COS symposium 2022
Building Functionality - The Relevance of Form Across Biological Scales

How structure, shape & architecture guide functionality is a fundamental open question in biology that expands through all biological scales: From the structure of the DNA to the morphology of organs. In this symposium, we aim to highlight common and differential features emerging from the molecular, cellular, tissue and organ architecture in the animal and plant kingdoms. We will also discuss how to engineer biological models to generate novel functions.

Thursday - 13.10.2022

13:00 - 14:00	Registration
14.00 - 14.15	Welcome note
14.15 - 15:45	Session 1: Architecture of cells & tissues
	Julie Gray – University of Sheffield *Stomata: Form and function of plant pores*
	Yohanns Bellaïche – Institut Curie *Regulation of epithelial tissue morphogenesis*
15:45 - 17:30	Coffee break & Poster session
17:30 - 18:30	Keynote Manu Prakash – Stanford University
20.00 - open end	Speaker's dinner – Bodega Don Jamón

Friday - 14.10.2022

9:00 - 10:30	Session 2: Organization of molecules and organelles
	Edith Heard – EMBL *Epigenetic mechanisms in Development and Disease: insights from the X chromosome*
	Mónica Bettencourt-Dias – Gulbenkian Institute *Centrioles in development, evolution and disease: tiny organelles, multiple and critical functions*
10:30 - 11:00	Coffee break

11:00 - 12:30	Session 3: Cellular and tissue mechanics Lazaro Centanin – COS *The relevance of form across biological scale – a fish perspective.* Gáspár Jékely – University of Exeter *Organismic biology in Platynereis: linking genes, brains and bodies to the environment*
12:30 - 14:00	Lunch
14:00 - 15:30	Session 4: Engineering biological systems Kerstin Goepfrich – MPI Medical Research *Synthetic cells: Building functionality with DNA nanotechnology* Linnea Hesse – Freiburg University *Taking a looking into living material systems: how plants can inspire technology*
15:30 - 16:00	Coffee break
16:00 - 16:30	Schmeil Award Ceremony
16:30 - 18:00	Session 5: Organismal form Toshihiko Fujimori – NIBB *Trans-Scale polarity formation in the mouse oviduct* Edwige Moyroud – University of Cambridge *One-size-fits-all? Understanding how flowering plants build communication devices on their petals*
18.00 - open end	COS Autumn party

More info:
https://www.cos.uni-heidelberg.de/en/centre-for-organismal-studies-heidelberg/scientific-events-at-cos/cos-symposium-2022

ABSTRACTS COS SYMPOSIUM 2022

O-glycosylation of WUSCHEL-RELATED HOMEOBOX 4 fine-tunes radial growth

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Stem cell proliferation is tightly coordinated with metabolism to maintain energy homeostasis. In animals, it is well-documented that perturbations in energy homeostasis trigger post-translational modification of nucleocytosolic proteins with O-linked N-acetylglucosamine (O-GlcNAc) to adjust stem cell activity. O-GlcNAc modification of proteins has previously been demonstrated in plants, but its role in regulating growth and development is unclear. By transcriptional profiling of O-GlcNAc-deficient mutants we found that the pathway is required for promoting meristematic activity. Indeed, the plant O-GlcNAc transferase SECRET AGENT positively regulates radial growth in Arabidopsis, which is driven by the vascular cambium. Glycoproteomic analysis revealed that key transcriptional regulators of vascular development are modified by O-glycosylation and identified WUSCHEL-RELATED HOMEOBOX 4 (WOX4) as a candidate for fine-tuning stem cell activity in the vascular cambium in response to metabolic perturbations. We show that SECRET AGENT physically interacts with WOX4 in vivo. O-glycosylation of WOX4 was confirmed using in vitro enzymatic assays and attributed to the disordered N-terminal domain. Fluorescence recovery after photobleaching indicates that the glycosylated region is crucial for protein trafficking. Together, our results suggest that cambium stem cells adapt proliferation to perturbations of plant energy homeostasis via dynamic glycosylation of WOX4.

An unexpected function of the highly conserved SERAT3 isoforms in the cytosol of Arabidopsis

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Cysteine biosynthesis takes place in all subcellular compartments capable of protein translation and is catalyzed by the consecutively acting enzymes: serine acetyltransferase (SERAT) and O-acetylserine(thiol)lyase (OAS-TL). In Arabidopsis, both enzymes are encoded by multi-gene families whose members are distributed in the cytosol, mitochondria, and plastids. Formation of the carbon nitrogen-containing precursor, O-acetylserine (OAS) by SERAT limits cysteine synthesis, which is achieved by replacing the activated acetyl-residue in OAS with sulfide by OAS-TL. The cytosolic SERAT1;1 and the organelle-localized SERAT2;1 and SERAT2;2 interact via a conserved C-terminal tail with OAS-TLs in a bi-enzyme protein complex called cysteine synthase complex (CSC). Since the C-terminal tails of SERATs bind into the active site of OAS-TLs (Francois et al., 2006), complex-associated OAS-TL is inactive, and the association of the CSC is controlled by the OAS-TL substrates, OAS and sulfide. Formation of the CSC activates SERATs of group 2 (Droux et al., 1998; Wirtz et al., 2012), strongly suggesting that CSC formation regulates the SERAT activity in response to the available sulfide supply.

Arabidopsis possesses two additional cytosolic SERAT isoforms (SERAT3;1 and SERAT 3;2),

which cannot interact with OAS-TL and display much lower specific SERAT activities than the major SERATs of groups 1 and 2 (Kawashima et al., 2005). The presence of at least one SERAT of group 3 appears to be conserved in vascular plants. The CSC-associating thee major SERATs contribute more than 90% of total SERAT activity in Arabidopsis. Nonetheless, the presence of a single SERAT3 in the quadruple SERAT mutants Q3;1 and Q3;2 or both SERAT3 isoforms in the serat tko mutant is sufficient for plant survival but results in decreased growth. In contrast, the remaining activity of SERAT1;1 in the Q1;1 mutant allows for wild-type like growth under non-stressed conditions (Watanabe et al., 2018), strongly suggesting that SERAT1;1 is the major SERAT in the cytosol. Explicitly transcription of SERAT3 genes is induced upon sulfur starvation, while the major SERATs and OAS-TLs are not transcriptionally regulated in response to sulfur supply.
In this study, we addressed the biological function of SERAT3 for cysteine biosynthesis by testing their physical interaction with cytosolic SERAT1;1 and OAS-TL A. Surprisingly, we found that both SERAT3 isoforms stably interact with SERAT1;1 in planta. We addressed the consequences of SERAT3;2 interaction with SERAT1;1 in a hetero-oligomeric SERAT complex in vitro and uncovered that SERAT3;2 inhibits the interaction of the hetero-oligomeric SERAT complex with OAS-TL A. Our data suggest that SERAT3;2 modulates the sensor function of the cytosolic CSC when sulfur supply is limited. The consequences of this modulatory impact on sulfur uptake and whole-plant sulfur distribution under sulfur-limiting conditions will be discussed.

References:
Droux, M., Ruffet, M.L., Douce, R., and Job, D. (1998). Interactions between serine acetyltransferase and O-acetylserine (thiol) lyase in higher plants-structural and kinetic properties of the free and bound enzymes. Eur J Biochem 255, 235-245.
Francois, J.A., Kumaran, S., and Jez, J.M. (2006). Structural Basis for Interaction of O-Acetylserine Sulfhydrylase and Serine Acetyltransferase in the Arabidopsis Cysteine Synthase Complex. Plant Cell 18, 3647-3655.
Kawashima, C.G., Berkowitz, O., Hell, R., Noji, M., and Saito, K. (2005). Characterization and expression analysis of a serine acetyltransferase gene family involved in a key step of the sulfur assimilation pathway in Arabidopsis. Plant Physiol. 137, 220-230.
Watanabe, M., Tohge, T., Fernie, A.R., and Hoefgen, R. (2018). The Effect of Single and Multiple SERAT Mutants on Serine and Sulfur Metabolism. Frontiers in Plant Science 9.
Wirtz, M., Beard, K.F.M., Lee, C.P., Boltz, A., Schwarzländer, M., Fuchs, C., Meyer, A.J., Heeg, C., Sweetlove, L.J., Ratcliffe, R.G., and Hell, R. (2012). Mitochondrial Cysteine Synthase Complex Regulates O-Acetylserine Biosynthesis in Plants. Journal of Biological Chemistry 287, 27941-27947.

Proteomic dynamic of the cnidarian extracellular matrix during development

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While the cellular process and dynamics during development have received much attention over the years, the influence of the extracellular matrix (ECM) on developmental processes remains largely unknown. Cnidarians are the earliest animals that possess all core components of the animal ECM combined with their simple body plan and complex, multi-stage life cycles

they are ideal model organisms to study the developing ECM. We present the first proteomic map of the ECM the sea anemone throughout multiple stages of its complex life cycle. Through quantitative proteomic studies of the isolated ECM from larvae, primary polyp and adult we identified both all structural and solved proteins that make up the ECM during development. We identified more than 50 candidates that are differentially detected throughout development. Among these a number of functionally undescribed proteins. Based on these candidates we currently perform knockdown screens to identify ECM components that are important for development. We use custom antibodies as well as elasticity measurements using atomic force microscopy to measure the morphological and physical effect of the knockdowns and correlate these to the observed developmental phenotypes.

Changes in timing of EMT affect epithelial dynamics during fly gastrulation

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During animal development, internalization of the mesoderm is one of the first morphogenetic processes taking place during gastrulation. While mesoderm formation is, in principle, conserved, observable differences exist between species and range from single cells internalizing individually to collective infolding of the entire tissue. Recent work has shown that both modes of mesoderm internalization exist in flies, providing an exciting opportunity to study the molecular innovations associated with changes in morphogenetic programs.
Here we focus on the role of epithelial-to-mesenchymal transition (EMT) during mesoderm internalization and whether differences in EMT timing may have led to different modes of mesoderm internalization over the course of evolution. In the fruit fly Drosophila melanogaster, the future mesoderm folds into the embryo as intact epithelium before it undergoes EMT. In contrast, in the midge Chironomus riparius the mesoderm internalizes as individual cells. Using a comparative approach between these flies we investigate the role of known EMT-driving proteins on mesoderm internalization. Among other genes, we have investigated Chironomus orthologs of snail and abelson tyrosine kinase (abl) and asked how their function defines cell and tissue behavior during mesoderm internalization. Our results raise the possibility that a reduction in abl activity increases cell-cell adhesion, delays the onset of EMT, and permits deeper tissue invagination.

Chromatin structure and remodeling factors during cambium development in Arabidopsis thaliana

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Organismal development is driven by differential gene expression in strictly regulated spatio-temporal patterns. Chromatin accessibility it is a fundamental component driving gene transcription and thus tissue differentiation. In this regard, cambium stem cells are an excellent model to follow chromatin rearrangement during tissue formation because they are the basis of radial plant growth and the main player in vascular tissue development and organization. To characterize cambium dynamics at the cellular level, we generated a series of plant lines expressing nuclear-localized fluorescent proteins under the control of cell type-specific

promoters. Through Fluorescence Activated Nucleus Sorting (FANS), we established a high-resolution gene expression map of cambium-associated tissues associated. Moreover, we coupled FANS with the Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq), investigating chromatin structure in stem cells and differentiating vascular cells. In particular, we are currently evaluating chromatin accessibility during phloem formation a vascular tissue in which key proteins, designated as SUPPRESSOR OF MAX2 1-LIKE, determine cell identity. We show that interaction between SMXL5 and the plant homeodomain (PHD) finger-protein OBERON3 (OBE3) is important for establishing a phloem-specific chromatin structure during early events in phloem formation.

Investigating the impact of mechanical stress on the division orientation of cambium stem cells

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Regulation of cell division plane orientation is essential for plant development. Mechanical forces have been proposed to serve as a directional cue in this context, but how these forces determine cell division orientation on the molecular level is still unclear. Here, we use the highly regular cell divisions of cambium stem cells as a model to investigate the role of inter-tissue forces in orienting cell division planes. Supporting a role of mechanical stress, we find that the division plane orientation of cambium stem cells is more regular when providing exogenous mechanical stimuli. Indicating a role of microtubules, spiral2 mutants, harbouring an increased microtubule dynamics, show a more regular cell division pattern. Through stem-cell specific transcriptome analyses, we identified three genes encoding microtubule-associated proteins whose downregulation likewise leads to a more regular cell division plane orientation. To probe microtubule dynamics, we moreover established a procedure for visualizing microtubule organization specifically in cambium stem cells by live-cell imaging and immunofluorescence. Our work provides tools for probing the control of cell division plane orientation, investigates the role of microtubule organization, and suggests that mechanical stress influences cell division plane orientation in the cambium.

Deciphering the genetic basis of the individual xenobiotic susceptibility in the Japanese rice fish medaka: a genome–wide association study

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In the course of life, humans are exposed to a plethora of pharmacologically active or toxic substances. However, xenobiotics affect individuals differently, with genetic predisposition playing an important role. Unravelling the underlying mechanisms will assist pharmacogenomics in the development of personalised medicine and provide a basis for the individualised assessment of environmental toxins.
We are conducting a large-scale genome-wide association study (GWAS) to decipher the causative gene-environment interactions (GxE) and to gain a mechanistic understanding of the biological pathways involved in xenobiotic response at the molecular level.
To overcome the limitations of the heterogeneity of the human genome, we have established an inbred panel from a natural population of the teleost fish medaka (Oryzias latipes). This Medaka Inbred Kiyosu Karlsruhe (MIKK) panel is the first vertebrate inbred panel derived from the wild and consists of 80 fully sequenced near isogenic lines. Medaka has proven itself in over 100 years of biological and genetic research and has many attractive features such as a high reproductive rate, high inbreeding tolerance and the availability of genome editing tools such as CRISPR/Cas9 and base editors. The transparency of the chorion and embryo enables non-invasive in vivo imaging. Its many orthologous genes to humans allow the transferability of results to humans.
So far, we have screened about 12000 embryos of the MIKK panel with two xenobiotics (ethanol and disulfiram) for changes in heart rate as a general physiological indicator. The heart rates were measured using a software we developed. Clear differences in the response to treatment were found. Not only was there a spectrum between lines that showed a large reduction in heart rate over time and lines that showed little or no change compared to untreated controls, but most lines also showed different response patterns depending on the drug. Preliminary analyses of genetic contributions to xenobiotic response traits have revealed clear genetic effects. Eight F2 crosses based on five extreme lines were generated for future mapping of quantitative trait loci, with the first F2 embryos being sequenced. These results will pave the way for the identification of contributing loci and their validation.

Embryonic progenitors undergo a behavioral change on the way to becoming adult stem cells in the medaka retina

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While a large number of vertebrate species grow life-long, in particular, mammals seem to have lost this capacity. Since distinct and dedicated stem cell populations facilitate this life-long growth, understanding their initial establishment and life-long maintenance is key to understanding the transition between the modes. The retinal stem cell niche of teleost fish, the ciliary marginal zone (CMZ), facilitates retinal growth and the proliferative behavior of stem and progenitor cells ensures full organ functionality throughout life. While stem and progenitor cells within the CMZ and their relative contribution to shape and function are well established, the origin of retinal stem cells has remained obscure. The adult retinal stem cells can be described as slow-proliferating and being able to continuously give rise to clonal descendants, named arched continuous stripes (ArCOS). In this study, we show that stem cell marker expression is still initiated in the CMZ upon defected eye morphogenesis, indicating that all retinal progenitors retain the potency of acquiring retinal stem cell identity. Moreover, we observe that the fast-proliferating retinal progenitors in the retinal periphery

undergo a switch to a slower proliferative behavior during the embryonic development of medaka. Using long-term clonal analysis and BrdU treatment enabled us to identify the timing when the retinal progenitor cells start giving rise to ArCOS, thereby changing its behavior to a stem cell one. The single-cell RNA sequencing data of the medaka retina provided us with further insights into the biology of stem and progenitor cells. Thus, we have been able to identify new unique markers for stem and progenitor populations and the differences in the transcriptomic profiles of these populations. Overall, this argues for a transition of embryonic retinal progenitor cells to adult retinal stem cells at a distinct time point in the CMZ of the medaka retina.

Non-muscle myosin II drives critical steps of nematocyst morphogenesis

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Actomyosin networks control multiple processes in eukaryotic cells that involve membrane shaping and remodeling. Although myosin II motor proteins are considered as key factors regulating Golgi membrane dynamics, they have not been implicated so far in the generation of large organelles deriving from the Golgi apparatus. Here, we show that a non-muscle myosin II homolog from the early diverging metazoan Hydra is essential for the formation of its stinging organelle. Nematocysts are generated by intracellular secretion of proteins into a giant post-Golgi vesicle. They consist of a capsule that elongates into a long tubule, which is coiled in the capsule matrix and gets expelled during discharge. The process of tubule initiation and elongation during organelle development has so far been elusive. Here, we show that a nematocyst-specific non-muscle myosin II (NNMII) condenses to a collar structure surrounding the apical constriction of the nematocyst vesicle. NNMII facilitates tubule growth as evidenced by blebbistatin treatment and genetic knockdown leading to a depletion of early morphogenetic nematocyst stages by tubule disintegration. Our data demonstrate an essential function for actomyosin-based processes throughout major developmental steps of the cnidarian stinging organelle.

WNT signaling controls chromosome stability in a cell fate dependent manner

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Pluripotent stem cells hold a great promise for regenerative medicine and mammalian embryonic development since they are able to self-renew and potentially give rise to all the cell types of the organism. However, they show in vitro a high degree of chromosomal instability (CIN), a major drawback for their transfer to the clinic. Beyond genotoxic stress, the extrinsic causes of CIN remain largely unknown. Here, we report that the WNT signaling pathway, which is critical in stem cell maintenance and lineage specification, has a moonlighting role in genome stability in specific cellular embryonic and adult stem cell states, including pluripotency. In particular, inhibition of WNT signaling triggers DNA damage and chromosome segregation defects in human induced pluripotent stem cells. We performed parallel sequencing of the genome and transcriptome of single human induced pluripotent stem cells after WNT inhibition. This powerful technique allowed us to track fate and copy number variations of the same cell. Given the important roles of WNT modulation in germ layer specification, we questioned its impact on genome integrity across early cell fate decisions. Importantly, we found that WNT inhibition had no impact on genome stability of mesoderm precursors but triggered aneuploidy in neuroectoderm lineages, including neural progenitor cells. We conclude that WNT signals coordinate cell lineage specification and genome maintenance in specific developmental lineages, which could be critical to our understanding of how aneuploidy arises during development and tissue homeostasis.

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Janina Hattemer[1], Anchel de Jaime-Soguero[1], Anja Bufe[1], Nicolas Böhly[2], Ulrike Engel[3], Holger Bastians[2], and Sergio P. Acebron[1]

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Control and maintenance of genome stability in stem cells is crucial to enable faithful cell lineage specification, organ development and tissue homeostasis [1,2]. However, how genome stability is maintained across different cellular lineages remains poorly characterised. Whereas common intrinsic mechanisms leading to genome instability have been extensively studied during the last years [3], we have little information on how extrinsic cellular sources impact genome maintenance. In the last years, rising evidence emerged that Wnt signalling is beside its important role in development, also crucial for faithful cell division by regulating chromosome alignment and segregation in mitosis [4,5,6,7,8]. Pluripotent stem cells are prone to chromosomal instability, which currently precludes their therapeutic application. Here, we show that Wnt inhibition triggers numerical and structural chromosomal defects in human induced pluripotent stem cells (hiPSCS), but not during early cell lineage specification. Additional functional analyses in different cellular lineages indicated that Wnt roles in chromosomal maintenance are uncoupled of its functions in cell fate determination. We propose that Wnt signalling safeguards chromosomal stability in pluripotent stem cells and other specific cellular lineages that represent developmental bottlenecks where genome stability is at risk.

Deciphering the Role of Jasmonate Signaling in Stem Cell Maintenance

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In Arabidopsis shoot apical meristem (SAM), stem cell maintenance is regulated by WUSCHEL(WUS)-CLAVATA3(CLV3) feedback loop. While the basic stem cell regulatory feedback system has been elucidated, how dynamic stem cell maintenance is achieved in response to stress-related signals still remains elusive. Jasmonate (JA) plays a crucial role in both stress signal perception and plant developmental regulation. According to our study, long-term JA treatment in SAM represses the expression of CLV3 and WUS, hence results in reduced meristem size. Exogenous JA treatment can not only de-repress the JA signaling in SAM but also activate WUS protein domain expansion. Moreover, JA signaling related protein JRWI1 is a potential WUS interactor. JRWI1 is specifically expressed in rib zone of SAM, overlapping with WUS expression domain. Interestingly, JRWI1 is a positive regulator of stem growth potentially by repressing JA signaling through interacting with WUS. Altogether, our study suggests a novel mechanism for stem cell maintenance in response to environmental signals, mediated by JA signaling. Furthermore, we reveal a new module of JA signaling network in plant developmental regulation.

Role of activation switch in microglia upon viral infection

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The latest pandemic brought to the fore that some viruses are potentially neuroinvasive; they can infiltrate the brain tissue and cause inflammation in the Central Nervous System (CNS). Consequently, some patients face temporary or even permanent cognitive impairments after being infected with viruses (Zhang, et al., 2022). Upon viral infection, microglia cells that typically sustain brain homeostasis, set in motion the brain immune response and thus play a crucial antiviral role (Chen, et al., 2019).
In our work, we aim to understand the role of the activation switch in microglia on neuroprotection or degeneration upon viral infection. We use in vitro culture, adult murine microglia (BV2), and a set of triggers to switch microglia through spectra of reactive states: from M1 or pro-inflammatory to M2 or anti-inflammatory, each having a different intracellular metabolic profile (Kelly & O'Neill, 2015). Along with biochemical assays, we focus on understanding intracellular phenotypic changes by use of soft x-ray tomography (Loconte, et al., 2021). Our preliminary analysis on a single-cell soft x-ray tomography of resting and reactive microglia demonstrates how organelles change upon activation, providing a basis for explaining how these immune cells will react to viral infections and the development of antiviral drugs.

References

Chen, Z., Zhong, D. & Li, G., 2019. The role of microglia in viral encephalitis: a review. Journal of Neuroinflammation, 09 04, 16(1), p. 76.

Kelly, B. & O'Neill, L. A., 2015. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. Cell Research, 07, 25(7), pp. 771-784.
Loconte, V. et al., 2021. Using soft X-ray tomography for rapid whole-cell quantitative imaging of SARS-CoV-2-infected cells. Cell Reports Methods, 22 11, 1(7), p. 100117.
Zhang, J., Li, Z., Lu, H. & Shi, J., 2022. Evidence of microglial immune response following coronavirus PHEV infection of CNS. Frontiers in Immunology, 10 01.Volume 12.

The Hox transcription factor Ubx ensures somatic myogenesis by suppressing the mesodermal master regulator Twist

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Early lineage-specific master regulators are essential for the specification of cell types. However, once cells are committed to a specific fate, it is critical to restrict the activity of such factors to enable differentiation. To date, it remains unclear how these factors are silenced. Using the Drosophila mesoderm as a model and a comparative genomic approach, we identify the Hox transcription factor Ultrabithorax (Ubx) to be critical for the repression of the master regulator Twist. Mesoderm-specific Ubx loss-of-function experiments using CRISPR/Cas9 and overexpression studies demonstrate that Ubx majorly impacts twist transcription. A mechanistic analysis reveals that Ubx requires the NK-homeodomain protein Tinman to bind to the twist promoter. Furthermore, we find these factor interactions to be critical for silencing by recruiting the Polycomb DNA binding protein Pleiohomeotic. Altogether, our data reveals that Ubx is a critical player in mediating the silencing of Twist, which is crucial for coordinated muscle differentiation.

Analysis of organ growth dynamics and hierarchical relationships amongst adult stem cells (aSC) in fish gills

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Most organs and tissues in vertebrates contain pools of adult stem cells (aSC) that proliferate and generate progeny that differentiate into specific cell subtypes. During post-embryonic stages, aSCs carry out two main functions: a) play a homeostatic role by replenishing those cells lost as a part of the normal biological activity of the organ they belong to, and b) they are the main actors involved in controlling organ size, which must grow together with the rest of the body in a coordinated way. Furthermore, organs are comprised of multiple differentiated cell types arising from different stem cells. Thus, their right post-embryonic growth and the function that they exert depend on an exquisite coordination among their aSC populations. However, little is known about how such coordination occurs in a molecular context.
Teleost fish and particularly their gills are an excellent model to address this question, as they display permanent growth during their entire life. The gills are composed of branchial arches, which in turn contain an ever-growing number of filaments. Using lineage-tracing tools, we have identified four different types of stem cells supporting gill growth, each contributing to a different lineage. We are currently studying gill stem cells in medaka fish (Oryzias latipes)

and other species from the Oryzias genus as a model for growth coordination from two points of view since: i) different fate-restricted stem cells have to coordinate their activity to sustain gill growth, and ii) the permanent growth of branchial arches and filaments has to match that of the entire body. Our results using blastomere transplantation in a same species (wild type and mutant cells) or inter species suggest a hierarchical organisation among the different stem cells types that, however, can be broken in order to adapt to certain environmental conditions. We are currently following single-cell omics approaches in order to investigate how such coordination among different stem cell populations takes place in our model.

Mechanical forces and cell differentiation in retinal organoids

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During the development of an embryo, morphological changes shape cells and impact on tissue structure. Niches are formed which provide environmental input to cells ranging from chemical signals to mechanical forces. In the embryo, the influence of single aspects of the mechanical and structural component impacting on tissue identity is difficult to study.
Here, we make use of the organoid system of the medaka fish (Oryzias latipes) retina to study the impact of forces and geometrical restriction on cell identity. The organoid system recapitulates early steps of retinal cell differentiation, but so far does not fully resemble the distinct layered shape and cell composition. Therefore, the retinal organoid system offers the perfect platform to systematically test the definition of cell types in the retina by the application of external forces.

Recording the Transcriptional History of Mammalian Cells

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In the field of developmental biology, great emphasis is placed on the study of cell signaling due to its roles in cell differentiation, cellular regeneration, organogenesis, and a plethora of other processes. Cell signaling can be analyzed either at low spatio-temporal resolution or using techniques that disrupt the tissues. While genetic reporters have become pivotal in analyzing the downstream effects of cellular signals, very few methods are available to analyze the cascades across long time periods. One such emerging technology is a molecular tool known as a DNA recorder. DNA recorders are attenuated to capturing specific signals and converting and storing them into DNA. That way the information can be reconstructed at any time point via Next Generation Sequencing. While Cas9 has been the pioneering biomolecule in developing these DNA recorders, my interests lie in another protein complex known as RT-Cas1 and Cas2 from the organism F. saccharivorans. What is unique about this protein complex is that it is capable of not just capturing a limited amount of signals but has the capability of recording the transcriptional history of a cell in chronological order. So far the ability to record information on this large of a scale and in sequential order has yet to be obtained in eukaryotic DNA recorders. In my research I will attempt to humanize these

bacterial protein complexes by adapting their function and localization in human culture cells, which are convenient models in which to establish and characterize their function. In addition I will stably transfect the fixed address into a safe genomic harbor of HEK293T cells via Cas9 knock in. Because the integration of new information at the fixed address is low, it cannot be detected by conventional PCR. I will instead use DNA amplification followed by sequencing using SENECA, a technique that selectively amplifies expanded CRISPR arrays. In the long term, I plan to optimize this tool to generate transgenic mouse tissues, such as in vitro culture intestines, and animal models. These biological "sentinel" models could be used for biomedical purposes such as characterizing injuries, determining the effects of therapeutic drugs, or identifying pollutants.

Elevated temperature fatally disrupts the orchestrated timing of nuclear divisions in the early Drosophila embryo

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Temperature is a key regulator for the speed of biological processes, especially in animals that can't actively regulate their body temperature. Previous work in insects suggests that spatiotemporal variations in temperature can locally influence the embryonic development. Yet, it is unclear if elevated temperatures affect the fidelity of embryonic development, and if so, how. Here we use early embryonic development of the fruit fly Drosophila melanogaster as a model to address consequences of elevated temperature on the fly embryonic development. We identified the syncytial blastoderm stage as a critical time window during the embryonic development that is particularly sensitive to elevated temperature, with cascading defects in later stages of development, and a significant increase in embryonic lethality.

During the syncytial blastoderm stage, the embryonic patterning is being set up, in parallel with 4 rounds of meta-synchronous nuclear divisions. Even though all nuclei divide together, there is a lag between the nuclear cycles at the anterior/posterior poles vs those in the middle of the embryo. We found that, in the embryos developing at elevated temperature, the lag between middle vs anterior/posterior increases progressively over the course of successive nuclear division cycles. We could also demonstrate that elevated temperature leads to local crowding of nuclei, subsequent failure of nuclear division in crowded regions, and a loss of cortical nuclei that failed to divide. We could show that the loss of cortical nuclei during syncytial- and cellular-blastoderm, in extreme cases, produced holes in the blastoderm epithelium. An early exposure to elevated temperature also increased the embryonic lethality. We propose that the loss of nuclei results in a loss of patterning information and the abrogation of proper embryonic development. We also identified candidate genes that potentially attenuate the effects of higher temperature, and functionally tested their capacity to rescue the embryonic lethality.

Our results reveal a vulnerability in developmental timing, which is exposed at elevated temperatures, and which can potentially be rescued by modulating the expression of just a few factors. We propose that the expression levels of the corresponding genes could be used as indicators to predict fitness effects on insect populations that are exposed to increasing temperature variations.

Reverse proteomics analysis of polar membranes domains associated with lateral root formation in Arabidopsis

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Roots anchor plants in their substrate, determine their capacity to uptake water and nutrients and thus their fitness. Plants cells are surrounded by a rigid cell wall and organ morphogenesis solely relies on a precise orientation of cell division and control of directional cell growth. An example of localized cell expansion associated with organ formation is the initiation of lateral roots (LRs) that determine root architecture. LRs formation occurs deep into the parental root and is marked by the asymmetric expansion of two abutting founder cells sharing a common interface. This common cell interface acts as an organizing center that polarize each cell and organizes LR morphogenesis. Despite its importance, the exact molecular composition of this polarizing interface remains poorly known. In other tissues than LR, auxin-dependent nanoclustering of proteins and lipid has been found to be fundamental for polarization and anisotropic growth.
In this project we aim to reveal the particular molecular identity of the polarizing domain using a proteomic-based approach built on proximity labeling and affinity purification. The main goals are to identify signalling components localizing to this interface and the composition of auxin induced specific membrane nanoclusters (MNC).
For this proposes, we fused the TurboID enzyme to proteins resident in this polar domain of LR founder cells (LRFCs) and targeted their expression using the LR specific promoter GATA23. This will allow us to identify by proximity biotinylation other proteins enriched in this domain. Besides, to elucidate the composition of auxin-dependent MNC we took advantage of the Split-TurboID approach by fusing the halves of the enzyme to proteins recruited to MNC in response to auxin. Finally, the role of the new proteins identified will be analyzed by studying the impact of their mutations on LR initiation.
Here we report the correct localization of the polar and non-polar baits in LRFCs and the setup for the LR initiation system used to maximize the number of primordia at the right stage of development for the study. On the other hand, we carried out a proof-of-principle assay in N. benthamiana in which we successfully tested the Split-TurboID system in plants.

Plant V-ATPase interacting proteins

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The vacuolar H+-ATPases (V-ATPases) are multisubunit complexes that are indispensable for pH homeostasis of various cellular compartments in all eukaryotic cells. The localization of this multifaceted enzyme is determined by the isoforms of the membrane integral subunit, VHA-a. The incorporation of VHA-a1 targets the V-ATPase to the trans-Golgi network/early endosome (TGN/EE) whilst the incorporation of VHA-a2 and VHA-a3 targets the V-ATPase to the tonoplast. Not much is known about proteins that recognize and maintain V-ATPases at their destinations, proteins that assist V-ATPases to perform their functions as well as proteins that are responsible for the removal of V-ATPases at the end of their life time. We have

employed the TurboID biotin ligase in proximity labelling experiments to shed light on these interactors.

Funcitonal analysis of DNA methylation in the invasive Marbled Crayfish

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Procambarus virginalis, also known as marbled crayfish, is a freshwater species that emerged from the German aquarium trade in the 90s and since then, has been able to colonise different ecosystems in Madagascar and Europe. This successful expansion is partially explained due to its mode of reproduction since it is an obligatory apomictic parthenogenetic animal and produces hundreds of descendants every year. However, this type of parthenogenesis makes marbled crayfish an all-female clonal population with no genetic variation, something that speaks against its remarkable adaptability. Therefore, in the absence of the usual genetic variation a population displays, epigenetics arose as a likely and important mechanism mediating the adaptability of these animals to new environments. Previous analysis in the lab showed that marbled crayfish presented a methylation pattern enriched in gene bodies and a complete DNA methylation toolkit (DNMT1, DNMT3 and TET) easily disturbed via RNAi, which successfully showed the subsequent reduction in DNA methylation. Therefore, we questioned this knockdown system looking for observable phenotypes related to the levels of DNA methylation. Both the immune and nervous systems play important roles in the relationship of the individual and the environment. Thus, knockdown animals for DNMT1, DNMT3 and both DNMTs were generated with the RNAi technology and one month after the treatment, (and after checking the DNA methylation levels with a newly developed PCR-based assay), the different proportions of hemocytes (innate immune cells) were analysed using Image Cytometry. DNMT3 KD and double KD animals presented higher levels of granular cells, the most mature cell and functional cell type of the crayfish immune system suggesting a role of de novo gene body DNA methylation in invertebrate cell differentiation. In addition, behavioural tests seemed to correlate the three groups of DNMTs KD with reduced levels of anxiety in the marbled crayfish. Both phenotypes, a more mature immune system and a more exploratory-like behaviour can be essential in adaptability to new ecosystems, and if controlled by the environment via DNA methylation, might suggest a major role of this epigenetic mark in invertebrate phenotypic plasticity that will require further mechanistic analysis.

Structural and functional mechanisms of Wnt cleavage by novel astacin protease

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Research groups are always on the look-out for novel Wnt-pathway regulators because of the essential role of Wnt in regulating cell differentiation, cell proliferation, and stem cell renewal in both embryonic and adult tissues (Steinhart and Angers, 2018). Furthermore, Wnt pathway dysregulation is a major factor in various cancers such as Colorectal cancer and Hepatocellular carcinoma (Bugter et al, 2021). A novel Wnt inhibitor known as Hydra astacin 7 (HAS-7) has

recently been discovered in the freshwater polyp Hydra, by the Özbek lab (Ziegler et al, 2021). HAS-7 is an astacin metalloprotease and has been shown to cleave recombinant Hydra Wnt3 (HyWnt3). siRNA knockdown of HAS-7 results in a double-axis phenotype, presumably due to unregulated HyWnt3 activation (Ziegler et al, 2021). My PhD thesis aims to dissect the interaction interface between HAS-7 and Wnts. For this, we require sufficient and stable amounts of HAS-7 and Wnt proteins. Therefore, I am currently expressing and purifying multiple constructs of HAS-7, various Wnts from different species, and a HAS-7 orthologue: Xenopus Hatching Enzyme-4 (XHE-4) using mammalian, insect and bacterial cell expression systems. These purified proteins can then be used in structural studies utilising X-ray crystallography, Cryo-EM and various kinetic and enzymatic assays. Since astacins are a well-conserved group of proteins, knowledge of the structural relationship between HAS-7 and XHE-4 with Wnts may be useful in the development of novel therapies against Wnt dysregulation related disorders.(Bond and Beynon, 1995).

References:

Bond, J. S., & Beynon, R. J. (1995). The astacin family of metalloendopeptidases. Protein science: a publication of the Protein Society, 4(7), 1247–1261

Bugter, J.M., Fenderico, N. & Maurice, M.M. Mutations and mechanisms of WNT pathway tumour suppressors in cancer. Nat Rev Cancer 21, 5–21 (2021)

Steinhart, Z., & Angers, S. (2018). Wnt signaling in development and tissue homeostasis. Development (Cambridge, England), 145(11), dev146589.

Ziegler, B., Yiallouros, I., Trageser, B. et al. The Wnt-specific astacin proteinase HAS-7 restricts head organizer formation in Hydra. BMC Biol 19, 120 (2021).

NR2F1 in neural crest cells: a novel approach to model Bosch-Boonstra-Schaaf Optic Atrophy Syndrome (BBSOAS)

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Bosch-Boonstra-Schaaf Optic Atrophy Syndrome (BBSOAS) is an autosomal dominant disease caused by mutations or deletions of the transcription factor NR2F1, associated with brain and face development. BBSOAS is a neurodevelopmental disease characterized by a wide range of clinical representations such as intellectual disability, developmental delays, craniofacial abnormalities, hearing impairment, and atrophy of the optic nerve. The craniofacial abnormalities observed in BBSOAS indicate a relationship with neurocristopathies; a group of pathologies associated with abnormal development of the neural crest cells (hNCC). These cells represent a transient population of embryonic cells that give rise to a wide variety of cell types, including craniofacial cartilage and bones, eye, inner ear, peripheral and enteric neurons. Since NR2F1 is not only highly expressed in neuronal lineages but also in hNCC, we hypothesize that some features such as facial, ear, and optic abnormalities observed in BBSOAS may occur due to hNCC malformation associated with aberrant NR2F1 expression.
To investigate this hypothesis, we explore hNCC derived from induced pluripotent stem cells (hiPSC) acquired from BBSOAS patients. Moreover, to dissect the role of NR2F1 in face and

brain development, we also differentiate these hiPSC lines into neural progenitor cells (hNPC) and cortical neurons and plan to determine the regulatory network orchestrated by NR2F1 in these cell types. Consequently, we postulate that NR2F1 underlies a differential tissue-specific expression that is controlled by a dynamic regulatory landscape. To this end, we also successfully generated novel CRISPR/Cas9 engineered hiPSC models, with deletions of NR2F1 and its potential regulatory elements.
Combining epigenomics, transcriptomics, and bioinformatics we will determine the impact of NR2F1 defects on face and brain development in BBSOAS. The cellular models we have developed will provide us with a unique opportunity to dissect the regulatory network controlled by NR2F1 during hNCC, hNPC, and neurons development. Once the network is determined, we will identify novel genetic variants within that network, that are not only associated with BBSOAS but also potentially with other disorders.

Patient-based models in medaka to study congenital disorders of glycosylation

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Glycosylation is one the main forms of protein modification that affects stability, transportation and function of proteins. Rare human diseases like congenital disorders of glycosylation (CDG) are a growing group of metabolic disorders of which the pathophysiology is poorly elucidated and therapeutical approaches are limited. Null alleles of the glycosylation machinery enzymes are usually lethal. In CDG patients therefore mutations presumably cause global protein hypo-glycosylation which interferes with embryonic development and phenotypically is most prominent in neural and musculoskeletal tissues.
The entire glycosylation machinery is highly evolutionarily conserved from plants to vertebrates which allows generating translational model organisms. Especially fish models offer a great opportunity as already the early embryogenesis can be studied trough the extrauterine and transparent developing embryos. To unravel the sensitivity of important signaling pathways to differing levels of glycosylation during development and tissue maintenance, we use advanced CRISPR/Cas9 based systems to generate patient-based mutations in medaka fish (Oryzias latipes). Additionally, an alternative approach termed "inception" is applied to reach previously inaccessible base editing sites for generation of mutations in a more flexible manner. Moreover, we designed a construct in which a split GFP sequence in the parental line allows GFP to be expressed in the homozygous offspring, identifying mutants in early stages without genotyping.

Patient-based models in medaka to study congenital disorders of glycosylation

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glycosylation which interferes with embryonic development and phenotypically is most prominent in neural and musculoskeletal tissues.
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Moreover, we designed a construct in which a split GFP sequence in the parental line allows GFP to be expressed in the homozygous offspring, identifying mutants in early stages without genotyping.

High-throughput screening assay for genetic contributors to retinal regeneration in a highly inbred vertebrates population

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In vertebrates the ability to regenerate the retina ranges from no repair in mammals to context- dependent repair in fish. Upon injury, zebrafish regenerate their retina whereas Japanese rice fish, medaka (Cab strain), only repair photoreceptors by differentiation of Müller Glia (MG) cells. In medaka, full regeneration is observed by sox2 overexpression in MG cells, indicating that retinal regeneration can be modulated by appropriate transcriptional programs.
To systematically delineate genes modulating retinal regeneration, we exploited a panel of genetically diverse medaka inbred strains. Firstly, we assessed retinal regenerative response and strikingly, distinct medaka inbred strains exhibit a variation in retinal regenerative response, suggesting the contribution of the underlying genetics. Secondly to screen the whole panel for regenerative ability, we established high throughput assays to evaluate retinal repair/regeneration in medaka larvae. In the assays the retinal injury is induced with intense light and visual recovery is assessed by an optomotor response assay as a readout for repair/regeneration. The screening of the panel with the setup will allow to elucidate the underlying genetics and address the immediate adaptive programs impacting on in light-induced retinopathies and subsequent repair or regeneration.

Modifying from within - Novel approach to manipulating the interior environment of retinal organoids

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The last decades have shown that fundamental biological processes such as the differentiation of cells as well as the formation of organs and entire organisms are heavily influenced by the biochemical and physical environments of cells in their specific niches. In vitro 3D cell culture systems like self-organizing organoids derived from pluripotent stem cells of different species have been proven to be of great potential to decipher the central properties of those environments by allowing for more manipulation and control compared to the study of the respective in vivo system. Finding new ways of further expanding the control over 3D cell culture systems promises to pave the way for a deeper understanding of the significance of those environments as well as to open up avenues to manufacture cellular organizations not found in nature.
Here, we introduce a novel methodology utilizing DNA-based hydrogel beads for more precise control of the biochemical and physical interior environment of retinal organoids across species in an interdisciplinary collaboration with material scientists from the Max Planck Institute for Medical Research Heidelberg.
We were able to establish a setup for microinjection of these DNA hydrogel beads into the interior of spherical retinal organoids derived from fish primary embryonic pluripotent cells as well as from mouse embryonic stem cells. Immunostaining and confocal microscopy revealed that microinjected DNA hydrogel beads retain their structural integrity in the interior of retinal organoids for several days, are juxtapositioned to the membranes of adjacent cells and do not impact retinal progenitor cell differentiation and retinal layering.
Going forward we hope to be able to show that these DNA hydrogel beads can be uniquely functionalized to drive cell fate and thus modify the overall structure and composition of retinal organoids. Prospective functionalizations include customization of the DNA hydrogel beads' stiffness, as well as their swelling, shrinking and breakdown for modifying the physical interior environment of retinal organoids in a spatial and temporal manner and tagging of these beads with small molecules/peptides for modifying the biochemical interior environment of retinal organoids.

Embryonic stem cells from medaka self-organize into 3D cardiac aggregates with rudimentary functionality

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Vertebrate organogenesis is a complex process governed by various factors intrinsic to the embryo and restrictions of the environment. Using in vitro systems such as stem-cell-derived 3D organoid cultures provides a convenient opportunity to investigate intrinsic factors more closely. Additionally, they allow for interfacing biological processes with modern engineering approaches. Cardiac organoids have been established for human and mouse stem cell culture in various levels of complexity during in vitro organogenesis. However, mammalian tissue culture has limitations in terms of culture conditions and duration of cardiac development. Previously, we have established a retinal organoid model derived from primary stem cells of the fish medaka (Oryzias latipes), mimicking their fast development in a highly robust protocol. We now investigate their ability to self-organize into cardiac organoids to uncover tissue

specification processes, electrophysiological characteristics, and differences in self-organization depending on genetic background. Cardiac organoids will then be used to study cell-environment interaction. Further, they may prove to be useful tools in microfluidics, soft robotics, and biomedical research applications due to their short generation time, high efficiency, a wide variety of available genetic backgrounds, and ease of genetic manipulation.

The Na,K-ATPase facilitates the unconventional secretion of Fibroblast Growth Factor 2

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FGF2 is a tumor cell survival factor that is exported from cells by an ER/Golgi-independent secretory pathway. This unconventional mechanism of protein secretion is based on direct translocation of FGF2 across the plasma membrane. FGF2 membrane translocation is thermodynamically driven by PI(4,5)P2-induced membrane insertion of FGF2 oligomers. PI(4,5)P2 grants the dynamic translocation intermediates of FGF2 molecules. Recently the Na,K-ATPase has been revealed as an upstream factor for FGF2 at the inner plasma membrane leaflet prior to PI(4,5)P2.
The Na,K-ATPase has previously been shown to play a role in this process, however, the underlying mechanism has remained elusive. Interestingly, the structural elements necessary for the direct physical interaction between FGF2 and the **α**1 subunit of the Na,K-ATPase have been defined. FGF2 **α**1-binding mutants were impaired regarding both recruitment at the inner plasma membrane leaflet and secretion to the outer leaflet. Ouabain, a Na,K-ATPase inhibitor, impaired both FGF2 secretion and the interaction with the Na,K-ATPase in cells.
The latest findings reveal the Na,K-ATPase as the initial recruitment factor for FGF2 at the inner plasma membrane leaflet, being required for efficient membrane translocation of FGF2 to cell surfaces. The combined findings of our group establish a novel type of self-sustained protein translocation across membranes. The molecular basis leading to the oligomerization of FGF2 protein molecules build up a unique structure, within evolves the higher functionality of an unconventional protein secretory pathway in the cell.

Light, specks and cell death: Optogenetic tools to control cell death in the zebrafish skin

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A key response to the infection of cells is the initiation of specific inflammatory cell death pathways and a signaling response to warn and activate surrounding cells. One of the well-conserved systems for an innate immune response is the formation of the inflammasome. The inflammasome is a large multiprotein signaling platform formed for the activation of pro-inflammatory caspases and is activated by oligomerization of pattern-recognition receptors. The ability to control programmed cell death (PCD) in time and space at high resolution can specifically aid the understanding of cell and tissue responses to PCD in vivo. To study this, we have developed a genetically encoded tool for activating PCD (pyroptosis and/or apoptosis) in zebrafish skin.
For the optimal spatial and temporal control of cell death, we constructed Opto-ASC, a fusion of the light-responsive element of Cryptochrome 2 (Cry-2olig) and the inflammasome adaptor

protein ASC (Apoptosis-Associated Speck-Like Protein Containing a CARD). We introduced a cassette of this construct under the control of heat shock element into zebrafish in which we can now induce ASC inflammasome (speck) formation in single cells of the skin.
We find that cell death resulting from ASC induced speck formation can differ in morphology and extrusion pattern. In the keratinocyte layer, some cells extrude apically and some basally. The apical extrusion in keratinocytes depends on caspb and involves a strong Ca2+ signalling response in neighbouring cells.

Curvature-mediated patterning of protein complexes

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Deformable geometries have been increasingly considered as a means of forming feedback loops in biological systems which lack the typical inhibitors expected in Turing systems. In my PhD project, I aim to explore the effects of curvature mediated interactions between diffusing components and expand self-patterning theories to include local curvature deformations. This approach will be constructed with a particular focus on the Nuclear Pore Complex as an example system. In this poster, I introduce my PhD project, and present results from a one-dimensional toy model system. Through this model, I demonstrate that density dependent patterning can arise from curvature-interactions on a free, fluid membrane when the curvatures induced at the protein edges are of opposite signs. I also outline future approaches to expand the model to two dimensions, with the aim to gain further insight into the organisation of curvature-mediated protein clustering on membranes.

Clonal dynamics in the murine male germline: From primordial germ cells to spermatogonial stem cells, and the next generation

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Embryonic primordial germ cells (PGCs) are the source of all adult gametes in most animals. Since gametes transmit genetic and epigenetic information to the offspring, dynamics of PGC lineages may contribute to the selection of hereditary information and the sustenance/evolution of species. Therefore, the lineage tracing from PGCs to gametes may help understand the selection process during germ cell development.
In mice, dozens of PGCs are induced at around embryonic day (E)6.5. PGCs migrate and enter bilateral genital ridges, then sex-specific gonad development starts. Male PGCs give rise to spermatogonial stem cells (SSCs) which continuously divide, maintain themselves and produce sperm through life. In these processes, the actual ratio of PGC lineages maintained until adult and the difference in contribution among lineages remain unknown.
We have traced the dynamics of clones derived from each PGC (PGC clones) by labeling PGCs with greatly diverse DNA barcodes. Our data suggest that most PGC clones after entering gonads produce SSCs and maintain themselves until puberty. The expansion of PGC clones is unequal from embryonic stages, and individual E6.5 PGC clones occupy from ~0.3% to ~10% of the whole germ cell pool. We also crossed E6.5-barcoded males with multiple

females for a year and analyzed barcodes that appear in their pups. It demonstrated that dozens of E6.5 PGC clones contribute to a few hundred pups, and N littermates normally derive from N/2 E6.5 PGC clones or more. On the other hand, the number of pups derived from individual E6.5 PGC clones was unequal. Comparing barcode frequency in pups and in the fathers' testes, we found that SSCs produce sperm proportionally with their clone size, and clone size in SSCs/sperm is reflected in their contribution to the offspring. Our results suggest that unequal clonal expansion of PGCs occurs from embryonic stages, and the clone size in SSCs impacts the number of offspring generated from each PGC clone. We propose that such unequal clonal expansion in the germline work for selective genetic/epigenetic inheritance.

Gene-environment interactions and interindividual variability in xenobiotic response – a genome-wide association study in medaka

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"Genetic predisposition influences susceptibility to xenobiotics and leads to different effects on individuals. In order to decipher the causative gene-environment interactions (GxE) and to gain a mechanistic understanding of the biological pathways involved in individual xenobiotic responses at the molecular level, we are conducting a large-scale genome-wide association study (GWAS). To overcome the limitations of the heterogeneity of the human genome, we make use of the high inbreeding tolerance of the teleost fish medaka (Oryzias latipes). This feature has led to the development of the Medaka Inbred Kiyosu Karlsruhe (MIKK) panel consisting of 80 fully sequenced and near-isogenic lines that were originally derived from the wild and therefore resemble a natural population.
The transparency of the medaka chorion and embryo enables non-invasive in vivo imaging. So far, we have screened about 18000 embryos of the MIKK panel with three xenobiotics (ethanol, disulfiram, caffeine) for changes in heart rate as a general physiological indicator. Significant differences in response to xenobiotics are found between the lines, ranging from absolute resistance to a substantial decrease in heart rate over time. Most lines also show different response patterns depending on the drug, indicating clear genetic effects.
F2 segregation analysis and mapping of quantitative trait loci will pave the way for the identification of contributing loci and their validation. Since medaka has many orthologous genes to humans, unravelling these will assist pharmacogenomics in the development of personalised medicine and provide a basis for the individualised assessment of environmental toxins."

MAPKs link injury to patterning in Hydra regeneration by default Wnt signaling

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"Wnt signal activation in response to injury is a shared trait between early metazoans and humans. Yet, the molecular cues triggered by injury resulting in Wnt expression have so far been elusive. The basal metazoan Hydra with its unlimited regeneration capacity that

depends on Wnt signaling is an ideal model to dissect the temporal sequence of molecular events leading from injury to tissue regeneration.
Using a combination of protein biochemical, genetic and in silico analyses, my work elucidated how generic wound signals are transmitted to position-specific axial regeneration in Hydra. My results showed that injury initially led to a release of reactive oxygen species (ROS) and calcium, which in turn activated ERK, p38, and JNK by increasing their phosphorylation levels. MAPK activation was then crucial to promote indiscriminate Wnt expression in early head and foot regenerates of dissected animals. This early injury-related role of Wnt signaling was required to drive the tissue into a regeneration-competent state, which later allowed patterning of the head tissue by the sustained activity of Wnt9/10c, Wnt3, and Wnt7 that was absent in the foot regenerate. We speculated that these stage-specific functions of Wnt resulted from the interplay of the wound signal with the"source density", a graded regenerative competence of the tissue along the oral-aboral axis of Hydra, which was postulated on the basis of classical regeneration experiments. Our hypothesis was validated experimentally by the transformation of presumptive foot tissue into head structures upon incubation with recombinant Wnt or ectopic stabilization of **β**-catenin. Given the high degree of conservation of the analyzed pathways in the animal kingdom, my work may contribute to a more profound understanding of injury induced signaling circuits that could also play a role in regeneration processes of higher vertebrates."

Immune surveillance of ciliary marginal zone of retina in medaka

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Teleosts have the capability of lifelong postembryonic growth which is possible due to the active postembryonic stem cells that help in maintaining the growth of the tissue. This continuous growth possesses a challenge to have a balance between the proliferation and differentiation ratio of the cells in order to avoid hyperplasia. In teleost fish, medaka, postembryonic retinal neurogenesis is facilitated by retinal stem cells (RSC) that generate neuronal and non-neuronal cell types which are ultimately integrated into the growing retina. In order to ensure proper growth and functionality of retina, there should be precise communication of RSCs with its environment. Immune cells have recently emerged to influence stem cell behavior in maintaining tissue integrity or by driving regeneration under stress. Preliminary research in our lab has shown that chemokine signaling plays a role in maintaining the homeostatic growth of retina in medaka. Immune cells expressing chemokine receptor 9a (ccr9a) were found to be located near ciliary marginal zone and migrate towards the RSCs expressing chemokine ligand 25 b (ccl25b) in order to initiate phagocytosis. These immune cells were characterized to be macrophages and it has been shown that ectopic stem cell numbers were produced in their absence. However, it is not known why these RSCs are being phagocytosed under homeostatic conditions and whether it is a stochastic or targeted process. We hypothesize that these phagocytosed RSCs might have tumor like characteristic and in order to investigate this, firstly, we will introduce the FUCCI system which will help in identifying the cell cycle phase, since an accelerating cell cycle is a typical tumor cell feature. Secondly, expression of certain oncogenes will be induced in a temporal manner to explore the behavior of macrophages towards these cells. Exploring the nature of RSC and whether this role of macrophage is limited only to these cells or is expanded to the retinal progenitor

population would be a potential area to explore. The results of this study will provide an insight into a novel role of macrophages that might be important to maintain the continuous growth of retinal tissue in medaka.

Secretory activity is required for oil body formation in Marchantia polymorpha

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Eukaryotic cells possess endomembrane organelles equipped with specific sets of proteins, lipids, and polysaccharides that are fundamental for realizing each organelle's specific function and shape. A tightly regulated membrane trafficking system mediates the transportation and localization of these substances. In addition to conserved organelles among eukaryotes, some eukaryotic lineages have acquired novel organelles during evolution through mechanisms that remain largely obscure. The existence of the unique oil body compartment is a synapomorphy of liverworts that represents lineage-specific acquisition of this organelle during evolution, although its origin, biogenesis, and physiological function are yet unknown. We found that a specific syntaxin-1 homolog in the liverwort Marchantia polymorpha is targeted to the oil body and that secretory-related mutants harbored abnormally shaped oil bodies. These findings highlight that the liverwort oil body is formed by the redirection of the secretory pathway inward the cell depending on cellular phase transition.

Fish retinal organoid culture as a system to study cell fate decisions during retinal development

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Proper visual perception is strictly dependent on coordinated differentiation and correct assembly of multiple tissues of developing eye. During vertebrate development, the neuroepithelium of the optic vesicle differentiate into three main tissues: neuroretina (NR), retinal pigmented epithelium (RPE), and a ciliary margin zone (CMZ) harboring retinal stem cells on their boundary. The mechanism of how a single progenitor gives rise to three different tissues, particularly to cells of CMZ, is not fully understood. Here we use retinal organoids derived from the teleost fish medaka (Oryzias latipes) as a model to study mechanisms that contribute to the bias of progenitors of the optic vesicle towards NR, RPE and CMZ cell fates. We have previously demonstrated that fish primary embryonic stem cells efficiently assemble into 3D retinal organoids carrying progenitors of the optic vesicle neuroepithelium. Here we show that this simple retinal neuroepithelium can be directed into NR, RPE and CMZ tissues fates by modulation of single signaling pathway, Wnt/**β**-catenin pathway. While RPE specification requires high levels of Wnt/**β**-catenin signaling activity, NR specification can occur only in its absence. Interestingly, intermediate levels of Wnt/**β**-catenin signaling activity result in acquisition of CMZ fate, indicating that the gradient of Wnt/**β**-catenin signaling built up during optic cup morphogenesis contributes to the acquisition of NR, RPE and CMZ fates and that possibly the interface between NR (with no Wnt activity) and RPE (with high Wnt activity) provides the niche for establishment of stem cell carrying CMZ.

The rhythm of life - genes involved in heart function

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Cardiovascular mortality is the leading cause of death worldwide. A majority of cardiovascular diseases have a complex etiology caused by a multitude of genetic and environmental factors. The cumulative impact of these factors complicates the correlation of genotype and phenotype. Genome-wide linkage studies provide the unique opportunity to investigate genetic and environmental causes of complex traits in an unbiased and systematic approach. Here, we utilize the Medaka Inbred Kiyosu-Karlsruhe (MIKK) panel to study the genetic contribution to complex heart phenotypes under different environmental conditions. The MIKK panel was established from a wild medaka (Oryzias latipes) population and reflects the genetical diversity of a naturally occurring population. By performing a large-scale phenotypic analysis, we aim
to link novel genetic variants to heart function and thus uncover cardiovascular disease relevant genetic profiles. We assayed 56 genetically distinct MIKK panel strains to determine strain-dependent differences in the heart rate at various temperatures. Strategic crossing of multiple phenotypic contrasting strains was done to perform an F2 segregation analysis. To link genetic determinants to the heart phenotypes in the F2 populations, whole genome sequencing (WGS) is performed at the moment. For the pending confirmation of quantitative cardiac trait loci (QTL) mapping results we have established mechanistic validation approaches using genetic tools including the CRISPR/Cas9 and base editing system. By genetic mapping of cardiac trait loci and their subsequent functional validation, we expect to identify novel genetic determinants with relevance for the cardiovascular system and disease susceptibility.

Effect of planar cell polarity on neighbouring muscle stem cells

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Epithelial tissues exhibit a polarity in the plane of the tissue surface defined as planar cell polarity (PCP). Genetic screens in Drosophila led to the "polarity" mutant Frizzled (Fz), which has a very unique, distinct, and reproducible mutant polarity pattern. Fz is a key component of the Frizzled/Starry night pathway, which is important for establishing molecular asymmetry in cells. Published single cell data show that fz is expressed in the epithelium of the wing disc of third instar larvae. The epithelium serves as a stem cell niche and provides asymmetric signals important for the organisation and maintenance of the two underlying populations of adult muscle progenitor cells (AMPs), that give rise to two types of adult flight muscles - direct and indirect flight muscles (DFM and IFM). However, whether and how epithelial PCP signalling affects AMPs remains unexplored. We used CRISPR/Cas9 to specifically knock out fz1 and fz2 in wing disc epithelium to determine the cell type-specific effect of PCP on underlying AMPs. Knockout flies show disoriented bristles and a reduced number of bristles, confirming published data on the role of Fz in the epithelium. Interestingly, adult knockout

flies exhibit wings with abnormal position, a phenotype referred to as the “held-up wing” phenotype, suggestive of a muscle defect. Conducting a flight assay revealed that less than 2 % of knockout flies can fly compared to 75 % of control flies. Immunofluorescence staining and subsequent manual counting showed that the absence of fz1 and fz2 had a small effect on the number of DFM AMPs located in the hinge region. In addition, phalloidin staining of actin filaments showed aberrant fibre morphology of IFMs, which would lead to flight inability. Single cell RNA sequencing was used to determine the transcriptome of cells of the wing disc and AMPs. Our plan is to study the transcriptome of the epithelium and both AMP populations in order to uncover the underlying cell type-specific molecular mechanism of PCP signalling. This will help to better understand the heterogeneity of the two AMP populations and furthermore contribute to a fundamental understanding of the specific interactions between stem cells and their niches.

Auxin methylation in root nodule symbiosis

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Leguminous plants attract symbiotic bacteria, called rhizobia, and create ""nodules"" on their roots. Nodule development consists of bacterial infection of root epidermis and subsequent primordium formation with cell division in root cortex. Cortical cell division occurs just below the site of rhizobial infection in epidermis, suggesting a spatiotemporal coordination between epidermis and cortex during nodulation. However, little is known about the molecular mechanism.
Lotus japonicus mutant “daphne” has uncoupled symbiotic events in epidermis and cortex, in that it promotes excessive bacterial infection in epidermis but does not produce nodule primordia in cortex. Therefore, daphne should be useful for exploring unknown signals that coordinate these events across tissues.
Here, we conducted time-course RNA-seq using daphne after rhizobial infection. We noticed that IAA carboxyl methyltransferase 1 (IAMT1), which encodes the enzyme that converts auxin (IAA) into its methyl ester (MeIAA), is transiently induced in wild-type roots at early stages of infection but shows different expression dynamics in daphne. Phylogenetic tree suggested that IAMT1 is duplicated in the legume lineage, and one of two paralogs (named IAMT1a) was mainly expressed in root epidermis. IAMT1a knockdown affected cortical events such as nodule and its primordium formation, but not epidermal infection. The finding that IAMT1a was mainly expressed in epidermis but its function is required in cortex suggests that IAMT1a may be involved in coordinated epidermal and cortical regulation. LC/MS-MS succeeded MeIAA detection in roots infected with rhizobia, and constitutive expression of MeIAA demethylase gene inhibited nodulation. These indicate the importance of auxin methylation in nodule development.
IAMT1 has previously been reported to serve shoot development of Arabidopsis. The gene duplication of IAMT1 in the legume lineage may have resulted in the evolutionary acquisition of auxin methylation in roots and its involvement in nodule symbiosis. Legumes have developed epidermal infection systems to escort rhizobia in the process of acquiring the current nodulation systems. And at the same time, they may have acquired an coordinated epidermal and cortical regulation system to take advantage of it. In this presentation, I would

like to discuss the function of auxin methylation in nodule symbiosis and its relevance to evolution.

Housekeeping at the tonoplast: uncovering the degradation mechanisms of tonoplast proteins

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The plant vacuole is indispensable for a plant cell's survival and function by acting as the major storage organelle for solutes, heavy metals and water. These compounds are accumulated inside the vacuole lumen with the aid of a plethora of proteins which are found on the limiting membrane of the vacuole (tonoplast). Its is known how tonoplast proteins are made and delivered to the vacuole but little is known about the precise mechanisms of their degradation. We have designed a genetic screen to identify components of the sorting machinery required for tonoplast membrane protein degradation. We aim to use the vacuolar accumulation of betalains as a read out to identify mutants of tonoplast degradation machinery.

The function of Wnt signaling in the Drosophila muscle stem cell niche

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Stem cells and their niches exchange important biological information through signaling pathways, which can control stem cell behavior. Wnt signaling is one of these key pathways and is involved in numerous functional processes. This evolutionary conserved pathway plays a crucial role in the larval Drosophila muscle stem cell niche. Thus, previous studies indicated the expression pattern of Drosophila Wnt ligands in the muscle stem cell niche and the importance of the Wnt ligand Wg in cell specification and proliferation of the muscle stem cells. Notably, the muscle stem cell niche is a heterogenous tissue with two adult muscle stem/progenitor cell (AMPs) populations, which are distinct in their population size. Nevertheless, the spatial-restricted gene expression pattern and function of each individual Wnt ligand, fz-receptors and co-receptors are mostly uncharacterized. Furthermore, it is unclear how Wnt signaling is connected to heterogeneity in this tissue. The goal is therefore, to uncover a specific Wnt code underlying stem cell heterogeneity. First, we validated the spatial-restricted gene expression pattern of Wnt candidates via RNA-FISH. In addition, genetic tools (siRNA/CRISPR) for a cell-specific knockdown/knockout (kd/ko) of Wnt components in the AMPs/epithelium are performed in order to study the functionality of these candidates. While fz1, fz2 and otk kd in the AMPs showed flies with normal wings, the otk2 kd flies showed flies with a held-out wing phenotype, suggesting a defect in the adult fly muscles. Furthermore, custom-specific computational approaches (e.g. Cellpose) and deep learning strategies enabled a successful 3D cellular segmentation of the muscle stem cell niche to precise count individual cell populations. Further kd experiments in combination with computational tool will allow us to understand how stem cell heterogeneity is achieved in our model tissue.

Auxin induces cambium stem cell identity during radial shoot growth

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Growth and development of plants is highly plastic, a feature which is based on the extraordinary flexibility of their cell identities. Radial thickening of plant shoots and roots is driven by the vascular cambium, a stem cell niche which produces tissues specialized for long distance transport in a bifacial manner. In shoots, cambium cells are initially only found in the center of vascular bundles. During the onset of radial growth, cambium cell identity expands into the area between vascular bundles – the so-called interfascicular region – thereby establishing a closed cylinder of stem cell activity. Interfascicular cambium formation has therefore the potential to serve as a showcase for induced trans-differentiation of cells and the re-establishment of stem cell attributes in a differentiated cellular context.
By using a linage tracing system, we identified starch sheath cells as the origin of the interfascicular cambium demonstrating an endogenously induced cell fate change. During cambium initiation, these cells carried identity markers of both starch sheath and cambium cells indicating a direct transition between both cell identities. Strikingly, induction of auxin biosynthesis specifically in starch sheath cells was sufficient for provoking this cell fate change and provided an experimental switch to analyze the sequence of events during the de novo initiation of cambium identity. Interestingly, genome-wide transcriptional profiling upon auxin-induction revealed similarities between the molecular signatures of cambium initiation and the auxin-dependent formation of lateral roots. These signatures included the induction of similar auxin signaling components and cell wall remodeling factors. Indeed, histological analysis of arf7;arf19 double mutants which are affected in the formation of lateral roots showed impaired induction of interfascicular cambium suggesting comparable mechanisms within both processes.