbed in zebrafish *rbx1* mutant larvae, pericardial edema is evident starting at 3.5 days post-fertilization. Taking advantage of high-resolution imaging, we observed that *rbx1*^{-/-} embryos exhibit a hypertrabeculation-like phenotype, or multi-layering of cardiomyocytes. This phenotype is not affected by a block in ErbB signaling but it fails to manifest itself in the absence of blood flow/ contractility. Interestingly, *rbx1* mutant ventricles contain reduced numbers of cardiomyocytes as well as reduced CM proliferation. Furthermore, *rbx1* mutants display ErbB independent Notch activity in the myocardium. We generated tissue-specific *rbx1* over-expression lines and find that endothelial, but not myocardial, specific *rbx1* expression normalizes the trabeculation phenotype. Collectively, our data indicate that Rbx1 functions in the endocardium to regulate the morphology of the myocardial wall in an ErbB2 independent fashion.

28 - A smart imaging platform for automated high-resolution imaging of zebrafish

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Whole organism model systems such as zebrafish embryos are increasingly employed in screening assays to address biomedical research questions, as they share high genetic similarities with human and offers great imaging modalities. Despite this widespread usage, the lack of dedicated workflows and image processing tools hampers the automated acquisition of high resolution datasets and the automated quantitative analysis of phenotypic screening data. The aim of this project is to develop a flexible tool-box for various whole organism imaging and screening applications, thus enhancing acquisition and analysis of large scale datasets. This tool-box will provide guidelines and generic workflows based on existing solutions from the image-processing, computer vision or data-processing fields like object-recognition or machine-learning for instance. There are 2 main directions in this project:

- (i) Smart Acquisition takes advantage of the feedback microscopy interface offered by the ACQUIFER screening microscope to directly use image-processing solutions during the acquisition. This allows to automatically detect regions of interests within larger objects, detect rare events or adjust the focus on a specific object.
- (ii) Smart Analysis deals with post-acquisition analysis of large datasets. The objective is to develop solutions to quantify a phenotype of interest (e.g. morphological alterations, variations gene expression patterns), and to categorize and classify results. This includes classical image processing approaches (e.g. segmentation, keypoints detection) completed by novel machine-learning algorithms for supervised-learning like Convolutional Neural Networks.

The project is collaboration between ACQUIFER and the University Children's Hospital. The developments will be interfaced with typical high content screening projects namely the study of toxicological and pharmacological effects of compounds on the developing pronephros in zebrafish disease models.

29 - Heterogeneity in cardiomyocyte maturation is retained in the adult zebrafish heart and conditions its regenerative response

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Contrary to the adult mammalian heart, zebrafish display an extraordinary capacity for heart regeneration after cardiac injury. This regenerative response relies on the ability of cardiomyocytes (CMs) to proliferate and replenish the lost tissue. However, CM heterogeneity in the developing and adult zebrafish heart has never been explored to have a full insight into the process of regeneration. Through comparative transcriptomic analysis of developing and adult zebrafish hearts, we identified *tnnc2* as an early marker of CMs which is mostly turned off after embryogenesis and *tnni4b.3* as a marker of more mature CMs which turns on at the end of embryogenesis. We used the promoter of these genes to develop new reporter lines, which helped us further investigate their expression pattern during development. *tnnc2*-driven reporters mark embryonic CMs as well as primordial layer CMs in the adult heart; *tnni4b.3*-driven reporters mark CMs starting at the larval stage and the majority of CMs in the adult stage. These spatio-temporal expression analyses indicate that the *tnnc2*- and *tnni4b.3*-driven reporters mark immature and mature CMs, respectively. Interestingly, during heart regeneration, some CMs in the injured area express the immature reporter. We are planning to carry out some lineage tracing experiments during cardiac regeneration to determine the proportion of new CMs that originate from *tnnc2* expressing CMs. In summary, our findings provide further evidence for CM heterogeneity in the adult zebrafish heart which may contribute differently to cardiac regeneration.

30 - In vivo analysis of cardiomyocyte proliferation during ventricular trabeculation

Veronica Uribe, Radhan Ramadas, Deepika Dogra, S. Javad Rasouli, Felix Gunawan, Hiroyuki Nakajima, Ayano Chiba, Sven Reischauer, Naoki Mochizuki, and Didier Y.R. Stainier

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Cardiomyocyte proliferation is critical for cardiac growth and patterning; however, few studies have investigated the behavior of dividing cardiomyocytes in vivo. Here, we use time-lapse imaging of beating hearts in combination with the Fucci system to monitor the behavior of proliferating cardiomyocytes in developing zebrafish. Confirming in vitro observations, sarcomere disassembly as well as changes in cell shape and volume precede cardiomyocyte cytokinesis. Unlike what is observed in mouse, cardiomyocytes in zebrafish embryos and young larvae mostly divide parallel to the myocardial wall in both the compact and trabecular layers, and cardiomyocyte proliferation is more frequent in the trabecular layer. While analyzing known regulators of cardiomyocyte proliferation, we observed that the Nrg/ErbB2 and TGF- β signaling pathways differentially affect compact and trabecular layer cardiomyocytes, indicating that distinct mechanisms drive cardiomyocyte proliferation in these layers. In summary, our data indicate that cardiomyocyte proliferation is essential for trabecular growth, but not initiation, in zebrafish, and set the stage to further investigate in vivo the cellular and molecular mechanisms driving cardiomyocyte proliferation.

31 - The role of neuropilin1a in the zebrafish Optic Tectum

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It has recently been postulated that axonal scaffolds in the optic tectum (OT) are dependent on signals from the nearby vessels (Ulrich et al., 2011). For this reason, we are interested in the molecules involved in the neurovascular link, setting focus on the branching of axonal terminals of retinal ganglion cells (RGC) in the optic tectum of zebrafish.

RGCs express a key protein in the neurovascular link signaling cascade: neuropilin1 (co-receptor with VEGFR2 for VEGF signaling as well as co-receptor with plexins for class 3 semaphorin signaling). In zebrafish, *nrp1* is a duplicated gene (*nrp1a* and *nrp1b*). Although both show a neurovascular expression pattern, *nrp1a* is more highly expressed in neurons while *nrp1b* is more predominantly expressed in vessels ((Bovenkamp et al., 2004). Therefore, we generated *nrp1a* KO zebrafish and we studied the axonal layering of the OT in the mutant and control larvae.

We found that, while *nrp1a* knockouts do not expose an obvious vascular phenotype, the sublamina layering in the OT was abnormal with axons aberrantly positioned between the SO and the SFGS layer. Moreover, and since the OT is the primary visual center in zebrafish, behavior experiments showed that the mislayering of the axons also translates in a defect in the visual function.

32 - Dynamics of neurovascular interaction in the zebrafish embryo

Bettina C. Kirchmaier, Blanca Peguera-Carre, Beatriz Furones Cuadrado,
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Vessels and neurons possess similar specialized structures (tip cells and growth cones) which, through filopodial extensions, sense the surrounding tissue for specific cues that direct their movements. Interestingly, emerging evidence suggests that axonal growth cones and capillary tip cells might use common repulsive and attractive signals in their environment that ultimately determine their directional navigation through the body. In our group, we study the unknown neurovascular function of guidance cues focusing on CNS vascularization and the control of the neurovascular unit. To investigate the importance of the molecules reelin, neuropilin and flrt in regulating communication at the neurovascular interface we use a combination of state-of-the-art inducible and cell type-specific genetics in mouse and a wide range of cellular assays and tissue *ex vivo* techniques. To complement the mouse data we will use zebrafish as a small vertebrate model to visualize in real time and dynamic spatial resolution the dynamic cell-to-cell contact interactions *in vivo*. We will concentrate our studies on the neurovascular communication during neuronal migration in the zebrafish brain (optic tectum) and in the trunk.

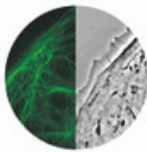


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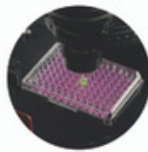
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