Joint Meeting of the German and Israeli Societies of Developmental Biology

17. – 20. February 2019
Vienna, Austria

WEBSITE: https://gfe2019.univie.ac.at

Organizers: Ulrich Technau and Eli Arama
Co-Organizers: Elly Tanaka, Fred Berger, Michael Brand, David Sprinzak, Peleg Hasson

Photo: Ruth Ashery-Padan

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Vienna, February 17-20, 2019

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GfE https://www.vbio.de/gfe-entwicklungsbiologie
IsSDB http://issdb.org

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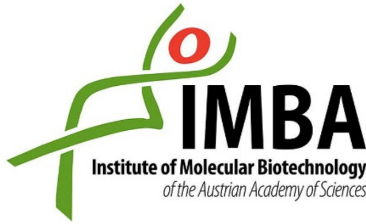
Sponsors



General information

Venue

Campus of the University of Vienna
Spitalgasse 2, 1090 Vienna
Enter GPS coordinates 48.217146, 16.353164 with a navigation app like Google Maps or similar or scan the QR code on the map below with your phone and follow the link.

Getting there

The venue is located in the “Old general hospital” or “Altes AKH” in the 9th district of Vienna. It is accessible by trams 5 and 33 from the “green” U4 subway line (station Friedensbrücke) and by trams 43 and 44 from the “brown” U6 subway line (station Alser Straße) or the “purple” U2 subway line (station Schottentor/Universität).

From the airport, you can take an ÖBB local train line S7 in the direction of Floridsdorf (travels at 18 and 48 minutes past every hour, the journey takes approximately 25 minutes, single ticket €4.20 including further transportation in Vienna) or CAT (travels at 09 and 39 minutes past every hour, journey time approximately 15 minutes, single ticket €12, return €21, additional ticket is required for the public transportation in Vienna) to Wien-Mitte. At Wien-Mitte, change to the "green" subway line U4 in the direction of Heiligenstadt. Exit at the Friedensbrücke station, change to tram 5 in the direction of Westbahnhof or tram 33 in the direction of Josefstädter Straße. Exit at Lazarettgasse or Lange Gasse and walk to the venue. The whole journey will take about an hour.

If you come by long distance train, it is advisable to get off at the station Wien-Meidling. Most trains stop at this much smaller station before continuing to Wien Hauptbahnhof. Change to the "brown" subway line U6 in the direction of Floridsdorf. Exit at Alser Straße and change to the tram 43 (more frequent) or 44 in the direction of Schottentor. Exit at Lange Gasse and walk to the venue. Journey time 25-30 minutes.
If you exit your train at Wien Hauptbahnhof, take tram line D (yes, a letter!) in the direction of Nußdorf. Exit at Schottentor and either change to tram 43 or 44 and exit at Lange Gasse or just walk directly from Schottentor (approximately 900 m). Journey time will also be around 30 minutes.

Taxi: It is advisable to pre-order a taxi (e.g. http://www.flextaxi-flughafentaxi.at/ or call +436603620746) for travel to or from the airport. It costs 25-30 EUR. A taxi taken directly in front of the airport will cost around 60 EUR. Travel time Airport – City center is between 30-45 min, depending on traffic.

If you come by car, and unless your hotel provides private parking, you are well-advised to leave it either in one of the Park and Ride lots such as the one near U4 Hütteldorf or to park in the 19th district, where it is free. In central districts, such as 9th, parking on the street will not be possible for you for longer than 2 hours from 07:00 till 22:00 and will require purchasing parking tickets at gas stations or at tobacconists.

Transportation in town. You can purchase your tickets in machines located at every railway or subway station. You will be able to choose English on the first screen. The options include single journey tickets (bus/tram/subway/S-Bahn changes are allowed; all Vienna is a single tariff zone) as well as 24, 48 and 72 hour tickets and week tickets (always start from Monday). Single journey tickets can also be bought at a slightly higher price in ticket machines on the trams. No tickets can be purchased in city buses.

Useful links for planning your journey

Austrian railway network planner: http://fahrplan.oebb.at/bin/query.exe/en or scan the QR below and follow the link.

Wiener Linien planner for transportation in town: https://www.wienerlinien.at/eportal3/ or scan the QR below and follow the link.

Registration

Registration will open at 14:00 on Sunday, February 17th in the lobby of the venue.

Cloak room

The venue has a cloak room available for you.

Information for speakers

The venue has a PC, which can be used for the presentations. In this case, please upload your talks in **.ppt**, **.pptx** or **.pdf** format latest in the break before the session. For morning talks, presentations have to be uploaded in the evening.

If you wish to bring your own laptop, please make sure that it has a VGA or HDMI port or bring a suitable adapter with you. This is especially relevant for Mac users.

In order to avoid delays by switching between computers, please remember that speakers using their own laptops must check that their computer recognizes the projector and that the projected picture is alright latest in the break before the session! There will be always someone to assist you with checking this.

Please have a backup copy of your presentation on a USB device with you in case something goes wrong.

Poster sessions

Poster sessions will be taking place in the hallway to the left and to the right of the main lecture hall and in the adjoining room behind it. Please observe the markings and consult the floor plan below. All posters can be put up at the beginning of the meeting according to the number of the poster in the abstract book. Please do not forget to put them down afterwards.

Internet access

The venue offers wireless internet access via eduroam.

Coffee breaks and welcome reception

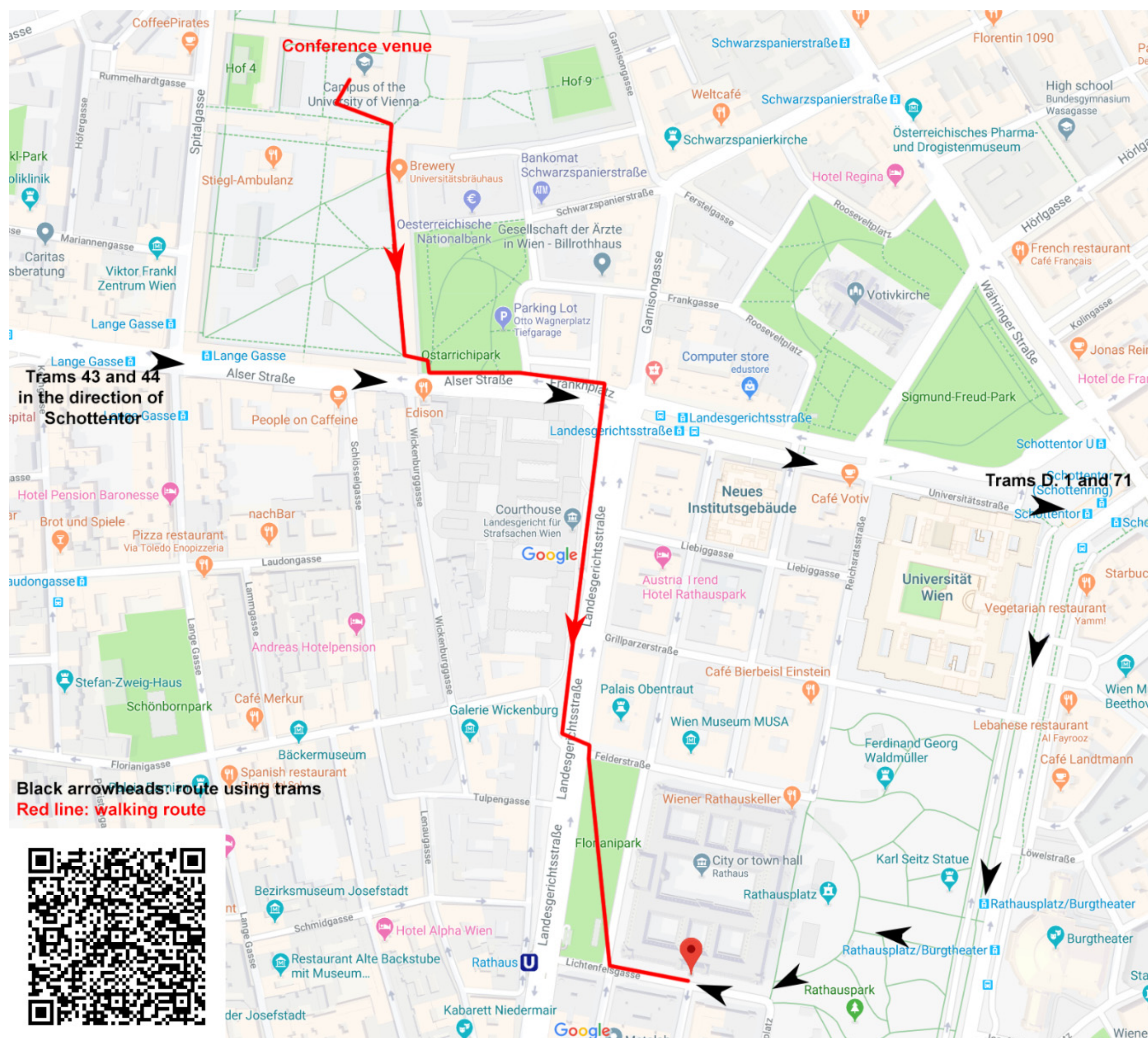
Coffee breaks and welcome reception will take place in the lower floor of the venue. This meeting has been certified as an "Eco-Event", a label from the City of Vienna. Accordingly, we make an effort to have this meeting as sustainable as possible. We therefore avoid any plastic and hence use only real plates and glasses. Please help us by keeping track of your glasses and cups and re-use them for refills.

Lunch breaks

Those of you who included lunch in the conference fee will receive lunch vouchers at the registration desk. The vouchers will be valid in the restaurant Gangl in the first courtyard (Hof 1) of the Altes AKH campus. For those of you who did not pay for lunch vouchers, there is a convenient BILLA supermarket in the same courtyard (closed on Sunday) as well as several restaurants. Both, Gangl and BILLA are marked in red on the map in the "Getting there section".

Conference dinner

If you registered for the conference dinner (Tuesday, February 19th), please be advised that it will take place in the City Hall (Rathaus), which is in the walking distance from the venue (approximately 1.3 km, see map below). Members of the organizing Department will assist you as guides for the walk. If you prefer to use public transportation, take tram 43 or 44 from Lange Gasse in the direction of Schottentor to the last stop (Schottentor) and change to trams D, 1 or 71 for one additional stop. Exit at Rathausplatz/Burgtheater and start walking around the neo-gothic City Hall building on its left side (when looking from the tram stop on the Universitätsring). The entrance will be through an archway approximately in the middle of the building.
Enter GPS coordinates 48.210149, 16.357097 with a navigation app like Google Maps or similar or scan the QR code on the map below with your phone and follow the link.



Invited Speakers Dinner

On Monday, February 18, invited speakers and board members of the GfE and IsSDB are invited for a dinner at the restaurant "Ellas", Judenplatz 9, 1010 Vienna. The restaurant is located in the 1st district and can be either reached by foot (15-20 min walk) or by taxi.

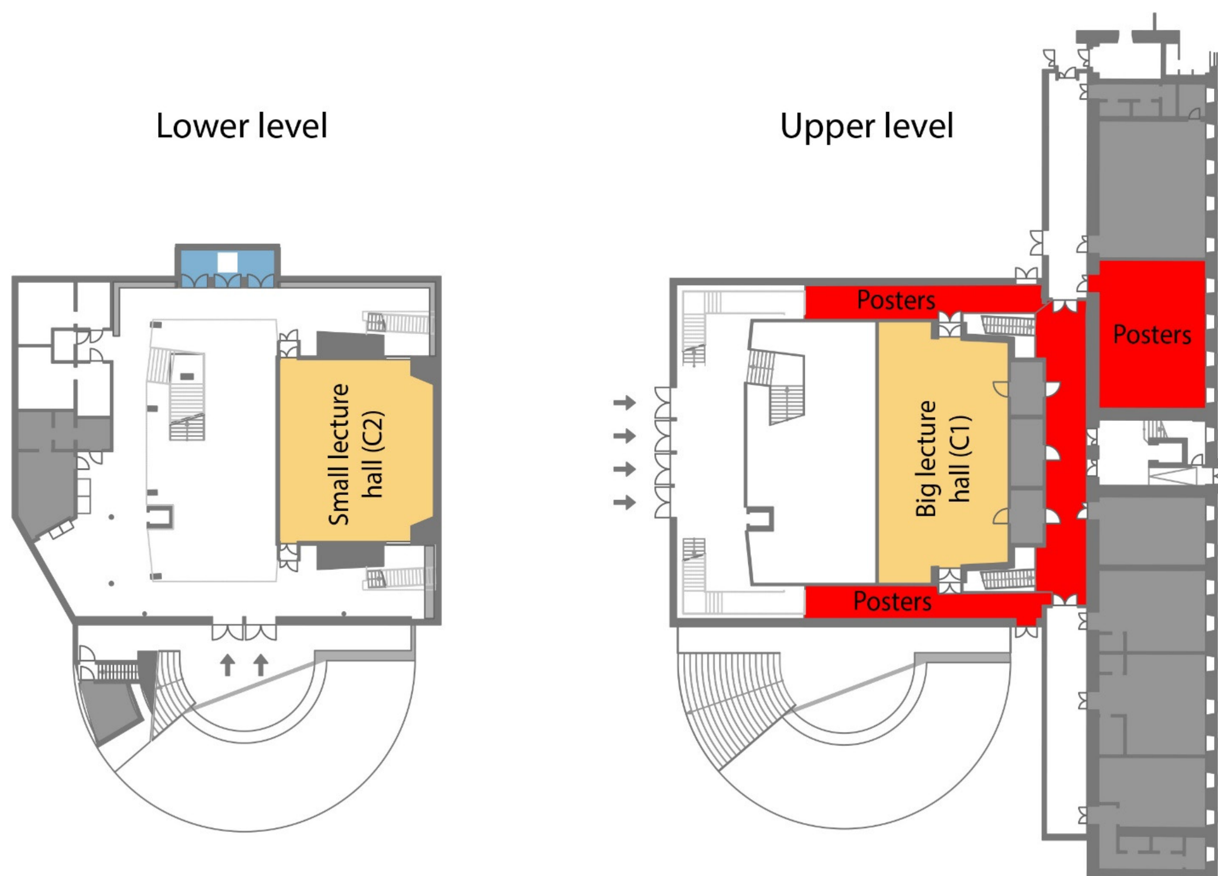
Awards

During the meeting, four prizes will be awarded: the IsSDB prize to Howard Cedar (Monday, February 18, at 18:30), the GfE Klaus Sander Prize to Herbert Jäckle (Tuesday, February 19, at 18:30), the GfE PhD Prize (Tuesday, February 19, at 14:15), and the Poster Prize (Wednesday, February 20, at 12:30).

GfE members meeting / IsSDB members meeting

GfE members meeting will take place on Tuesday at 13:30 in the big lecture hall. IsSDB members are welcome to use the small lecture hall for their members meeting if required at the same time.

Floor plan of the venue



Overview of the sessions

Sunday

starts	Big hall (C1)
14:00	Registration
16:00	Welcome
16:10	Developmental Cell Biology
17:30	Coffee
18:00	Developmental Cell Biology
18:55	Keynote
19:45	Reception

Monday

starts	Big hall (C1)	Small hall (C2)
09:00	Keynote	
09:35	Neural development	
10:40	Coffee	
11:10	Neural development	
11:50	Stem cells, organoids and disease	
12:55	Lunch	
14:30	Single cell approaches	Biophysics of development
15:50	Coffee	
16:20	Poster session I	New techniques
18:30	IsSDB prize talk	
19:15	Invited speakers dinner	

Tuesday

starts	Big hall (C1)	Small hall (C2)
09:00	Genomic approaches to development	
10:45	Coffee	
11:15	Regeneration	
12:35	Lunch	
13:30	Society meeting GfE	
14:15	GfE PhD Prize award	
14:30	Polarity and axis formation	Germline / early cleavages
16:00	Coffee	
16:30	Poster session II	
18:30	Klaus Sander Prize talk	
19:15	Conference dinner	

Wednesday

starts	Big hall (C1)
09:00	Keynote
09:35	Organogenesis
10:40	Coffee
11:10	EvoDevo and emerging models
12:30	Poster Prize / End

Scientific program

Sunday, February 17th

Big lecture hall (C1)	
14:00	**Registration opens**
16:00	**Opening remarks by Uli Technau**
16:10 – 17:30	**Developmental Cell Biology (chair: Peleg Hasson)**
16:10	*Benjamin Podbilewicz* Evolution of developmental cell fusion
16:35	*Jiri Friml* Evolution of efficient root gravitropism during conquest of land by plants
17:00	*Felix Gunawan* Uncovering the development and maturation of vertebrate heart valves at single cell resolution
17:15	*Tom Schultheiss* A novel hedgehog-regulated molecular module that shapes epithelial cell and tissue morphogenesis to position the ventral embryonic midline
17:30	**Coffee break**
18:00 – 18:55	**Developmental Cell Biology (chair: Estee Kurant)**
18:00	*Frank Schnorrer* Measuring molecular tension at developing muscle attachment sites
18:25	*Dorothee Bornhorst* Intra-organ communication within the developing zebrafish heart involves mechanical coupling of myocardium and endocardium
18:40	*Alina Kolpakova* The two mitofusin genes are functionally interchangeable, but both are required for the full fusion of the Drosophila spermatid mitochondria
18:55	**Keynote: *Benny Shilo* (chair David Sprinzak)** Dynamics of morphogen shuttling in the Drosophila embryo
19:45	**Welcome reception**

Monday, February 18th (morning session)

Big lecture hall (C1)	
09:00 – 11:50	**Neural development (chair: Thomas Hummel)**
09:00	**Keynote: *Andrea Brand*** **Time to get up: awakening stem cells in the brain**
09:35	*Christian Klämbt* Development of the Drosophila blood-brain barrier
10:00	*Anna Kicheva* Coordination of progenitor specification and growth in the developing spinal cord
10:25	*Wolfgang Driever* Dynamic control of stemness and neurogenesis in neural proliferation zones of the larval zebrafish brain
10:40	**Coffee break**
11:10	*Dalit Sela-Donenfeld* Hindbrain boundaries - new sites of neural stem/progenitor cells during embryonic development
11:25	*Chaya Kalcheim* The dynamics of dorsal neural tube development: from Neural Crest to definitive Roof Plate
11:50 – 12:55	**Stem cells, organoids and disease (chair: Lazaro Centanin)**
11:50	*Jürgen Knoblich* Cerebral organoids: modelling human brain development and tumorigenesis in stem cell derived 3D culture
12:15	*Prisca Liberali* Self-organization and symmetry breaking in intestinal organoid development
12:40	*Erika Tsingos* Taking turns: Quiescent and active stem cells coexist in homeostatic growth of the retinal pigment epithelium of medaka
12:55	**Lunch**

Monday, February 18th, (afternoon session)

Big lecture hall (C1)		Small lecture hall (C2)	
14:30 – 15:50	**Single cell approaches (chair: Elly Tanaka)**	**14:30 – 15:50**	**Biophysics of development (chair: Patrick Müller)**
14:30	*Barbara Treutlein* Dissecting human and chimpanzee cerebral organoids using single-cell transcriptomics	14:30	*Naama Barkai* Designing a robust biological timer
14:55	*Naomi Habib* From single cells to landscapes of brain regeneration and degeneration	14:55	*Stephan Grill* Integrin-mediated attachment of the blastoderm to the vitelline envelope impacts gastrulation in Tribolium castaneum
15:20	*Omri Wurtzel* A foxF regulatory program specifies planarian muscle formation revealed by single cell analysis	15:20	*Albert Thommen* Physiological basis of metabolic rate scaling in planarian flatworms
15:35	*Alison Cole* Development and Homeostasis in a sea anemone: a multi-faceted approach to characterize Cnidarian stem cells	15:35	*Adi Salzberg* Alteration in ECM composition affects sensory organ mechanics and function
15:50	**Coffee break**		
16:20 – 18:30	**Poster session I (even numbers)** **area around the big lecture hall**	**16:20 – 16:50**	**New techniques (chair: Alexander Klimovich)**
		16:20	*Florian Raible* A combined, versatile depigmentation and clearing method (DEEP-C) for studying animal nervous systems across scales
		16:30	*Wouter Masselink* Broad applicability of a streamlined Ethyl Cinnamate-based clearing procedure
		16:40	*Tinatini Tavhelidse* Efficient single-copy HDR by 5′ modified long dsDNA donors

Big lecture hall (C1)	
18:30	**IsSDB prize talk *Howard Cedar* (chair: Chaya Kalcheim)** **DNA methylation and the unending process of development**
19:15	**Dinner on your own / invited speakers dinner**

Tuesday, February 19th (morning session)

Big lecture hall (C1)	
09:00 – 10:45	**Genomic approaches to development (chair: Oleg Simakov)**
09:00	*Kikue Tachibana-Konwalski* New insights into the causes of egg aneuploidy at advanced maternal age
09:25	*Enrico Coen* Resolving Conflicts: The Genetic Control of Plant Morphogenesis
09:50	*Stephan Mundlos* 3D chromatin conformation of Pitx1 locus defines forelimb vs hind limb identity
10:15	*Anna Kögler* Light-controlled perturbation of transcription factor function during embryogenesis
10:30	*Michael Borg* Paternal resetting of H3K27me3-silenced states primes early plant development
10:45	**Coffee break**
11:15 – 12:35	**Regeneration (chair: Monika Hassel)**
11:15	*Kerstin Bartscherer* Regeneration initiation – from planarians to African spiny mice
11:40	*Eldad Tzahor* Signaling Mechanisms in Heart Regeneration
12:05	*Christian Lange* Thyroid hormone signaling controls adult brain regeneration in zebrafish
12:20	*Prayag Murawala* Single-cell analysis uncovers convergence of cell identities during axolotl limb regeneration
12:35	**Lunch**

Tuesday, February 19th, (afternoon session)

Big lecture hall (C1)	
13:30	**Society meeting GfE**
14:15	**GfE PhD Prize award: *Jaydeep Sidhaye*** **Cellular dynamics in zebrafish optic cup morphogenesis**

Big lecture hall (C1)		Small lecture hall (C2)	
14:30 – 16:00	**Polarity and axis formation (chair: Urs Schmidt-Ott)**	**14:30 – 16:00**	**Germline / early cleavages (chair Tamar Lotan)**
14:30	*Elisabeth Knust* Crosstalk between the Drosophila Crumbs polarity complex and the apical cytocortex orchestrates epithelial polarity	14:30	*Hila Toledano* Draper isoforms in cyst cells regulate phagocytosis and aging of germ cell debris
14:55	*Marja Timmermans* Pattern formation by mobile morphogen-like small RNA signals	14:55	*Maria Torres Padilla* Epigenetic mechanisms in early mammalian development: impact of heterochromatin dynamics
15:20	*Gregor Bucher* Double abdomen in a short germ insect: Zygotic control of axis formation revealed in the beetle Tribolium castaneum	15:20	*Roland Dosch* Functional equivalence of the zebrafish germ plasm organizer Bucky ball with the unrelated Drosophila Oskar
15:45	*Jörg Grosshans* The emergence of the subapical domain depends on polarization of cortical actin	15:45	*Andrea Pauli* Bouncer and SPACA4 - small proteins with big roles
16:00	**Coffee break**		
16:30 – 18:30	**Poster session II (odd numbers)** **area around the big lecture hall**		

Big lecture hall (C1)	
18:30	**GfE Klaus Sander Prize talk *Herbert Jäckle* (chair: Uli Technau)** **From Gradients to Disease, from Fly to Man**
19:15	**Conference dinner at the City Hall**

Wednesday, February 20th

Big lecture hall (C1)	
09:00 – 10:40	**Organogenesis (chair: Andrea Vortkamp)**
09:00	**Keynote: *Olivier Pourquié*** **Deconstructing the human segmentation clock *in vitro***
09:35	*Ruth Ashery-Padan* Investigating the transitions from multipotent precursors to stably differentiated cell types of the eye
10:00	*Miltos Tsiantis* The genetic basis for diversification of leaf form: from understanding to reconstructing
10:25	*Wiebke Herzog* Wnt7-catenin signaling regulates VE-cadherin-mediated anastomosis of brain capillaries by counteracting S1pr1 signaling
10:40	**Coffee break**
11:10 – 12:30	**EvoDevo and emerging models (chair: Smadar Ben-Tabou de-Leon)**
11:10	*Kai Rathje* Bacteria interactions cause tumorigenesis in Hydra
11:25	*Stefan Schulte-Merker* Different ways to make a spine - notochord sheath cells, but not the sclerotome, drive axial spine metamerisation in zebrafish
11:40	*Matt Gibson* Functional interrogation of an axial Hox code in the sea anemone, Nematostella vectensis
12:05	*Sabine Zachgo* Marchantia polymorpha: Insight into land plant evolution form a liverwort perspective
12:30	**Poster Prize and concluding remarks**
13:00	**End of the meeting. Do not forget your posters!**

Numbers, presenting authors and titles of the posters

A | Developmental Cell Biology

A01	*PILLEMER Graciela*	Retinoic acid signaling reduction recapitulates the effects of alcohol on embryo size
A02	*YASEEN-BADARNE Wesal*	Fibroblast fusion at the muscle fibers termini facilitates muscle-tendon junction development.
A03	*PUKHLYAKOVA Ekaterina*	Cadherin switch marks germ layer formation in the diploblastic sea anemone Nematostella vectensis
A04	*URBANSKY Silvia*	Revealing the mechanism that optimized the rate of body axis elongation in flies.
A05	*RUHLAND Naima*	From extension to infolding: uncovering the role of MyoII in the origin of novel tissue behaviour
A06	*TÖPFER Uwe*	Characterization of Drosophila Nidogen/entactin reveals roles in basement membrane stability, barrier function and nervous system patterning
A07	*SOKOLOVA Natalia*	Neuroepithelial flow during optic cup formation in medaka and molecules influencing it
A08	*EASA Yathreb*	The role of Fat4-Dchs1 interactions in regulating downstream Hippo signaling
A09	*MAMISTVALOV Rose*	Elucidating the role of endothelial cadherins on Notch signaling during angiogenesis.
A10	*EAFERGAN Natanel*	Towards quantitative analysis of the Notch transcriptional response
A11	*HIRSCHHÄUSER Alexander*	The role of CK2 on the activity of the WAVE regulatory complex controlling cell shape and cell migration of Drosophila macrophages
A12	*COHEN Yonatan*	The wave complex regulates epidermal proliferation and morphogenesis
A13	*SUN Xin*	Epithelial-Mesenchymal Transition of the Embryonic Epicardial Cells is Regulated by Extracellular Matrix Protein Agrin
A14	*BAGAEVA Tatiana*	RTK/MAPK/ERK- signaling regulates the behavior of the endodermal cells during gastrulation in the sea anemone Nematostella
A15	*KNABL Paul*	Identification of a novel modulator of BMP signaling in a sea anemone
A16	*KISHI Kasumi*	Regulation of notochord size and shape in mouse development
A17	*FLORES-BENITEZ David*	Crumbs organizes the apical transport machinery by negatively regulating Pten in Drosophila larval salivary glands
A18		
A19	*GÜCÜM Sevinç*	Disease Modeling in O.latipes: Using CRISPR/Cas9 to Generate N-Glycosylation Hypomorphs

A20	*RIOS Daniel*	The endocytic pathway balances membrane organization during subcellular tube formation
A21	*LÜBKE Stefanie*	Signaling transduction during Drosophila myoblast fusion
A22	*BAJUR Anna*	Dynamic pool of Crumbs in the Drosophila embryonic epidermis is regulated by the actomyosin cortex and endocytosis
A23	*BEN-HUR Sharon*	MVBs function to degrade sperm mitochondria after fertilization in Drosophila
A24	*TVERIAKHINA Lena*	The extracellular domains of DLL1 and DLL4 mediate differential receptor selectivity in vitro and divergent ligand function in vivo
A25	*WACHNER Stephanie*	Tissue invasion of Drosophila embryonic macrophages – BMP-activation leads the way
A26	*RUS Stefanie*	The roles of BMP and Wnt signalling in dorsal neural tube development
A27	*HOLZ Oliver*	Separate signaling pathways control cortical and basal actomyosin organization in epitheliomuscular cells in Hydra
A28	*HASSEL Monika*	Tools to detect Rho activity and PIP- signaling in Hydra
A29	*SAPOZHNIKOV Lena*	Irradiation-induced cell migration (ICM) involves EMT and cell migration, the latter of which is regulated by caspases
A30	*BRAUN Tslil*	Role of caspases during spermatid terminal differentiation in Drosophila
A31	*NGUYEN Hong Nhung*	The Tip60 chromatin remodeling complex is involved in the maintenance of adult midgut precursor cells in the Drosophila midgut
A32	*DRAUT Heidrun*	The role of retinoic acid in the development of zebrafish pelvic fins
A33	*HOF Silvana*	Cell competition and innate immunity signaling in the Drosophila testis niche
A34	*FINDEIS Daniel*	Cell focussing: Pattern formation by dancing cells
A35	*SHCHERBATA Halyna*	Stress-dependent regulation of a liquid droplet component, Rbfox1
A36	*ELKOUBY Yaniv*	A centrosome organizing center coordinates early oogenesis in zebrafish
A37	*ZILOVA Lucie*	Emergence of retinal stem cells in fish retina
A38	*D'ANGELO Valentina*	Regulation of the MAST kinase Drop out in D. melanogaster
A39	*VALOSKOVA Katarina*	A highly conserved MFS regulates O-GalNAc glycosylation to optimize Drosophila macrophage migration and tissue invasion
A40	*BISCHOFF Marcus*	Studying a dynamic contractile actomyosin network during Drosophila abdominal morphogenesis
A41	*EMTENANI Shamsi*	Investigating the function of a novel nuclear protein in tissue penetration of Drosophila melanogaster macrophages

A42	*CORNEAN Alex*	Deficient Protein O-mannosylation Affects Signalling Pathways In Vertebrates
A43	*BECKER Clara*	Progenitor amplification is the rate-limiting step in determining retinal size in medaka
A44	*GERLITZ Offer*	Study of Human Ovarian Development & Dysgenesis Mechanisms in a Drosophila Model
A45	*GUTIERREZ PEREZ Paula*	Exploring the functional conservation of a deeply conserved animal microRNA
A46	*BOCANEGRA-MORENO Laura*	Epithelial rearrangement dynamics during mouse neural tube development
A47	*SCHEJTER Eyal*	Balanced Rho activation and inhibition regulates exocytosis by large secretory vesicles
A48	*LISCHIK Colin Q.*	Elevated Wnt-signaling leads to differentiation and restriction of potency of single stem and progenitor cells in vivo
A49	*QUINONEZ Claudia*	Elucidating the functional role of Hif during brain development

B | Neural Development

B01	*HUMMEL Thomas*	Breaking Symmetry: Glial signaling induces lateralization of a Drosophila central brain circuit
B02	*THUMBERGER Thomas*	Subfunctionalization of rx genes in Medaka
B03	*KAUR Rashmit*	Split-Brain in a fly: Developmental mechanism underlying bi-lateral nervous system organization
B04	*LOTAN Tamar*	GABAB Signaling regulates early development and neurogenesis in the sea anemone Nematostella vectensis
B05	*RUMPF Sebastian*	Coordinated cytoskeleton disassembly pathways during dendrite pruning in Drosophila
B06	*YARON Avraham*	Elucidating the multi-functionality of Semaphorin3A/Plexin-A4 signaling
B07	*VARSHAVSKY Stas*	Role of FGF signaling in regulating the stem/differentiation state of hindbrain boundary cells
B08	*KUZMICZ-KOWALSKA Katarzyna*	Regulation of neural tube growth by the morphogens Shh and BMP
B09	*DAVID Charles N.*	Immunostaining for neuropeptides identifies the neural circuits controlling behavior in Hydra described by Dupre and Yuste (2017)
B10	*WOLTERHOFF Neele*	PP2A-29B regulates actin disassembly during dendrite pruning in Drosophila
B11	*HOLLEMANN Thomas*	TRIM29, a E3-ligase involved in neural tube closure and neural crest specification

C | Stem cells, organoids and disease

C01	*GROMBERG Elena*	Mechanism of Floor Plate Induction in the Neuroepithelial Organoids
C02	*COSACAK Mehmet Ilyas*	Single cell analysis reveals a novel mechanism controlling neural stem cell plasticity in Alzheimer's disease model of adult zebrafish
C03	*LAYER Paul*	Far advanced network formation in retinal spheroids from chick embryo depends on cholinergic and glutamatergic differentiation
C04	*SCHLESINGER Sharon*	Genome scale mapping of histone H3.3 turnover rate in mouse embryonic stem cells and during early differentiation
C05	*LUST Katharina*	Ependymoglia behavior during postembryonic growth of the axolotl telencephalon
C06	*BÖKEL Christian*	Niche signalling in the Drosophila testis - cell fate choice or micromanagement?
C07	*BAJOGHLI Baubak*	T-cell lineage decision is determined by temporal residency in specialized thymic microenvironments
C08	*KLEIN Sabine*	Efficiency of in vivo Transfection of Primordial Germ Cells in Chickens at two Stages of Embryonic Development
C09	*KRAMMER Teresa*	Unveiling the molecular mechanisms of neural crest migration and formation of dorsal root ganglia using three-dimensional neural tube organoids
C10	*DIVVELA Satya Srirama Karthik*	Atoh8 a novel regulator of TGF-ß signaling.
C11	*NACHMAN Iftach*	Prediction and control of symmetry breaking in embryoid bodies by environment and signal integration
C12	*SHOSHKES CARMEL Michal*	Subepithelial telocytes constitute the intestinal stem cell niche

D | Single cell approaches

D01	*ZIMMERMANN Bob*	Ancient animal genome architecture reflects cell type identities
D02	*WÖLK Michaela*	Single-cell transcriptome analysis elucidates similarities and differences between the population of intestinal stem cells and their progenitors
D03	*BAGERITZ Josephine*	Gene expression atlas of a developing tissue by single cell expression correlation analysis
D04	*STEGER Julia*	Molecular profiling of cells in the sea anemone Nematostella vectensis

E | Biophysics of Development

E01	*MICHEL Marcus*	Increase in mechanical tension and E-Cadherin mobility facilitate cell extrusion in Drosophila epithelia
E02	*EL-SHERIF Ezzat*	Speed regulation of genetic cascades allows for evolvability and robustness in the body plan specification of insects
E03	*SAVIR Yonatan*	The role of cellular replicative lifespan and stem cell dynamics on corneal epithelium homeostasis and pattern formation
E04	*AKHMANOVA Maria*	Modeling the mechanics of an epithelial sheet deformed by a migrating cell
E05	*BISCHOFF Maik Chrsitian*	Regulators of the migration of Drosophila testis nascent myotubes
E06	*ROGERS Katherine*	Mechanisms underlying the spatiotemporal organization of BMP-dependent target genes

F | New Techniques

F02	*RAIBLE Florian*	A combined, versatile depigmentation and clearing method (DEEP-C) for studying animal nervous systems across scales
F04	*MASSELINK Wouter*	Broad applicability of a streamlined Ethyl Cinnamate-based clearing procedure (2Eci)
F06	*TAVHELIDSE Tinatini*	Efficient single-copy HDR by 5’ modified long dsDNA donors

G | Genomic and system approaches of development

G01	*SCHMIDBAUR Hannah*	Did genome re-organization drive the formation of new regulatory units in cephalopods?
G02	*MEADOWS Lisa*	Resources and Services at the Vienna Drosophila Resource Center (VDRC)
G03	*GULERAY Naz*	Investigation of Genetic Causes in a Developmental Disorder: Oculoauriculovertebral Spectrum
G04	*ZHOU Qi*	Evolution and Development of Avian Limbs and Digits
G05	*HAMMOUDA Omar*	A Change of Heart: Medaka as a model for Human Cardio-Vascular Diseases & GWAS

H | Regeneration

H01	*SCHWARZER Simone*	Neurogenesis in the zebrafish inner ear: a NeuroD/Nestin-positive progenitor pool as a source of new neurons during growth, homeostasis and regeneration.
H02	*BERTEMES Philip*	No head regeneration here: regeneration capacity and stem-cell dynamics of Theama mediterranea (Polycladida, Platyhelminthes).

H03	*MATOSEVICH-LEPAR Rotem*	A dynamic pattern of auxin sources orchestrates root regeneration
H04	*LOU Wilson Pak-Kin*	The dynamics of neural stem cells and neurons in axolotl spinal cord regeneration
H05	*HEIGWER Jana*	Development of a high-content in vivo screening platform using automated laser-assisted photoablation in an acute kidney injury model in zebrafish
H06	*GROSSER Lidia*	Testing regeneration potential of mouse skin fibroblasts
H07	*LIN Tzi-Yang*	Testing the regenerative potential of limb blastema cells in post-metamorphic Xenopus laevis
H08	*POLIKARPOVA Anastasia*	Skin and muscle connective tissue cells in bone fracture healing in axolotl
H09	*STOCKINGER Alexander*	A cellular profiling approach to dissect the regulation of regeneration in a marine annelid
H10	*LANGE Christian*	Single cell sequencing reveals diversity of newborn neurons in the adult zebrafish brain

I | Polarity and axis formation

I01	*PLÖGER Ruben*	The secreted tyrosine kinase PKDCC and the Wnt pathway during gastrulation in the rabbit embryo
I02	*PIEPER Tobias Karl*	An early chick embryo culture device for studying molecular and morphological left-right patterning
I03	*SASTRADIHARDJA Tania*	Three pathways regulate spindle directions in three dimensions: a new function for FGF in the C. elegans embryo
I04	*STOCK Jessica*	Toddler signaling is essential for cell polarization during gastrulation

J | Germline and early cleavages

J01	*MÜLLER Arno*	The MAST Kinase Drop out controls Dynein-mediated transport and polarised membrane growth in Drosophila cellularisation
J02	*ECKMANN Christian*	The TRIM32-related ubiquitin ligase, GRIF-1, reprograms primordial germ cells to ensure germ cell immortality

K | Organogenesis

K01	*PU Qin*	Fgf8 mRNA and Protein relay for a long-range FGF8 concentration gradient
K02	*STRICKER Sigmar*	The transcription factor Osr1 marks embryonic progenitors of brown adipose tissue (BAT) and adult adipose stem/precursor cells (ASPCs), and is essential for BAT formation
K03	*VORTKAMP Andrea*	Epigenetic regulation of chondrocyte differentiation

K04	*BREUER Marlen*	Caveolin 1a is required for muscular and neuronal integrity in Xenopus laevis
K05	*LIEBENSTEIN Thomas*	Outgrowth of zebrafish gill filaments is regulated by an interplay between the RA- and BMP-signalling pathways.
K06	*KIMMEL Robin*	Dynamic Cell Motility in Pancreatic Islet Morphogenesis
K07	*KISPERT Andreas*	DIVIDING THE EARLY METANEPHRIC FIELD - THE ROLE OF Tbx18 IN URETER SPECIFICATION
K08	*BARTLE Jakob*	Drosophila Twist - a myogenic switch?
K09	*GROß Karen*	Same same, but different - the anterior lateral line
K10	*ROSE Marcel*	Twist affects lineage reprogramming and transdifferentiation of syncytial alary muscles during Drosophila metamorphosis
K11	*DREES Leonard*	Tracheal apical extracellular matrix maturation in Drosophila melanogaster is mediated by evolutionary conserved serine proteases
K12	*KALEV-ALTMAN Rotem*	Matrix metalloproteases 2 and 9 are fundamental for neural crest and skeletal development in the mouse embryo
K13	*MUSTER Helena*	The zinc finger transcription factor DBcl11/CG9650 is required for proper somatic and cardiac muscle development in Drosophila
K14	*DOMSCH Katrin*	The Hox Transcription Factor Ubx stabilizes Lineage Commitment by Suppressing Cellular Plasticity
K15	*SCHAUB Christoph*	Org-1 drives direct muscle lineage reprogramming through negative regulation of Hippo signalling

L | EvoDevo

L01	*DENNER Andreas*	Characterization of putative stem cells in Nematostella vectensis
L02	*DNYANSAGAR Rohit*	The role of brachyury in “mesoderm” determination in metazoans
L03	*HASEL Eva*	Functional diversification of interleukin-1 during vertebrate evolution
L04	*LEMKE Steffen*	Release from yolk sac is required for extraembryonic envelope formation in the scuttle fly Megaselia abdita
L05	*MORAN Yehu*	The evolution of the microRNA pathway and its essential role in cnidarian development
L06	*KLINGLER Martin*	Supernumerary segments forming in a short germ insect through repair of a disrupted initial pattern suggest autonomous patterning capabilities downstream of the segmentation clock
L07	*KAUL-STREHLOW Sabrina*	The evolution of animal muscle cell types: insights from the diploblast Nematostella vectensis
L08	*TSIKOLIA Nikoloz*	Ancestral character of primate gastrulation.
L09	*GIRSTMAIR Johannes*	Evolution of Life Cycles in Polyclad Flatworms (Platyhelminthes)

L10	*GILDOR Tsvia*	Comparative studies of expression kinetics reveal developmental constraints and plasticity
L11	*MATT Ann-Sophie*	Uncovering the role of taxon-restricted genes in the neurons of the freshwater polyp Hydra.
L12	*NOESKE Viola*	A new gene buds out and takes over an essential role in tall blastoderm formation in higher flies
L13	*KLIMOVICH Alexander*	Ancestral complexity and function of the nervous system: insights from single-cell transcriptomics in Hydra
L14	*GAT Uri*	A New Gene Family of short Collagens in the Development and Regeneration of the Sea Anemone Nematostella vectensis
L15	*SELEIT Ali*	Tinkering with Development: The lateral line as a model to study pattern formation and evolution
L16	*TAUBENHEIM Jan*	Extrinsic and intrinsic factors regulate body size in Hydra by conserved signaling pathways
L17	*HE Jinru*	Towards understanding an ultimately simple metaorganism: impact of symbiotic microbes on developmental processes of Hydra
L18	*BEN-TABOU DE-LEON Smadar*	Echinoderms adapted the VEGF-driven vascularization program to generate calcite skeletons

Abstracts of talks (in order of presentation)

Developmental Cell Biology

Evolution of developmental cell fusion

Benjamin Podbilewicz
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Cell-cell fusion is vital for fertilization and organogenesis. In *C. elegans*, one third of all the somatic nuclei are within syncytia that form by cell-cell fusion in the epidermis and organs in the digestive, reproductive and excretory systems. EFF-1 and AFF-1 are type I membrane glycoproteins that fuse and sculpt cells during development. When expressed ectopically, these essential fusion proteins (fusogens) induce fusion between cultured cells as well as between enveloped viruses and cells, provided that they are present in both fusing membranes (bilateral). EFF-1 is structurally related to class II viral fusion proteins and to HAP2(GCS1) proteins that are essential for gamete fusion in plants and protists. We found that, similarly to EFF-1, *Arabidopsis* HAP2 bilateral expression can induce fusion in mammalian cells as well as between pseudotyped vesicular stomatitis virus and cells. Furthermore, virus-cell fusion can be mediated by HAP2–EFF-1 heterotypic trans-interactions. We named this superfamily of sexual, somatic and viral fusion proteins Fusexins: FUSion proteins essential for sexual reproduction and EXoplasmic merger of plasma membranes. We will show data regarding the evolution of fusexins and their diversity of functions in gamete and somatic cell fusions during development. Fusexins present an intriguing evolutionary history that generates questions like: were fusexins captured by the viruses from ancient hosts or were they viral inventions captured by early eukaryotes to facilitate sexual reproduction? We will present new evidence suggesting that fusexins were an archaeal invention, and are currently working on cellular, genetic and structural characterization of archaeal fusexins. Our hypothesis is that fusexins originated in ancestral archaea to mediate sexual cell-cell fusion, later incorporated by unicellular protists for mating and lastly, adopted by animals for fusion of somatic cells during morphogenesis. We will discuss how fusexins in archaea may have also contributed to the origin of the first eukaryote.

Evolution of efficient root gravitropism during conquest of land by plants

Jiri Friml, Yuzhou Zhang
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The ultimate conquest of the dry land by plants started at the Late Devonian period (about 382.7 million years ago) when the early diverging seed plants underwent dramatic evolutionary radiations and became the dominant group in most habitats, including the dry environment. The evolution of seed organs has been thought to be the key evolutionary innovation for the adaption to dry land since it allowed plants to break their dependence on water for reproduction and embryo development. Another, largely overlooked key adaption during colonization of land by plants is the efficient gravitropic growth of roots, which enabled to reach water and nutrients and firmly anchor plants in ground.

Here, we provide first insights into evolution of root gravitropic mechanism of the seed plants. Architectural innovation with root tip-constrained gravity perception along with a novel, shootward transport route for the phytohormone auxin appeared only at the onset of the seed plant advancement. Interspecies complementation and protein domain swapping revealed positive and later purifying selection-driven, two-step functional innovation within the family of PIN auxin transporters leading to evolution of gravitropism-specific PIN2. The PIN2 unique apical, subcellular localization was the major evolutionary invention that enabled connecting the anatomically separated place of gravity perception and growth response by the mobile auxin signal. Because of these evolutionary innovations of not only above-ground but also below-ground parts of the seed plants, they were able to overcome the past environmental restrictions and successfully colonize the dry land where their predecessor failed to arrive.

Uncovering the development and maturation of vertebrate heart valves at single cell resolution

Felix Gunawan, Alessandra Gentile, Didier Stainier, Anabela Bensimon-Brito
Max Planck Institute for Heart and Lung Research, Bad Nauheim, GER

The heart serves an indispensable function of pumping blood that delivers nutrients and oxygen throughout the body. Heart valve leaflets, which are cardiac structures in the junctions between heart chambers, close the lumen to prevent retrograde blood flow and ensure optimal circulation. They comprise valve interstitial cells (VICs), a specialized group of cells that secrete extracellular matrix (ECM) that confers biomechanical strength, and valve endocardial cells (VECs), the outer lining of the leaflets. It is challenging to analyze cellular and molecular dynamics that drive valvulogenesis *in vivo*, in particular the differentiation and maturation of the VICs which arise during later valve development. A significant proportion of hereditary human congenital heart defects stems from defective heart valves, further highlighting the need to elucidate the mechanisms behind valve development.

Using the zebrafish atrioventricular (AV) heart valve as a model system, we characterized valve development at single cell resolution from the embryonic to the adult stages. In the embryonic heart, collective migration of VECs in the AV canal establishes a pre-valvular leaflet structure. By specifically labelling the migrating VECs through photoconversion, we observed that they become the first VICs as they undergo endothelial-to-mesenchymal transition, invade the VEC layers, and downregulate their endocardial identity. Lineage tracing experiments show that this initial invasion of endocardial cells is followed by a smaller contribution of neural crest cells, which persist throughout development. Subsequently, a period of peak VIC proliferation at the juvenile stage expands the VIC layer and leads to further valve leaflet elongation into the cardiac lumen. This increase in cell number is accompanied by maturation of the ECM components and molecular stratification of the VICs.

Using reporter lines that monitor the expression of the NFκB-related transcription factor Nfatc1, as well as activation of Wnt and Notch pathways, we uncovered molecular heterogeneity within the valve cell population. In the developing VICs, Nfatc1 and the Wnt pathway are highly expressed, whereas the Notch pathway is downregulated. Using CRISPR-mediated mutagenesis, we mutated the *nfatc1* gene and discovered a requirement for this transcription factor in the establishment of Wnt-positive VICs in the developing heart valve leaflets. Mutations in *nfatc1* results in reduction of the number of VICs and severe valve malformations, leading to retrograde blood flow.

In summary, our study uncovers vertebrate heart valve development at single cell resolution, and establishes the molecular signature of VIC establishment in developing valves. Using heat shock transgenic lines, we are further investigating the effects of modulating Nfatc1, and Wnt and Notch pathways in the later stages of valve leaflet elongation and proliferation. We are also characterizing the transcriptomic profiles of Nfatc1-positive, valve-forming cells and Nfatc1-negative, non-valve-forming cardiac cells purified through fluorescence activated cell sorting to identify novel regulators of valve development.

A novel hedgehog-regulated molecular module that shapes epithelial cell and tissue morphogenesis to position the ventral embryonic midline

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Much recent process has been made towards understanding how epithelial morphogenesis is shaped by intra-epithelial processes such as cortical contractility, cell polarity, and cell adhesion;however, much less is known regarding how these cellular processes might be regulated and coordinated across the tissue or embryo by extra-epithelial signals. The current study uses the chick embryo coelomic epithelium (CE) as a model system for studying epithelial morphogenesis in an *in vivo* context. During embryonic development, the CE's of the two sides of the embryo converge medially, generating the dorsal mesentery (DM), which normally becomes located in the embryonic midline. We find that during convergence, CE cells transition from a densely packed, pseudostratified columnar morphology to a flatter, less densely packed arrangement. Hedgehog (Hh) signaling from the adjacent endoderm modulates the shape of CE cells and regulates CE convergence towards the midline. Bilaterally unbalanced hh signaling in the two CE's causes asymmetric convergence of the CE's and mis-positioning of the DM such that it becomes misaligned with the general body midline axis. Using RNAseq and functional studies, Sec5 (ExoC2), a component of the exocyst complex, and RhoU (Wrch-1), a small GTPase, are identified as hedgehog-regulated genes that modulate epithelial cell morphology and CE convergence, at least in part through their effects on cellular N-Cadherin and apical actin distribution, respectively. These results provide a molecular mechanism linking extra-epithelial Hh signaling, CE cell morphogenesis, and DM midline positioning, thus providing a model for how transcellular signaling can modulate specific intracellular processes to shape tissue morphogenesis.

Measuring molecular tension at developing muscle attachment sites

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During muscle development, muscle cells establish integrin-mediated attachments to tendon cells that allow the generation of mechanical tension across developing muscle fibers. This tension has been demonstrated to be important for myofibrillogenesis and the formation of regularly spaced sarcomeres within myofibrils. However, to date, tension at the molecular level cannot be directly measured in the living organism during muscle development.

Therefore, we adapted a Förster resonance energy transfer (FRET)-based tension sensor from cell culture and introduced it into the *Drosophila* genome by CRISPR/Cas9-mediated genome editing. By inserting the tension sensor module into the endogenous locus of the integrin adaptor protein Talin, that localizes to muscle attachment sites, we ensure proper expression levels and timing in all tissues, including the indirect flight muscles. The flies generated this way are viable and able to fly, showing that the Talin tension sensor fusion protein is fully functional and allows the flight muscles to work properly.

We established a protocol for fluorescence lifetime imaging (FLIM) and data analysis, which enables us to measure FRET in a reproducible manner in developing muscles of living pupae. We tested three different sensor modules to identify the best-suited sensor for the range of forces present in the muscle tendon system. We then applied this sensor to determine how molecular tension across Talin at muscle attachment sites changes during muscle attachment formation and maturation, the latter coinciding with myofibrillogenesis, in the living organism.

We are now quantifying the effects of genetic perturbations on tension levels and myofibrillogenesis during muscle development. Together, this allows us to study how force is transduced molecularly at muscle attachment sites, and thus provides insights into the molecular mechanism how tension build-up and myofibrillogenesis are functionally linked during muscle morphogenesis.

Intra-organ communication within the developing zebrafish heart involves mechanical coupling of myocardium and endocardium

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Over the course of development, intra-organ communication guides morphogenetic processes that are essential for an organ to carry out complex physiological functions. In the heart, the growth of the myocardium is tightly coupled to that of the endocardium, a specialized endothelial tissue that lines the interior of the heart. Several molecular pathways have been implicated in the communication between these tissues including secreted factors such as Wnts, BMPs, FGFs, or components of the extracellular matrix, and cell-cell communication via the Notch, ErbB2, or Ephrin signaling pathways. Yet, it is unknown which modes of communication coordinate the growth of the endocardium with that of the myocardium.

In zebrafish, endocardial chambers grow by proliferation with neither accretion of cells from external sources, nor from cellular intermingling over the chamber boundary at the atrioventricular canal. In striking contrast, during the process of cardiac ballooning at 30-54 hours post fertilization (hpf), the myocardium grows mostly through an accretion of cells to the chamber poles and due to cell size increases. In this study, we show that mechanical coupling is one means of communication between the two tissues and coordinates cardiac chamber growth of endocardium and myocardium. We used two different genetic conditions that cause an exaggerated expansion of myocardial atrial and a reduction of ventricular chamber dimensions. Comparing these two extreme pathophysiological conditions to WT revealed that an increased expansion of myocardial atrial chamber dimensions is compensated by increased endocardial proliferation and cell numbers. We find that an increased expansion of the atrial chamber volume generates higher junctional forces within endocardial cell membranes. This leads to biomechanical signaling involving the endothelial-specific adherens junction protein Cadherin-5 (VE-cadherin), triggering nuclear localization of the Hippo pathway transcriptional regulators Yap1/TAZ and endocardial proliferation. Our work suggests that the growth of the endocardium is a direct result of cardiac chamber volume expansion and ends when the tension on the tissue is relaxed.

The two mitofusin genes are functionally interchangeable, but both are required for the full fusion of the *Drosophila* spermatid mitochondria

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Mitochondria are highly dynamic organelles constantly undergoing fission and fusion. This dynamics is important for several cellular processes including, cell survival, proliferation, and migration. Mitochondrial fusion involves the fusion of both inner and outer membranes, mediated by two conserved protein families. The fusion of the inner membrane requires the dynamin family GTPase, OPA1, while fusion of the outer membrane requires another GTPase, Mitofusin. Interestingly, whereas mitochondrial fusion usually forms a branched tubular network in most cell types, in the *Drosophila* post-meiotic round spermatids, all the cell mitochondria aggregate and fuse to form a giant oval mitochondrion called Nebenkern. Both the significance of this unique mitochondrial formation for sperm development and function, and the molecular mechanisms underlying this phenomenon are largely unclear. Although *fuzzy onions* (*fzo*), which is required for Nebenkern formation, is the first mitochondrial fusion gene identified in any organism, we still do not know how this specific Mitofusin homolog and its somatic paralog Marf promote such distinct mitochondrial formations in the soma and the male germ cells.

To address this question, we first performed rescue studies to test the ability of *Marf* to compensate for the loss of *fzo*. This analysis revealed that despite sharing only around 60% similarity in their protein sequence, their function is highly similar if not identical. We next examined *opa1*, and showed that it is also required for Nebenkern formation.

To identify possible factors that might uniquely contribute to Nebenkern formation, we carried out a candidate screen for genes involved in this process. Using RNAi lines to systematically knockdown several genes 228 encoding mitochondrially targeted proteins and cytoskeleton-related proteins. We identified 4 poorly studied genes in *Drosophila* that are essential for Nebenkern fusion, 2 of which encode the homologs of two mammalian proteins knowns to be involved in Opa1 processing, whereas the other 2 are members of the Heat Shock protein family, likely involved in the correct folding and targeting of mitochondrial proteins.

Therefore, our analysis suggests that the canonical fusion machinery is essential for Nebenkern formation. The difference between mitochondrial fusion in the soma and in the germ cells might lay in other factors, such as factors responsible for the aggregation of the mitochondria in the male germ cells and/or differences in the distribution of the proteins that mediate the fusion on the mitochondrial membrane surface.

Dynamics of morphogen shuttling in the *Drosophila* embryo

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Establishment of morphogen gradients in the early *Drosophila* embryo is challenged by a diffusible extracellular milieu, and rapid nuclear divisions that occur at the same time. The Spaetzle gradient, activating the Toll pathway, patterns the embryo along the dorso-ventral axis. This gradient is generated by a process of ligand shuttling that concentrates the morphogen towards the ventral midline. As a dynamic readout for Toll pathway activation, we followed the generation of graded nuclear Dorsal protein in live embryos. We show that a sharp morphogen gradient is formed by extracellular, diffusion-based shuttling that progresses through several nuclear division cycles. Re-entry of Dorsal into the nuclei at each cycle refines the signaling output, by guiding graded accumulation of the zygotic *T48* transcript that drives patterned gastrulation. We conclude that diffusion-based ligand shuttling, coupled with dynamic readout, establishes a refined pattern within the diffusible environment of early embryos.

Neural Development

Time to get up: Awakening stem cells in the brain

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Neural stem cells can generate new neurons in the brain in response to a range of stimuli, including exercise, nutrition and injury. In this way stem cells meet the needs of the organism during growth and in response to damage. A key control point is the decision between stem cell quiescence and proliferation. Drosophila neural stem cells enter quiescence in late embryogenesis and are reactivated post-embryonically in response to nutrition. We found that feeding induces the expression of insulin-like peptides within the brain itself. We showed that insulin signalling is essential for the stem cells to exit quiescence and resume proliferation. Insulin signalling can also promote proliferation in vertebrate neural stem cells, suggesting that the mechanisms controlling stem cell reactivation may be conserved. We are investigating the systemic and local signals that regulate neural stem cell quiescence and reactivation. Understanding the signals that instruct stem cells to produce new neurons at will raises the prospect of future therapies for brain repair after damage or neurodegenerative disease.

Development of the *Drosophila* blood-brain barrier

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The blood-brain barrier (BBB) is crucial to allow normal function of the nervous system. It is established early during development and needs to stay intact during the subsequent growth of the brain. In invertebrates, the occluding junctions of the BBB are established by septate junctions. In Drosophila, the subperineurial glia forms the BBB and during development these cells grow enormously and become polyploid, which is controlled by the N-end rule pathway component Öbek. During cell growth, septate junction strands must stay intact. We show that in the BBB septate junctions are preassembled in a folded manner during embryonic stages and are stretched out as the animal grows. Only little protein turnover is observed and only few new septate junction strands are generated during larval stages. The formation of long septate junction strands connecting the cell vertices requires the G-protein coupled receptor Moody. Interestingly, the impairment of junctional integrity can be compensated by extensively interdigitating cell-cell contacts, which resembles the main barrier mechanism found in many other animal clades. To further understand septate junction strand formation, we identified several novel septate junction proteins. One of the newly identified proteins, Undicht, acts in a non-cell autonomous manner and is able to coordinate septate junction formation within a tissue.

Coordination of progenitor specification and growth in the developing spinal cord

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As the spinal cord grows during embryonic development, an elaborate pattern of molecularly distinct neuronal precursor cells forms along the DV axis. This pattern depends both on the dynamics of a morphogen-regulated gene regulatory network, and on tissue growth. We study how these processes are coordinated. Our data revealed that during the first day of mouse neural tube development, neural progenitors integrate signaling from opposing morphogen gradients to determine their identity. This happens via a mechanism equivalent to maximum likelihood decoding, which allows accurate assignment of position along the patterning axis and can account for the observed precision and shifts of pattern. During the subsequent developmental phase, cell-type specific regulation of differentiation rate, but not proliferation, elaborates the pattern.

Dynamic control of stemness and neurogenesis in neural proliferation zones of the larval zebrafish brain

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Proper growth of the vertebrate brain requires well balanced neural stem cell proliferation, progenitor plasticity, and neurogenesis. The larval zebrafish brain is an excellent model to study the regulation of these processes when neural proliferation zones develop from proliferating neuroepithelium, and later organize into stem cell niche-like structures. While fundamental mechanisms controlling these processes have been discovered, including the roles of SoxB1 transcription factors in neural stem cells, of Delta/Notch signaling and HES/HER bHLH transcription factors in inhibiting neurogenesis and maintaining neural stem cells, and of Ascl and other proneural factors in neural progenitors, the regulation of the different cell populations in vivo in stem and proliferation zones is not well understood.

The Hairy and enhancer of split (HES/HER) genes are crucial to inhibit neurogenesis and maintain stem cell pools. HES/HER expression itself may be regulated either in a Notch-signaling dependent or in a Notch-independent way. Zebrafish have at least two Notch-independent HER genes, *her6* and *her9* (homologs of mammalian Hes1), and several clusters of Notch-dependent HER genes, the *her2 - her15.1 - her15.2* cluster and the *her4.1-4.5 - her12* cluster (homologs of mammalian Hes5), which are all expressed in larval neural proliferation zones. Notch-independent HER genes are mostly expressed in the ventricular layer of Sox2-positive stem cells only, while Notch-dependent HER gene expression can also be detected in progenitors adjacent to ventricular stem cells. To dissect the contributions of Notch-dependent and -independent Her genes, we have used CRISPR/Cas9 genome editing to generate deletions of individual *her* genes and of whole *her* gene clusters. We generated composite mutant genetic strains to investigate neural development when either Notch-dependent or Notch-independent Her function, or both, were absent. Analysis of proliferation zones and stem cell niches revealed that Notch-independent Her genes can largely compensate loss of Notch-dependent Her genes, but not vice versa. Progenitor pools expressing proneural genes are affected predominantly in the *her6 / her9* double mutant embryos. We characterized a severe neurogenesis phenotype in the quadruple mutant for all four *Hes1*- and *Hes5*-type *her* genes and gene clusters studied.

We further generated fluorescent reporter lines for Sox2, Ascl1a, Neurog1, and Her6 expression to investigate the dynamics of their protein expression during transitions from the stem to progenitor cell compartments. Using a transgenic zebrafish line expressing an anti-PCNA chromobody (gift of P. Panza and C. Söllner), we correlate cell proliferation dynamics with proneural gene expression in vivo. We also obtained (Sox2) or generated (Her6) antibodies to validate their temporal expression profiles, and show that anti-Her6 immunofluorescence and fluorescence of the Her6 reporter generated by CRISPR/Cas9 mediated homologous knock-in into the endogenous *her6* locus correlate well. Using in vivo SPIM long-term imaging of proliferation zones, we reveal Her6 protein dynamics during transition from stem to progenitor zones in individual cells as well as coherent cell groups. We then used our genetic models to modulate Her, Sox2 and Ascl1 activities, and analyzed the effects on cellular composition as well as regulatory impact on stem cell and neurogenesis factor expression. We will present a model on how the dynamics of Notch-dependent and Notch-independent her gene expression may regulate stem cell states and progenitor plasticity in larval zebrafish neural proliferation zones.

Hindbrain boundaries- new sites of neural stem/progenitor cells during embryonic development

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Neural stem cells (NSCs) are self-renewing progenitors that line the embryonic neural-tube and generate all the central nervous system (CNS) via the process of neurogenesis. Although the main principles of neurogenesis are similar along the neural-tube, NSCs originating from spinal-cord or forebrain differ in their quiescence/proliferation state and exhaustion or persistence in designated niches throughout adulthood.

The brainstem is the ancient-most part of the brain. It is a key relay hub linking bilaterally the spinal-cord and supra-brain centers via extensive circuits that regulate breathing, vestibular, auditory and motor networks. During development, the brainstem originates from the hindbrain, which is subdivided into repetitive units, termed rhombomeres. Rhombomeres are separated from each other by well-defined boundaries, which share specialized molecular characteristics. Although existing in all vertebrates, the role of hindbrain boundaries is elusive. Moreover, it is not clear whether the development of hindbrain-NSCs reflects the spinal-cord or the upper brain ontogeny.

We found that hindbrain boundaries consist of pools of slow-proliferating cells that express the NSC markers Sox2, Nestin, GFAP and the extracellular-matrix (ECM) proteoglycan Chondroitin Sulphate (CSPG) in chick and mouse embryos. Various in-vivo and live imaging analyses revealed the contribution of boundary cells to rapid-amplifying progenitors that either undergo direct neurogenesis in the boundary ventricular-mantle axis, or migrate to adjacent rhombomeres as $Sox2^{+}$ progenitors. The ability to form neurospheres confirmed the typical behavior of boundary cells as a multipotent and self-renewing NSC-population ex vivo. Manipulating Sox2 activity shifted the balance between stem-to-differentiation state of these cells and influenced hindbrain development. The ECM within the boundaries was found to be essential r to maintain the boundaries as stable niches of non-differentiating NSCs in-between differentiating rhombomeres.

These findings reveal for the first time that hindbrain boundaries contain repetitive reservoirs of NCSs in-between rhombomeres that possess, both, spinal-cord and upper brain-like features, highlighting the embryonic brainstem as an evolutionary hallmark of CNS development.

The dynamics of dorsal neural tube development: From Neural Crest to definitive Roof Plate

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Within the dynamic context of a developing embryo, the multicellular patterns formed are remarkably precise. Through cell-cell communication, neighbouring progenitors coordinate their activities, sequentially generating distinct tissues. We focus on an aspect of development that illustrates these principles: the ontogeny of the dorsal neural primordium which first generates Neural Crest (NC) cells, precursors of most of the peripheral nervous system, and then becomes the definitive Roof Plate (RP) of the central nervous system. We ask how peripheral and central branches of the nervous system arise and separate from each other during embryogenesis.

We discovered first, that an interaction between BMP and its inhibitor noggin, in coordination with somitic signals, triggers a molecular cascade that regulates the timely onset of epithelial-to-mesenchymal transition (EMT) of NC cells followed by cell emigration. Second, that RP progenitors arise in the NT ventral to the premigratory NC pool and progressively relocate dorsalward to reach their final midline localization upon termination of NC emigration. Third, whereas NC cells depend on BMP signaling for undergoing EMT, the nascent RP becomes refractory to this factor. This may be partly accounted for the activity of RP-specific Hes/Hairy that inhibits NC traits and BMP activity thus allowing the lineage transition. Fourth, we performed a transcriptome analysis of premigratory NC vs. definitive RP progenitors and uncovered the differential expression of multiple genes to each stage, respectively. We are currently investigating the spatio-temporal dynamics of expression of these differentially expressed genes and their precise functions in ending the production of NC and/or in promoting RP generation. Furthermore, we find that not only changes in BMP signaling, but also in Wnt, Hippo/Yap and Retinoic acid pathways play a role in NC-to-RP transition. A tentative molecular network that integrates the activities of these signaling factors with that of selected genes uncovered in our screen will be discussed.

Stem cells, organoids and disease

Cerebral organoids: Modelling human brain development and tumorigenesis in stem cell derived 3D culture

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The human brain is unique in size and complexity, but also the source of some of the most devastating human diseases. While many of these disorders have been successfully studied in model organisms, recent experiments have emphasized unique features that can not easily be modeled in animals. We have therefore developed a 3D organoid culture system derived from human pluripotent stem cells that recapitulates many aspects of human brain development. These cerebral organoids are capable of generating several brain regions including a well-organized cerebral cortex. Furthermore, human cerebral organoids display stem cell properties and progenitor zone organization that show characteristics specific to humans. We have used patient specific iPS cells to model microcephaly, a human neurodevelopmental disorder that has been difficult to recapitulate in mice. This approach reveals premature neuronal differentiation with loss of the microcephaly protein CDK5RAP2, a defect that could explain the disease phenotype. More recently, we have been able to generate organoid based models for human brain cancer and demonstrated their feasibility for drug testing. Our data demonstrate an *in vitro* approach that recapitulates development of even this most complex organ, which can be used to gain insights into disease mechanisms.

Self-organization and symmetry breaking in intestinal organoid development

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Intestinal organoids are complex three-dimensional structures that mimic cell type composition and tissue organization of the intestineby recapitulating the self-organizing capacity of cell populations derived from a single stem cell. Crucial in this process is a first symmetry-breaking event, in which only a fraction of identical cells in a symmetrical sphere differentiate into Paneth cells, which in turn generates the stem cell niche and leads to asymmetric structures such as crypts and villi. We here combine a quantitative imaging approach with single-cell gene expression to characterize the development of intestinal organoids from a single cell. We show that intestinal organoid development follows a regeneration process driven by transient Yap1 activation. Cell-to-cell variability in Yap1, emerging in symmetrical spheres, initiates a Notch/Dll1 lateral inhibition event driving the symmetry-breaking event and the formation of the first Paneth cell. Our findings revealhow single cells exposed to a uniform growth-promoting environment have the intrinsic ability to generate emergent, self-organized behavior resulting in the formation of complex multicellular asymmetric structures.

Taking turns: Quiescent and active stem cells coexist in homeostatic growth of the retinal pigment epithelium of medaka

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Quiescence is believed to maintain a reserve population of adult stem cells in a non-cycling state that can be rapidly reactivated in response to injury, and has also been implicated in the long-term survival of therapy-resistant cancer cells that go on to generate metastases. In this work, we characterize cell dynamics of the stem cell niche of the retinal pigment epithelium (RPE) during post-embryonic growth of the teleost fish medaka (*Oryzias latipes*). We uncover that quiescent and actively cycling RPE stem cells coexist and alternate their roles during normal homeostasis of the organism.

The hemispherical RPE forms a cell monolayer around the neural retina (NR). Both tissues lack cell death and movement; during growth of the organism, new cells arise from a ring-shaped stem cell niche. Our previous computational model of retinal growth [bioRxiv: 437269] predicted that as a consequence of the coordinated growth of NR and RPE, some RPE stem cells should display longer periods of label-retention compared to the underlying NR.

Surpassing our expectations, we experimentally detected extremely frequent and long-lasting label retention indicating that a large proportion of RPE stem cells stay quiescent for months at a time. Another fraction of RPE stem cells maintains the actively cycling state. These two populations coexist in adjacent territories and dynamically switch roles throughout the life of the organism. An extension of our computational model explains this phenomenon by factoring in short-term memory of stem cells. Thus, some cells remember to persist in the quiescent state while their adjacent sister stem cells are actively cycling. Stochastic local interactions can then lead to a role reversal where quiescent cells turn active and active cells enter a quiescent state. The interplay between different stem cell populations increases lineage diversity within the stem cell niche, potentially reducing the burden of clonal mutations. This model of homeostatic stem cell quiescence opens the door to mechanistic studies of this elusive cell state.

Single cell approaches

Dissecting human and chimpanzee cerebral organoids using single-cell transcriptomics

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Cerebral organoids have emerged as powerful models of human brain development, and offer the potential to study uniquely human brain evolution. However, the extent to which cerebral organoid systems recapitulate fetal gene expression networks has remained unclear. Here we use high-throughput droplet microfluidics based single-cell transcriptomics to dissect and compare cell composition and progenitor-to-neuron lineage relationships in human and chimpanzee cerebral organoids and fetal human neocortex. We find that human and chimpanzee organoid cortical cells use gene expression programs remarkably similar to those of the fetal tissue in order to organize into cerebral cortex-like regions. We identify genes that are differentially expressed in human progenitors and neurons relative to chimpanzee, and highlight modern human genetic changes that can be studied in organoid cultures.

From single cells to landscapes of brain regeneration and degeneration

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The prevalence of aging-related neurodegenerative diseases has dramatically increased over the past years. Since at the time of disease detection there is already substantial neuronal loss and damage, a combined therapeutic approach which will stop disease progression and induce repair is needed. In my lab we study how the cellular environment, different cell types and their interactions, can promote regeneration or degeneration. A better understanding of cellular circuits and molecular mechanism driving such processes, will enable the discovery of new and efficient therapeutic strategies. The enormous cellular diversity and complexity in the brain is challenging such molecular research, however,the emergence of new RNA-sequencing technologies, as the single nucleus RNA-seq (sNuc-Seq) method we have developed, provides a unique opportunity to study cellular circuits and molecular mechanisms in high-throughput.Specifically, we have applied sNuc-Seq to chart the RNA dynamics of adult neurogenesis in the hippocampus and identify neurogenesis in the adult intact spinal cord. We are now charting the cellular environment of the Alzheimer’s brain in mouse models and post-mortem human tissue. We find distinct molecular and cellular features of the AD brain compared to the healthy brain, showing multiple cell types involved in the disease and distinct cellular pathways associated with each cell type. The systemic charting of the unique cellular and molecular landscape of the diseased brain, highlight unexpected cell types and molecular pathways that might play an active role in disease progression, and suggest novel therapeutic approaches that can have a combined effect on degeneration and repair.

A foxF regulatory program specifies planarian muscle formation revealed by single cell analysis

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Planarians are flatworms capable of whole-body regeneration, a process that requires positional information for specifying the newly formed body regions. Planarian muscle is the major source of positional information. Yet, the regulatory programs that specify different muscle cell types and their roles in regeneration are poorly understood. Here, we used single cell RNA sequencing to define the transcriptional signatures of non-body wall muscle fibers, and identified their functions in regeneration. We found that foxF-1, a gene encoding a broadly conserved Fox-family transcription factor, is a master regulator of all non-body wall muscle. We functionally analyzed putative muscle lineages derived from foxF-1+ progenitors, and identified muscle lineages forming the dorsal-ventral muscle, intestinal muscle, and pharynx muscle. The medial and lateral dorsal-ventral muscle fibers were specified by nk4 and gata4/5/6-2, respectively. gata1/2/3b was identified as the transcriptional regulator of the intestinal muscle. These muscle cell types expressed genes encoding distinct types of positional information, and had critical roles in regeneration. We found that the dorsal-ventral muscle is crucial for correct medial-lateral patterning, and that injured animals with reduced dorsal-ventral muscle fibers regenerated cyclopic heads. Intestinal muscle and the medial subset of the dorsal-ventral muscle were critical for maintaining and regenerating branched intestinal morphology. Our study suggests that FoxF has an ancient and broadly conserved role in specification of mesoderm derivatives, prominently visceral muscle, in the Bilateria.

Development and homeostasis in a sea anemone: A multi-faceted approach to characterize cnidarian stem cells

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Stem cell research in Cnidaria is well established in the genus *Hydra*, where multi-potent interstitial cells (i-cells) give rise to a variety of differentiated cell types. However, i-cells have not been described outside of the Hydrozoa, and may therefore not represent the ancestral condition within the Cnidaria. The anthozoan *Nematostella vectensis* also demonstrates regenerative abilities and shows no signs of senescence, suggesting the presence of a multipotent stem cell population that maintains tissue homeostasis. To identify these putative stem cells in *Nematostella*, we generated unbiased single cell transcriptome libraries from different developmental stages. These data are used to assess gene usage across cell states, identify differentiation trajectories, and detect populations with characteristics indicative of multi-potent progenitor cells. In parallel, we conducted an in situ hybridization screen of genes whose homologs are known to have a conserved role in stem cell biology in both vertebrates and *Hydra*. We have generated transgenic lines expressing fluorophores under the control of gene-specific promoters for a number of these candidate genes, and find support for the stemness and progenitor state of fluorescent cell populations through in vivo lineage tracing. Ongoing molecular characterization of these transgenic lines, through the generation of cell type-specific transcriptomes of labelled cell populations, will help us understand how stemness is regulated and maintained in Cnidaria during embryogenesis, adult homeostasis and regeneration. Our data show that the combination of unbiased single cell transcriptomes and gene-directed validations permit the identification of stem- and progenitor- cell populations, and their corresponding differentiation trajectories, in the absence of prior morphological knowledge. Comparison with other species will reveal conserved gene regulatory networks underlying stem cell biology in metazoans.

Biophysics of Development

Designing a robust biological timer

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Biological timers synchronize patterning processes during embryonic development. In the Drosophila embryo, neural progenitors (neuroblasts; NBs) produce a sequence of unique neurons whose identities depend on the sequential expression of temporal transcription factors (TTFs). The stereotypy and precision of NB lineages indicate reproducible TTF timer progression. We combine theory and experiments to define the timer mechanism. The TTF timer is commonly described as a relay of activators, but its regulatory circuit is also consistent with a repressor-decay timer, where TTF expression begins when its repressor decays. Theory shows that repressor-decay timers are more robust to parameter variations than activator-relay timers. This motivated us to experimentally compare the relative importance of the relay and decay interactions in vivo. Comparing WT and mutant NBs at high temporal resolution, we show that the TTF sequence progresses primarily by repressor-decay. We suggest that need for robust performance shapes the evolutionary-selected designs of biological circuits.

Integrin-mediated attachment of the blastoderm to the vitelline envelope impacts gastrulation in Tribolium castaneum

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During gastrulation, physical forces reshape the simple embryonic tissue to form a complex body plan of multicellular organisms. These forces often cause large-scale asymmetric movements of the embryonic tissue. In many embryos, the tissue undergoing gastrulation movements is surrounded by a rigid protective shell. While it is well recognized that gastrulation movements depend on forces generated by tissue-intrinsic contractility, it is not known if interactions between the tissue and the protective shell provide additional forces that impact gastrulation. Here we show that a particular part of the blastoderm tissue of the red flour beetle Tribolium castaneum tightly adheres in a temporally coordinated manner to the vitelline envelope surrounding the embryo. This attachment generates an additional force that counteracts the tissue-intrinsic contractile forces to create asymmetric tissue movements. We show that this localized attachment is mediated by a specific integrin, and its knock-down leads to a gastrulation phenotype consistent with complete loss of attachment. Our findings reveal a mechanism whereby a pattern of tissue adhesion to the vitelline envelope provides controllable counter-forces that shape gastrulation movements. Our findings suggest a general principle by which the protective shell provides boundary conditions and external forces to shape intrinsic processes of self-organization that depend both on regulation and mechanics.

Physiological basis of metabolic rate scaling in planarian flatworms

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Across the animal kingdom, body mass differs by more than 16 orders of magnitude and thus represents a hallmark of animal diversity. Despite this enormous size span, the metabolic rate follows a general scaling relationship across different species known as the Kleiber’s law, whereby the metabolic rate scales with the 3/4 power of body mass. Although described already more than 80 years ago, the physiological basis underlying Kleiber’s law remains unknown. A major challenge in studying the underpinnings of the Kleiber’s law is the lack of a suitable experimental system. Here, we make use of the reversible and life history-independent body size changes of adult planarians of the species *Schmidtea mediterranea*. Using microcalorimetry, we show that adult planarians follow Kleiber’s law over a 100-fold change in body size. Strikingly, combining experimental and theoretical analysis of the organismal energy balance reveals that contrary to a common assumption, the average cellular metabolic rate is independent of body size. Instead, the Kleiber’s law in planarians is caused by a size-dependent increase in mass per cell resulting from the increased accumulation of energy stores.

Our work highlights planarians as a promising model system to further explore the molecular and physical processes governing Kleiber’s law, and to ultimately understand the fundamental principles underlying the 3/4 scaling exponent.

Thommen, A., Werner, S., Frank, O. *et al*. BioRxiv (accepted in *eLife*), doi: https://doi.org/10.1101/332916

Alteration in ECM composition affects sensory organ mechanics and function

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Proprioception, the ability to sense the posture and movement of body parts based on signals from within the body, is critical for the ability of the organism to move in a coordinated manner. In Drosophila, proprioception is largely mediated through specialized stretch-responsive sensory organs termed chordotonal organs (ChO) and specific subclasses of multiple dendritic neurons. Proprioceptive mechanosensing requires the transduction of mechanical strain generated by muscle contractions into sensory neuron impulses, via stretch-sensitive ion channels. However, in the ChO, the sensory neuron is not directly connected to the muscle. Rather, the interaction is mediated by specialized connective structure composed of accessory cells and associated extracellular matrix (ECM) that propagate the deformations to the neuron.

In this work, we use the fly ChO, a multicellular proprioceptor, to investigate how the mechanical properties of the connective element affect mechanosensing. We took advantage of chordotonal-specific fluorescent reporters that allow us to visualize, for the first time, the shape and length changes of the accessory cells during larval locomotion in wild type larvae and in larvae lacking the collagen-IV-like Pericardin, a major constituent of the ChO ECM.

We show in an *in-vivo* setup that the loss of Pericardin alter the mechanical properties of the ChO accessory cells resulting in short-wavelength buckling of the cells upon muscle contraction and low compressive strain within the organ. We further demonstrate that the transition from compression to bending as a result of ECM compositional change, interferes with the ability of the accessory cells to propagate muscle-generated deformations correctly to the neuron. This, in turn, interferes with proper sensing and hence, with coordinated locomotion. We explain these results using a simplified theoretical mechanical model of an elastic beam (which represents the ChO sensory organ) interacting with an elastic network (ECM) under a compressive force (generated by muscle contraction).

IsSDB award

DNA methylation and the unending process of development

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DNA methylation serves as an mechanism for annotating the genetic text, thereby providing information on how to regulate gene expression. This methylation pattern is initially erased in the early embryo and then re-established in a highly programmed step-wise manner, serving as a device for stabilizing key developmental decisions. We have attempted to understand what happens to these methylation patterns after birth when the organism becomes exposed to a variety of environmental influences. These studies indicate that a large number of enhancer-like sequences undergo significant changes in their levels of methylation in response to factors such as diet, trauma, pregnancy and exercise. This process takes place in a programmed manner which ultimately enables cells to alter expression patterns, thereby undergoing adjustment to fit the new needs of these cells. In this way, DNA methylation represents an epigenetic mechanism that allows cells to adapt to their environment. Recent studies have suggested that environmental inputs acting on parents may even work transgenerationally by influencing cell physiology in the offspring. New experiments in our lab now indicate that this phenomenon may also be mediated by altering DNA methylation patterns during early embryogenesis. Understanding the mechanism of this process has revealed new and important information about the gamete-embryo axis and its role in development.

Genomic and system approaches of development

New insights into the causes of egg aneuploidy at advanced maternal age

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Maternal age is the most important risk factor for trisomic pregnancies (Hassold & Chiu, 1985). Most trisomies are caused by chromosome missegregation in the meiotic divisions of oocytes (Hassold & Hunt, 2001). Aberrant meiosis generates aneuploid eggs and, upon fertilization, aneuploid fetuses. The increase in meiotic division errors occurs over different time scales in mouse (months) and human (decades), suggesting that physiological rather than chronological ageing of oocytes is important. However, the underlying "clock mechanism" is not known. Epidemiological studies have shown that the risk of trisomic pregnancies and miscarriages, which are most commonly caused by aneuploidy, decreases with prolonged use of hormonal contraception (Ford et al., 1995; Mikkelson et al., 2013; Nagy et al., 2013; Horanyi et al., 2017). The results were interpreted as being due to an increased oocyte pool, although other work has demonstrated that a greater oocyte pool does not protect against trisomic pregnancies (Rowsey et al., 2013). We noted that hormonal contraception, as well as pregnancy and lactation, suppress ovulations. We therefore propose that incessant ovulations determine the physiological ageing of mammalian oocytes, which manifests as meiotic chromosome missegregation and aneuploidy. We provide evidence that reducing ovulation frequency by at least two independent approaches suppresses maternal age-related egg aneuploidy in mice. Ovulation reduction by successive pregnancies caused a reduction in anaphase I chromosome bridges. Crucially, precocious sister centromere separation and chromosomal aneuploidy are decreased in live and fixed metaphase II eggs of aged mated compared to aged virgin females. To discern whether pregnancy/lactation or ovulations determine physiological ageing of oocytes, we generated *Gpr54* knockout mice, which remain in a pre-pubescent state with little or no ovulations. Remarkably, egg aneuploidy is reduced ˜3-fold in aged virgin *Gpr54*$^{-/-}$ compared to aged *Gpr54*$^{+/+}$ females. These results strongly support a model in which incessant ovulations, possibly through release of reactive oxygen species which damage proteins and nucleic acids, lead to age-related chromosome missegregation and egg aneuploidy. Our findings have critical implications for human female reproductive biology, suggesting that ovulation-suppressing conditions, including pregnancy and hormonal contraception, can reduce age-related egg aneuploidy and infertility.

Resolving conflicts: The genetic control of plant morphogenesis

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The mechanisms by which domains of gene activity lead to the generation of tissues with intricate three-dimensional shapes are poorly understood. We have been using a combination of genetic, morphological, computational and imaging approaches to address this problem in plants. Our findings suggest that spatiotemporal patterns of gene activity control shape by introducing conflicts at the subcellular, cellular and tissue levels. Resolution and interactions between these different levels of conflicts may underlie the enormous diversity of forms that have evolved in plants and animals.

3D chromatin conformation of Pitx1 locus defines forelimb vs hind limb identiy

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Pitx1 is a master regulator of hindlimb development. Here we investigate how the hindlimb specific regulationof Pitx1 is achieved. We define the regulatory domain of Pitx1 and show that dynamic changes in chromatin conformation can modulate and restrict the activity of enhancer elements. Inconsistent with its hindlimb-restricted expression,
Pitx1 is controlled by an enhancer (Pen) that shows activity in both,
fore- and hindlimb. By capture HiC and 3D modeling of the locus, we demonstrate that fore- and hindlimbs have fundamentally different chromatin configurations, whereby Pen and Pitx1 interact only in hindlimbs and are physically separated in forelimbs. Structural variants are able to convert the inactive into the active conformation, thereby inducing aberrant Pitx1 expression in the forelimb, causing partial arm-to-leg transformation in mice

Light-controlled perturbation of transcription factor function during embryogenesis

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Embryonic development relies on the precise regulation of gene expression in space and time. Studying the factors governing this regulation is often complicated by their spatial and temporal dynamics, as well as their essential and pleiotropic nature. While great advances have been made in observing these dynamic processes, particularly with “omics” and imaging technologies, we are still lacking tools to perturb their function at the required spatial and temporal resolution in whole animal models.

Using *Drosophila melanogaster* embryogenesis as a model, we aimed to overcome these limitations by conditionally and reversibly modulating the levels of nuclear factors, including key developmental transcription factors (TFs), within their endogenous contexts. To this end, I established a blue light-inducible system for rapid and reversible translocation of nuclear proteins to the cytoplasm in living *Drosophila* embryos. This optogenetic system, composed of a small genetically encoded tag, was fused endogenously to the TF Twist (Twi), a master regulator of mesoderm development. I show that blue light illumination of respective embryos leads to a rapid ($t_{1/2} < 30$ sec) and fully reversible mislocalization of Twi protein to the cytoplasm. This nuclear depletion of Twi results in a reliable perturbation of Twi function and disrupts Twi-dependent developmental and regulatory processes. Blue light-induced Twi depletion is compatible with microscopy-based investigation in living embryos as well as with bulk experiments using fixed embryos. Moreover, the blue light response can be tuned using variants of the optogenetic tag, leading to different light sensitivities, depletion efficiencies and recovery times.

The described tool provides a new level of perturbation: Nuclear proteins can now be depleted within their endogenous context in developing embryos with unprecedented temporal (and spatial) resolution. We envision that this will shed new light on many developmental processes, disentangling distinct roles of pleiotropic regulators and dissecting the temporal requirement of key nuclear factors.

Paternal resetting of H3K27me3-silenced states primes early plant development

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In mammals, epigenetic marks are reprogrammed in the germline and later in sperm through nucleosome replacement with protamine to reset genomic potential in the next generation. However, it remains unclear when and how chromatin marks are reprogrammed during plant development. Moreover, flowering plants retain histone-based sperm chromatin, raising questions about whether the paternal genome undergoes epigenetic reprogramming. In *Arabidopsis*, we show that the epigenetic mark H3K27me3 is selectively and globally lost from sperm chromatin through a multi-layered mechanism, which includes deposition of a sperm-specific histone H3 variant that is immune to lysine 27 methylation. Erasure of H3K27me3 coincides with establishment of active states that prime gene expression during spermatogenesis and in the next generation, revealing novel cycles of genome-wide reprogramming that coordinate early plant development. Thus, in contrast to global resetting of epigenetic marks in animals, plants have evolved mechanisms to simultaneously differentiate the male germline and reprogram the paternal epigenome.

Regeneration

Regeneration initiation - from planarians to African spiny mice

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The ability to regenerate lost or severely damaged tissues is rare, yet it occurs in all animal phyla. Why regeneration is initiated in some animals but not in others remains enigmatic, and it is not clear why even closely related species may have different responses to the same type of injury.
We identified the MAPK/ERK pathway as an important trigger of tissue regeneration in planarian flatworms and zebrafish and are currently exploring its role in mammalian regeneration using a comparative approach with a new naturally regenerating model organism, the African spiny mouse.

Signaling mechanisms in heart regeneration

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The Tzahor team studies novel mechanisms of mammalian cardiac regeneration and repair following injury, a major challenge in current biomedical research (Tzahor and Poss, Science 2017) using mouse, rats and pigs' models. We are focusing on the role of NRG1-ErbB2 signaling pathway in cardiac regeneration after revealing that ErbB2 is both necessary and sufficient to promote cardiac regeneration in adult mice (D'Uva et al., NCB 2015). We also study the cardiac microenvironment as a mean to enhance the regenerative potential of the heart (Yahalom et al., eLIFE 2015). We recently revealed that the proteoglycan, Agrin can serve as an inducer of mammalian heart regeneration, with a great therapeutic potential for the treatment of ischemic heart disease (Bassat et al., Nature 2017). Current studies examine the therapeutic effects of Agrin, whose administration into injured heart of pigs elicits a significant regenerative response. Our findings together have significant implications for improving current strategies to treat heart disease in human.

Thyroid hormone signaling controls adult brain regeneration in zebrafish

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Zebrafish can regenerate their brain after traumatic brain injury through induced reactive proliferation and neurogenesis from radial glia type stem cells. We are studying the molecular mechanisms underlying this paradigm of successful brain regeneration, with a view to developing strategies towards rekindling brain repair ability also in mammals. Thyroid hormone is an important factor in brain development and can function in adult neurogenic brain regions in mammals. We discovered a role of thyroid hormone in zebrafish brain regeneration after injury. By studying the role of thyroid hormone signaling for radial glia proliferation in the adult zebrafish telencephalon, we found that thyroid hormone signaling is upregulated in radial glia during brain regeneration. Genetic and pharmacological blockade of thyroid hormone generation shows its requirement for inducing radial glia proliferation and reparative neurogenesis after injury. Conversely, treating unlesioned adult fish with thyroid hormone is sufficient to increase proliferation of radial glia, apparently without triggering inflammation or apoptosis. Analysis of thyroid hormone receptor activation reveals that radial glia are primary targets for thyroid hormone signaling in the adult zebrafish brain. Thus, our data reveal a novel role of thyroid hormone as a physiological cue that is required and sufficient for the induction of radial glia proliferation and neural regeneration in adult zebrafish brain. These results suggest that manipulating thyroid hormone signaling may be a promising therapeutic strategy for brain repair in mammals, since manipulation of this pathway is already clinical practice.

Single-cell analysis uncovers convergence of cell identities during axolotl limb regeneration

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Amputation of the axolotl forelimb results in the formation of a blastema, a transient tissue where progenitor cells accumulate prior to limb regeneration. However, the molecular understanding of blastema formation had previously been hampered by the inability to identify and isolate blastema precursor cells in the adult tissue. We have used a combination of Cre-loxP reporter lineage tracking and single-cell messenger RNA sequencing (scRNA-seq) to molecularly track mature connective tissue (CT) cell heterogeneity and its transition to a limb blastema state. We have uncovered a multiphasic molecular program where CT cell types found in the uninjured adult limb revert to a relatively homogenous progenitor state that recapitulates an embryonic limb bud–like phenotype including multipotency within the CT lineage.Together, our data illuminate molecular and cellular reprogramming during complex organ regeneration in a vertebrate.

GfE PhD award

Cellular dynamics in zebrafish optic cup morphogenesis

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A common theme in organogenesis is epithelial morphogenesis, where sheets of cells undergo rearrangements to form complex architectures – organ precursors, which subsequently develop into mature organs. Timely development of the characteristic architectures of the organ precursors is crucial for successful organogenesis and is determined by the choice of epithelial rearrangements that organise the constituent cells in space and time. However, for many organogenesis events the cellular dynamics underlying such epithelial rearrangements remain elusive. Using zebrafish, I investigated the morphogenesis of the hemispherical retinal neuroepithelium (RNE) that serves as an organ precursor of the neural retina. I will present my findings that the zebrafish RNE is shaped by the combined action of two different epithelial rearrangements – basal shrinkage of the neuroepithelial cells and involution of cells at the rim of the developing optic cup. The basal shrinkage of the neuroepithelial cells bends the neuroepithelial sheet and starts the process of invagination. However, my results show that the major player in RNE morphogenesis is rim involution – a novel mode of collective epithelial migration. I will present the cellular dynamics observed during rim involution and its role in
RNE morphogenesis as the coordinator of the cellular location and the timing of RNE fate determination. Overall, I will illustrate how spatiotemporal coordination between morphogenic movements and fate determination critically influences organogenesis.

Polarity and axis formation

Crosstalk between the *Drosophila* Crumbs polarity complex and the apical cytocortex orchestrates epithelial polarity

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Polarity is a fundamental property of many cell types, and is manifested by the asymmetric distribution of proteins and lipids. Epithelial cells are polarised along the apico-basal axis, with the apical surface facing the outside and the basal domain contacting the basal lamina. In all epithelia studied so far, establishment and maintenance of a polarised phenotype depends on the integrated activity of several evolutionarily conserved proteins. One of them is the Crumbs protein complex. The central component of this complex is the transmembrane protein Crumbs, which controls a variety of functions, including maintenance of epithelial cell polarity, cell and tissue morphogenesis, growth control and prevention of retinal degeneration. Many of these functions are conserved from *Drosophila* to human. Recent data allow to conclude that several of these processes depend on mutual regulation between *Drosophila* Crumbs and the apical cytocortex.

Pattern formation by mobile morphogen-like small RNA signals

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Plants have the remarkable ability to generate new organs, such as leaves, throughout their lifetime, which can span hundreds or even thousands of years. Lateral organs have their origin in the meristem, a specialized stem cell niche at the growing shoot tip. Here, an autoregulatory loop involving the phytohormone auxin generates regular patterns of gene expression that drive organogenesis. Among them components of the adaxial-abaxial polarity network, which directs the flattened outgrowth and patterning of leaves. I will present our most recent life imaging data on how adaxial- and abaxial-promoting transcription factors become expressed in complementary domains on the top or bottom side of the initiating organ, respectively. The positional information needed to delineate these domains is provided by small RNAs. These form opposing mobility gradients across the leaf that generate sharply defined domains of target gene expression through an intrinsic and direct threshold-based readout reminiscent of classical morphogens. Small RNA mobility is precisely regulated via a gating mechanism that acts at defined cell-cell interfaces and functions independent of mechanisms controlling protein movement. Gating of small RNA mobility generates directional movement between neighboring cells that helps safeguard functional domains within the dynamic stem cell niche and mitigates a ‘signaling gridlock’ in contexts where developmental patterning events occur in close spatial and temporal vicinity.

Double abdomen in a short germ insect: Zygotic control of axis formation revealed in the beetle Tribolium castaneum

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The distinction of anterior versus posterior is a crucial first step in animal embryogenesis. In the fly *Drosophila*, this axis is established by morphogenetic gradients contributed by the mother. This strictly maternal contribution regulates zygotic target genes. This principle has been considered to hold true for insects in general but is fundamentally different from vertebrates where zygotic genes and Wnt signaling are required.

We investigated symmetry breaking in the beetle *Tribolium castaneum*, which among insects represents the more ancestral short germ embryogenesis. In order to identify novel components, we mined the data gathered by the genome wide RNAi screen iBeetle. We found that maternal *Tc-germ cell-less* is required for anterior localization of maternal *Tc-axin*, which represses Wnt signaling and promotes expression of anterior zygotic genes. Both, RNAi targeting *Tc-germ cell-less* or double RNAi knocking down the zygotic genes *Tc-homeobrain* and *Tc-zen1* led to the formation of a second growth zone at the anterior, which resulted in double abdomen phenotypes. Conversely, interfering with two posterior factors, *Tc-caudal* and Wnt, caused double anterior phenotypes.

These findings reveal that not only maternal but also zygotic mechanisms including Wnt signaling are required for establishing embryo polarity and induce the segmentation clock in a short germ insect.

Ansari* S, Troelenberg*, Dao, Richter, **Gregor Bucher#** and Martin Klingler# (2018) Double abdomen in a short-germ insect: Zygotic control of axis formation revealed in the beetle Tribolium castaneum. **PNAS** doi.org/10.1073/pnas.1716512115

Schmitt-Engel, C., et al. (2015). The iBeetle large-scale RNAi screen reveals gene functions for insect development and physiology. **Nat. Commun.** 6, 7822

The emergence of the subapical domain depends on polarization of cortical actin

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The subapical domain is a characteristic feature of epithelial cells defining the position of the adherens junctions, for example. The specification of the subapical domain and the mechanism of pattern formation for cortical domains is not well understood. Here we investigate the formation of the cortical domain during cellularization of Drosophila, when the subapical domain emerges for the first time in embryonic development positioned between apical and lateral domains. Using a live imaging assay for segregation of subapical, lateral and basal markers, we identified a function of the unconventional GEF complex ELMO/Sponge in restricting Canoe/Afadin to the prospective subapical domain. Furthermore we identified a critical relocalization of ELMO/Sponge from the actin caps in syncytial interphases to a ring-like pattern during onset of cellularization. The relocalization suggests that the information of the rim of actin caps is employed for positioning of the subapical domain. Based on this idea we identified a function the Formin Dia in polarization of actin caps. The actin caps are polarized with (+)-ends of actin filaments at the rim. This polarization is lost in dia mutants, which show a uniform distribution of (+)-ends. Furthermore, we found that the emergence of the subapical domain depends on *dia*, since the subapical markers do not become restricted during cellularization in *dia*mutant embryos. Combining these two phenotypes of *dia*mutants, we propose that the orientation of the actin filaments serves as a molecular template to place the determinants for subapical domain to the interface of apical and lateral region.

Germline and early cleavages

Draper isoforms in cyst cells regulate phagocytosis and aging of germ cell debris

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Highly regenerative tissues generate a vast number of functional cells throughout adult life. This process, however, is often concomitant with cell death. As such, efficient clearance of debris is required to prevent the accumulation of chemical and physical obstacles in the way of regeneration. In Drosophila, two isoforms of the engulfment receptor Draper (Drpr)/MEGF-10 are expressed on the cell membrane of phagocytes to generate an “on/off” phagocytic switch. Drpr-I promotes recognition and degradation of debris, while Drpr-II terminates phagocytosis. Here, we show that in the testis, debris that appears following germ cell death (GCD) is cleared by cyst cells that express both Drpr isoforms and act as non-professional phagocytes. Ex vivo live imaging of testis from young adults shows rapid generation and clearance of GCD debris on the scale of minutes, processes which are derailed in aged adults, thus leading to GCD debris accumulation. We further show that the opposite trend in terms of Drpr isoform expression occurs during ageing and that this underlies impairment in phagocytic efficiency. Therefore, we conclude that compromised phagocytosis is a major risk in tissue degeneration during ageing.

Epigenetic mechanisms in early mammalian development: Impact of heterochromatin dynamics

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Transposable elements are the largest individual constituent of mammalian genomes. These elements are highly diverse, a consequence of the multiplicity of genomic habitats that they inhabit and of the complex evolutionary histories that they have developed therein. In mammals, the terminally differentiated sperm and oocyte fuse to create a totipotent zygote upon fertilisation. The mechanisms underlying the epigenetic reprogramming towards totipotency that follows fertilisation are not fully understood, and the molecular features of totipotent cells remain scarce. Embryonic cells remain totipotent only for a restricted time window. During this time, embryonic cells are characterised by an atypical chromatin structure and reactivation of specific families of retrotransposons. We will discuss the consequences that the surge of transposable element transcription during mammalian preimplantation development have towards the establishment of totipotency and pluripotency, and towards the activation of the embryonic genome.

Functional equivalence of the zebrafish germ plasm organizer Bucky ball with the unrelated *Drosophila* Oskar

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Reproduction is a fundamental principle of biological systems. The reproductive cells or primordial germ cells (PGCs) of zebrafish are specified by a RNP granule termed germ plasm. So far, only two proteins are known to act as germ plasm organizers: Bucky ball (Buc) in zebrafish and Oskar (Osk) in *Drosophila*. Both proteins recapitulate germ plasm activities, but seem to be unique to their animal groups.

We discovered that Osk and Buc show conserved activities during germ cell specification. Overexpression of *Drosophila* Osk in zebrafish induces additional PGCs. Surprisingly, Osk and Buc do not show homologous protein motifs that would explain their related function. Nonetheless, we detect that Osk and Buc contain long amino acid stretches of intrinsically disordered regions (IDRs). We show that these IDRs aggregate Buc to induce a liquid-liquid phase separation suggesting that zebrafish germ plasm forms a liquid hydrogel. Moreover, IDRs are known to rapidly change their sequence during evolution and hence, might obscure biochemical interaction motifs conserved between Osk and Buc. Indeed, we discover that Buc binds to the known Oskar interactors Vasa protein and *nanos* mRNA providing a molecular mechanism for their related function. These results identify a conserved core-complex in the germ plasm of vertebrates and invertebrates controlled by two proteins with unrelated sequence but similar activities. Furthermore, our data appear to contradict the sequence-structure-function paradigm of the key-lock principle postulated by Emil Fishcher in 1894.

More importantly, Osk and Buc might represent a first example of a widespread phenomenon. Additional protein-pairs might exist in genome databases, which are currently listed as novel or species-specific, but with similar function across distant species.

Bouncer and SPACA4 - small proteins with big roles

Andrea Pauli
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Fertilization is fundamental for sexual reproduction, yet the molecular mechanism is poorly understood. We discovered Bouncer, a short, unannotated protein in zebrafish that is essential for fertilization. Bouncer is required for sperm-egg binding and sperm entry into the egg. Remarkably, Bouncer functions as the gate-keeper of the egg by ensuring species-specificity of fertilization: it allows conspecific sperm to enter while keeping heterospecific sperm out (Herberg et al., 2018).

While fish express Bouncer exclusively in the egg, the mammalian orthologue of Bouncer, Spaca4, is restricted to the male germline. Consistent with its testis-specific expression in mammals, functional analyses in mice reveal severely reduced fertilization rates in mutant male but not female mice. Thus, our study highlights Bouncer and Spaca4 as previously unknown fertilization factors that function either in female (fish) or male (mammalian) gametes.

GfE Klaus Sander Award

From gradients to disease, from fly to man

Herbert Jäckle
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Segmentation of the Drosophila embryo is under the control of a gene regulatory cascade which acts along the anterior-posterior axes. The anterior segment region is established in response to the maternal Bicoid transcription factor. Bicoid forms an anterior-to-posterior gradient which is thought to act as an anterior morphogen, i.e. it provides distinct threshold values along the anterior body region above which head and thorax segmentation genes are activated. We revisited the bicoid morphogen model. We found that the spatial limits of bicoid target gene expression also depend on antagonizing repressors under the control of the terminal torso signaling pathway. Our model suggests that Bicoid provides only one critical threshold value above which the head/thorax segmentation genes are activated and their spatial limits are set in response to torso-dependent repressors. Thus, in our view, the Bicoid gradient does not act according to the definition of a morphogen: Bicoid needs to provide only one threshold value that defines up to where anterior segmentation genes are activated along the anterior-posterior axis, and the spatial limits, i.e. the sharp cell-to-cell on/off response of target gene expression, are initially provided by torso-dependent repressors which act as Bicoid antagonists.

In the second part of the talk I will focus on energy homeostasis and how Drosophila can be helpful to understand obesity, a rapidly progressing life style pandemic disease of human.

Organogenesis

Deconstructing the human segmentation clock in vitro

Olivier Pourquié
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The spine is characterized by the periodic arrangement of vertebrae along the anterior-posterior (AP) axis. This segmental or metameric organization is established early in embryogenesis when pairs of embryonic segments called somites are rhythmically produced by the presomitic mesoderm (PSM). The tempo of somite formation is controlled by a molecular oscillator known as the segmentation clock. While this oscillator has been well characterized in model organisms, whether a similar oscillator exists in humans remains unknown. We have previously shown that human embryonic stem (ES) cells or induced pluripotent stem (iPS) cells can differentiate in vitro into PSM upon activation of the Wnt signaling pathway combined with BMP inhibition. Single cell RNA-sequencing comparison of the differentiating iPS cells with mouse PSM reveals that human PSM cells follow a similar differentiation path and exhibit a remarkably coordinated differentiation sequence. We show that these human PSM cells exhibit Notch and YAP-dependent oscillations of the cyclic gene HES7 with a 5-hour period. We also used this experimental system to dissect the role of Notch and FGF signaling in the control of oscillations.

Investigating the transitions from multipotent precursors to stably differentiated cell types of the eye

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Normal vision depends on the retinal pigmented epithelium (RPE), consisting of cells that reside between the blood vessels of the choroid and the light-sensitive outer segments of the photoreceptors. The RPE is required for the development and function of these adjacent cell types. Accordingly, mutations in RPE genes and epigenetic changes that occur during aging cause monogenic as well as multifactorial retinal diseases. Importantly, the RPE is readily generated from stem cells, and these stem cell-derived RPE cells are currently being tested in clinical trials for transplantation in cases of retinal dystrophies; they also constitute an important model to study developmental processes in vitro. To elucidate the molecular mechanisms regulating RPE differentiation and mediating interactions with adjacent photoreceptors and choroid blood vessels, we conducted functional and biochemical studies of transcription factors in vivo in mice, and in stem cell-derived RPE. The results reveal early and late gene regulatory networks of RPE-expressed genes and point to the signaling pathways mediating interactions with adjacent photoreceptors and choroidal vasculature. These findings contribute to uncovering gene regulatory networks that control the gradual and coordinated differentiation occurring during organogenesis of the eye in mammals and thus further advance research and therapy of retinal disorders.

The genetic basis for diversification of leaf form: From understanding to reconstructing

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A key challenge in biology is to understand how diversity in organismal form is generated. While key regulators that shape the body plans of model organisms have been identified, less is known about how the balance of conservation versus divergence of relevant developmental pathways influences cell growth to generate morphological diversity. To help address this issue, we developed the *Arabidopsis thaliana* relative *Cardamine hirsuta* into a versatile system for studying morphological evolution. We use a combination of genetics, advanced imaging and computational modelling to understand the mechanisms through which leaf morphology evolved in these species, resulting in simple leaves in *A. thaliana* and complex leaves with leaflets in *C. hirsuta*. This presentation will describe progress on identifying such mechanisms and in conceptualizing how they regulate the number, position and timing of leaflet production.

Wnt7-catenin signaling regulates VE-cadherin-mediated anastomosis of brain capillaries by counteracting S1pr1 signaling

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Canonical Wnt signaling is crucial for vascularization of the central nervous system and blood-brain barrier (BBB) formation. BBB formation and modulation are not only important for development, but also relevant for vascular and neurodegenerative diseases. However, there is little understanding of how Wnt signaling contributes to brain angiogenesis and BBB formation.

By combining high resolution *in vivo* imaging with temporally and spatially controlled manipulation of Wnt signaling, we were able to dissect different requirements for Wnt signaling.

In the absence of Wnt signaling, premature Sphingosine-1-phosphate receptor (S1pr) signaling reduces VE-cadherin and Esama at cell-cell junctions. Wnt signaling most likely suppresses S1pr signaling during angiogenesis to enable the dynamic junction formation during anastomosis, whereas later S1pr signaling regulates BBB maturation and VE-cadherin stabilization.

We could show that Wnt signaling blocks the S1pr signaling cascade upstream or at the level of Rac1 activation and continue to identify the molecules that mediate this interaction.

Our data provides a novel link between brain capillary angiogenesis and BBB formation and identifies Wnt signaling as coordinator of the timing and as regulator of anastomosis.

EvoDevo and emerging models

Bacteria interactions cause tumorigenesis in *Hydra*

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All animals are multiorganismal associations of a multicellular host and its specific microbes interacting with a given environment. An intricate balance between cell dynamics within the host, associated microbiota, and their proper adjustment to the environment maintain the integrity of such a metaorganism. Studies on mammals and human suggest a link between the disturbance of this cross-talk and diseases, such as dermatitis and cancer, yet the proof of causality is generally missing. Furthermore, the fundamental mechanisms, coupling the host homeostasis and microbiota and the degree of their conservation, remain poorly understood.

Recent studies revealed that even the most evolutionary old multicellular organisms, such as cnidarians, are prone to develop cancer. With these models we may provide deep insights into the fundamental rules governing the host-microbiota interdependency particularly with regard to microbes driven tumorigenesis.

Here, we demonstrate that the tumor growth in the freshwater polyp *Hydra* is caused by the altered composition of its commensal microbiota. While *Pseudomonas* bacteria dominate the microbiota in the healthy *Hydra oligactis*, they are largely displaced by a *Turneriella* spirochete in the tumorous polyps. By manipulating the microbiota, we proved the Koch's postulates and demonstrated that the elimination of spirochetes remediates tumor growth. Introduction of a single *Turneriella* strain, conversely, induces the tumor phenotype – elicits the accumulation of aberrant apoptosis-resistant germline stem cells, causes the cytoskeleton disorganization in the epithelial cells, and seriously affects the polyp's fitness.

Remarkably, our data suggest that the invading environmental bacterium (*Turneriella*) potentiates the normal commensal microbe (*Pseudomonas*) to disrupt the tissue homeostasis in the host. Interestingly, similar behavior of a symbiont turning to a pathogen, and therefore termed pathobiont such as *Bacteroides fragilis* may drive tumorigenesis in mice and human. Taken together, our study uncovers an evolutionary conserved role of the commensal bacteria in guarding the host's tissue homeostasis. Once perturbed, the microbiota may fuels the tumor growth.

Different ways to make a spine - notochord sheath cells, but not the sclerotome, drive axial spine metamerisation in zebrafish

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Metamerisation of the axial skeleton in amniotes depends on the segmentation clock which patterns the paraxial mesoderm, resulting in segmentally aligned sclerotome units that will eventually form the mineralized vertebral bodies, or centra. Whether this mechanism also operates in teleost embryos is unclear, since mutants exist in which the centra are largely normal, but where hemal and neural arches as direct derivatives of the sclerotome are misshapen and irregular. Using combinations of zebrafish *her1*, *her7*, *hes6* and *fused somites/tbx6* mutants we disrupt paraxial segmentation, but observe a largely autonomous segmentation process in sheath cells of the notochord. These axial notochord sheath cells express the mineralizing enzyme *entpd5* in a segmental manner, providing direct demonstration that the notochord, and not the sclerotome, is responsible for initial mineralization of the centra. These observations reveal for the first time the dynamics of notochord segmentation in teleosts, consistent with an autonomous patterning mechanism that is influenced, but not determined by adjacent paraxial mesoderm. Remarkably, sheath cells show plasticity in response to altered paraxial segment numbers and spacing. This behavior is captured by a simple reaction-diffusion model in which an instability invasion process is biased by weak features in the environment.

A question resulting from these genetic and molecular studies is whether the mechanism of notochord patterning is an ancient property shared by all vertebrates or, alternatively, whether it constitutes an acquired property specific to some fish. Interestingly, some basal fish have persistent notochords but lack chordacentra.

Functional interrogation of an axial Hox code in the sea anemone, *Nematostella vectensis*

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Hox genes encode conserved developmental transcription factors that govern anterior-posterior (A-P) pattering in diverse bilaterian animals. Although Hox genes are also present within Cnidaria, these animals lack a cannonical A-P axis, leaving it unclear how and when a functionally integrated Hox code arose during evolution. Here I will present data describing our development of short hairpin RNA (shRNA)-mediated knockdown and CRISPR-Cas9 mutagenesis to interrogate gene function in the sea anemone *Nematostella vectensis*. Using these tools, we demonstrate that a Hox-Gbx network controls radial segmentation of the larval endomesoderm during early development. Loss of Hox-Gbx activity also elicits marked defects in tentacle patterning along the directive (orthogonal) axis of primary polyps. On the basis of our results, we propose that an axial Hox code may have controlled body patterning and tissue segmentation before the evolution of the bilaterian A-P axis. Ongoing studies are focussed on understanding the genetic netowrks governing morphogenetic segmentation downstream of Hox gene function.

Marchantia polymorpha: Insight into land plant evolution form a liverwort perspective

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Land plants evolved over 500 MYA from an ancestral charophycean alga and had a major impact in transforming our terrestrial environment. The liverwort Marchantia polymorpha exhibits several features that make it an ideal basal land plant model organism that is currently intensively exploited to understand the evolution of novel biochemical, developmental and cellular attributes mediating the adaptation to a life on land. The Marchantia life cycle alternates between a multicellular haploid and diploid generation and enables to obtain quickly clonal vegetative propagules and also sexually reproduced plants. The availability of transformation protocols and establishment of genome editing tools as well as a sequenced genome encoding around 19.000 protein-coding genes allows investigating the diversification of different protein families. Notably, Marchantia has not undergone paleoploidisation events and exhibits a low genetic redundancy in most regulatory pathways, which often hinders functional studies in other land plant organisms. Together, these attributes enable analyses in this novel liverwort model to unravel the dramatic adaptations and body plan innovations that occurred during the water-to-land transition.

Poster abstracts

A | Developmental Cell Biology

A01 | Retinoic acid signaling reduction recapitulates the effects of alcohol on embryo size

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Intrauterine Growth Restriction (IUGR) is commonly observed in human pregnancies and can result in severe clinical outcomes. IUGR is observed in Fetal Alcohol Syndrome (FAS) fetuses as a result of alcohol exposure during pregnancy. To further understand FAS, the severe form of Fetal Alcohol Spectrum Disorder, we performed an extensive quantitative analysis of the effects of ethanol on embryo size utilizing our *Xenopus* model. Ethanol-treated embryos exhibited size reduction along the anterior-posterior axis. This effect was evident primarily from the hindbrain caudally, while rostral regions appeared refractive to alcohol-induced size changes, asymmetric IUGR. Interestingly, some embryo batches also exhibited an alcohol-dependent reduction of the anterior head domain, symmetric IUGR. To study the connection between ethanol exposure and reduced retinoic acid levels we treated embryos with the retinaldehyde dehydrogenase inhibitors, DEAB and citral. Inhibition of retinoic acid biosynthesis recapitulated the growth defects induced by ethanol affecting mainly axial elongation from the hindbrain caudally. To study the competition between ethanol clearance and retinoic acid biosynthesis we demonstrated that, co-exposure to alcohol reduces the teratogenic effects of treatment with retinol (vitamin A), the retinoic acid precursor. These results further support the role of retinoic acid in the regulation of axial elongation.

A02 | Fibroblast Fusion at The Muscle Fibers Termini Facilitates Muscle-tendon Junction Development.

Wesal Yaseen-Badarne, Ortal Kraft-Sheleg, Shelly Zaffryar-Eilot, Peleg Hasson
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Junctions between tissues are critical for the functioning of our bodies yet the mechanisms these junctions are formed and maintained have remained elusive. We have previously demonstrated that LoxL3 is expressed in and is required for muscle tendon junction (MTJ) formation. To dissect how LoxL3 expression is localized to the muscle tips, along the MTJ, we set to monitor the localization of its mRNA. Surprisingly, we find that *LoxL3* mRNA is not expressed by the myofibers, the cells that express the protein, but, is highly expressed in fibroblasts with the highest expression along the MTJ. Unexpectedly, we find that a subpopulation of fibroblasts also express *MyoD*, a key myogenic transcription factor. Our preliminary data further demonstrates that fibroblasts can fuse into the growing myofiber and contribute mRNAs to the fiber. We hypothesize that LoxL3 is localized to muscle fiber tips through a fusion process of this unique sub-population of fibroblasts which we find express myogenic and fibroblastic mRNAs along the tips of the growing fiber. Our results propose for a novel mechanism of protein localization that has not been characterized before and defy the current dogma which states that myofibers are formed completely of myogenic origin. We propose this mechanism enables the formation of a seamless myotendinous junction that transitions from myofibers into the tendons.

A03 | Cadherin switch marks germ layer formation in the diploblastic sea anemone *Nematostella vectensis*

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Morphogenesis is a process of tissue and organ formation during organism development. Morphogenesis is driven by coordinated cell shape changes, cell migration, cell proliferation and cell death and cell adhesion. Cadherins play a crucial role in morphogenesis and germ layer formation by regulating cellular recognition, segregation and migration. Cadherins are the main components of *adherens* cell junctions present throughout the animal kingdom. Classical cadherins are characterized by typical intracellular domains connecting the adhesion complex to the cytoskeleton. While the role of cadherins has been studied in model bilaterian species, very little is known in diploblastic organisms, such as cnidarians. Most of our knowledge on cell adhesion molecules in cnidarians is restricted to genome analyses. Here we characterize the expression and possible function of classical cadherins Cdh1 and Cdh3 during development of *Nematostella vectensis*. To visualize the localization of the proteins we generated specific anti-cadherin1 (Cdh1) and anti-cadherin3 (Cdh3) antibodies. We show that classical cadherins of *Nematostella*, Cadherin1 (Cdh1) and Cadherin3 (Cdh3), form the adherens junctions of the epithelium of both germ layers. Germ layer differentiation in *Nematostella* is marked by a cadherin switch, where Cdh3 is downregulated in the inner, endodermal layer, while Cdh1 is upregulated and remains the only cadherin expressed in the endoderm. Unexpectedly, we found that in addition to the apical adherens junctions both Cdh1 and Cdh3 are also involved in cell junctions between cells on the basal side. Knockdown experiments of *cdh1* and *cdh3* indicate important roles of cadherins in cell adhesion and tissue morphogenesis of *Nematostella vectensis*. While Cdh1 knockdown experiments reveal its importance for the mesentery and the apical organ formation, Cdh3 knockdown blocks embryo development before gastrulation. Moreover, cell adhesion is dramatically reduced in Cdh3 morphant cell aggregates: they are not able to form *adherens* junctions *de novo* and fall apart. Thus, despite the non-conventional extracellular domain structure, the dynamically expressed classical cadherins play a central and conserved role in cell adhesion, morphogenesis and germ layer specification during the embryonic development of *Nematostella vectensis*. The invaginating endoderm during gastrulation shows partial EMT, accompanied by a cadherin switch. The evolutionarily recurring mechanism of a cadherin switch suggests that the evolution of germ layer formation and tissue morphogenesis was facilitated by the differential expression of cadherins.

A04 | Revealing the mechanism that optimized the rate of body axis elongation in flies.

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Body axis elongation represents a widely conserved process in vertebrate and invertebrate development. At the cellular level, a key conserved contribution to body axis extension is cell intercalation. Despite global conservation of this cellular process, however, the rate of body axis elongation varies dramatically between different fly species, where we found that the *Drosophila melanogaster* germband extended at a rate of about 12 µm/min and in *Chironomus riparius* at 4 µm/min. We propose that optimization in cell neighbor exchange contributed to the speed-up of germband extension as observed in *Drosophila*. To test this hypothesis, we aim to characterize the regulation of cell neighbor exchange in *Chironomus* and compare it with *Drosophila*.

In *Drosophila*, cell neighbor exchange is characterized at the cellular level by convergence of dorsoventral (vertical) junctions (T1-T2 transition) and extension of new anteroposterior (horizontal) junctions (T2-T3 transition). At the molecular level, this process is regulated by the distribution of junctional versus medial apical non-muscle Myosin II. With the start of germband extension but before first converging junctions can be identified, myosin is enriched at vertical junctions, whereas high Bazooka/Par3 concentrations can be detected at horizontal junctions. Here the genetic regulation of junction remodeling and planar cell polarity is governed by a network of so-called pair-rule transcription factors, including Even-skipped and Runt. As germband extension continuous and membranes extent along A-P a re-distribution of myosin to the medial apical site took place. Folded gastrulation as a member of the GPCR signaling cascade, is described to play a role in medial apical myosin activation.

To characterize cell neighbor exchange in the *Chironomus* germband, we started to quantify timing, distribution, rate, and directionality of cell-cell intercalation. We could show, that T1-T2 and T2-T3 transitions are significantly slower in *Chironomus* but not lower in number. However, we could not identify as many rosette (higher order transitions) formations compared to *Drosophila*.

Further we will quantify convergent extension after knock down of pair-rule patterning genes by RNAi and overexpression of GPCR signaling cascade, by mRNA injections of for example *folded gastrulation* and characterize actin and myosin distributions within the cells. We hypothesis to identify changes of myosin distributions during fly evolution, which will allow us to correlate dynamics of cell exchange with the degree of cell polarity in the epithelial plane versus medial apical enrichment.

A05 | From extension to infolding: Uncovering the role of MyoII in the origin of novel tissue behaviour

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Epithelial tissues display astonishing plasticity during development, which ultimately gives rise to the phenotypic diversity visible throughout animals today. Studies of gastrulation, the first morphogenetic event in animal embryos, have shed light on the cellular and molecular mechanisms of epithelial plasticity and shape changes. These changes can occur in a 2D fashion, such as tissue elongation due to cell intercalation in the ectoderm, or in 3D, e.g. during mesoderm invagination via apical constriction . In the *Drosophila* embryo, tissue extension and invagination occur simultaneously, but spatially separated in two adjacent tissues. In other flies, such as the midge *Chironomus riparius*, the processes are less set apart, resulting in a tissue, that undergoes internalisation and cell intercalation at the same time.

The driving motor of both processes is the constriction of a F-actin and non-muscle Myosin II network, suggesting that they originated either independently by convergent evolution or that one of the two processes is derived from the other. We have found circumstantial evidence that mesoderm invagination by apical constriction is a recent morphogenetic innovation, which originated from the related, but older process of convergent extension. At the cellular level, we propose that cell intercalation and apical cell constriction compete for a common pool of activated MyoII. By a relatively simple re-localisation of non-muscle Myosin II to a medioapical position within the cell, cell intercalation is inhibited in the *Chironomus* mesoderm. At the same time apical constriction appears more coordinated, both in a temporal and spacial manner.

For the first time, we could genetically and experimentally dissect evolutionary differences in epithelial morphogenesis at a true cell-biological level.

A06 | Characterization of Drosophila Nidogen/entactin reveals roles in basement membrane stability, barrier function and nervous system patterning

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Basement membranes (BMs) are specialized layers of extracellular matrix (ECM) mainly composed of Laminin, type IV Collagen, Perlecan and Nidogen/entactin (NDG). Recent *in vivo* studies challenged the initially proposed role of NDG as major ECM linker molecule by revealing dispensability for viability and BM formation. Here, we report the characterization of the single *Ndg* gene in *Drosophila.* Embryonic *Ndg* expression was primarily observed in mesodermal tissues and the chordotonal organs, whereas NDG protein localized to all BMs. While loss of Laminin strongly affected BM-localization of NDG, *Ndg* null mutants exhibited no overt changes in the distribution of BM components.

Although *Drosophila Ndg* mutants were viable, loss of NDG led to ultrastructural BM defects compromising barrier function and stability *in vivo*. Moreover, loss of NDG impaired larval crawling behavior and reduced responses to vibrational stimuli. Further morphological analysis revealed accompanying defects in the larval peripheral nervous system especially in the chordotonal organs and the neuromuscular junction (NMJ). Taken together, our analysis suggests that NDG is not essential for BM assembly but mediates BM stability and ECM-dependent neural plasticity during *Drosophila* development.

A07 | Neuroepithelial flow during optic cup formation in medaka and molecules influencing it

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The eye is one of the major sensory systems in the animal kingdom. In higher vertebrates eyes are formed from two primordia on the lateral sides of the forebrain, which then transform into optic vesicles. These vesicles invaginate into optic cups, which grow into mature eyes. During this process, retinal progenitor cells acquire their final fate as neural retina (NR) or retinal pigmented epithelium (RPE). Previously, both of these cell types were thought to originate from predefined areas of the optic cup - the inner layer of the optic cup was thought to give rise to the NR and the outer layer to the RPE[1]. However, Heermann et al. showed that in zebrafish almost the entire optic vesicle gives rise to the NR, while the RPE is formed by a small domain of lens-averted epithelial cells[2]. This determination of cell fate was shown to occur via neuroepithelial flow of cells from the outer layer of the vesicle into the inner layer around the rim of the forming optic cup, a process termed "optic flow". BMP signaling was shown to play a crucial role in this process, arresting the movement of cells upon overactivation.

We will show data that neuroepithelial flow can also be observed in medaka (*Oryzias latipes*) during optic cup formation. This led to the hypothesis that this mode of eye formation may be common for all teleosts including the last common ancestor of zebrafish and medaka, which lived approximately 110 million years ago[3]. Similarly as in zebrafish, BMP can also influence optic flow in medaka. Experiments testing this hypothesis are currently being performed.

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A08 | The role of Fat4-Dchs1 interactions in regulating downstream Hippo signaling

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The FAT/Ds pathway is a highly conserved pathway, involved in planar cell polarity and growth control. The FAT and Ds families contain large transmembrane protocadherins which take part in heterophilic interactions on the boundary between cells. Recent work suggested that the mammalian Fat4 regulates Hippo pathway by sequestering the transcription factor YAP1 to the cell membrane, however, it is unknown whether this sequestration depends on the interaction between Fat4 and its heterophilic partner Dchs1. It is also unknown how Fat4 regulates YAP1 localization and activity. To address these questions, we developed a cell culture assay that can determine the effect of Fat4/Dchs1 interactions on Hippo signaling activity. Our Preliminary results shows that FAT4 repress hippo pathway activity but that this repression does not depend on binding of Fat4 to Dchs1

A09 | Elucidating the role of endothelial cadherins on Notch signaling during angiogenesis.

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Angiogenesis is the process by which new blood vessels are formed from pre-existing ones. This process is governed by the differentiation of quiescent endothelial cells into two distinct cell types, tip cells, which are characterized by long filopodia and high motility, and stalk cells which follow the tip cells to form the new sprout. This differentiation process is mediated by Notch signaling, as Dll4 is highly expressed in tip cells and activates Notch on adjacent cells to induce stalk cell fate. It has been shown that this differentiation process is highly dynamic, as tip cells change their fate into stalk cells, and vice versa, during the angiogenic process. However, it remains unclear how fate switching occurs and what factors affect this transition. We hypothesize that fate switching is controlled by interaction between Notch components and endothelial cadherins VE-cadherin (VE-cad) and N-cadherin (N-cad), which were shown to affect angiogenesis in a number of mechanisms. Here, we show that endothelial cells over-expressing VE-cadherin are more likely to become tip cells when they compete with wildtype cells in a sprouting assay, and that this bias is Notch dependent. We also show that VE-cad and N-cad knock-down resulted in enhanced Dll4 activity, and reduced Notch activity. VE-cad over expression induced the complementary effect, supporting Dll4. This effect is non-transcriptional, as cadherin manipulations did not alter Notch components RNA levels. We propose that this differential effect of VE-cad on Notch components leads to the observed bias in sprouting assay, and may play an important role in Tip/Stalk cell dynamics.
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A10 | Towards quantitative analysis of the Notch transcriptional response

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Notch signaling is a highly conserved pathway that plays a crucial role during normal development and in various diseases, such as cancer and Alzheimer. Notch signaling is transduced by the interaction between Notch receptors on one cell and Notch ligands on a neighboring cell. This interaction leads to the cleavage of the Notch intracellular domain (NICD), which then translocate to the nucleus to create an activating complex with RBPj, its DNA binding co-factor. Despite this molecular knowledge we lack a basic understanding of how the Notch transcriptional response depends on the occupancy of NICD on the promotor and the architecture of the promoter. Here, we report the development of a method to quantitatively measure the dynamics of NICD occupancy on a target promoter. We use live imaging of Halo-tagged NICD in cells that contain synthetic promoters with different number and orientation of RBPj binding sites. For large enough number of binding sites, we can observe a bright puncta corresponding to accumulation of NICD at the promoter. The ultimate goal of this project is to obtain a quantitative understanding of the relation between NICD occupancy and transcriptional response. These studies will improve our understanding and provide insights on how Notch signaling operates reliably in different contexts, both during normal development, and in disease states.

A11 | The role of CK2 on the activity of the WAVE regulatory complex controlling cell shape and cell migration of *Drosophila* macrophages

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The WASP family verprolin homologous (WAVE) protein is a key activator of the Arp2/3 complex and coordinates actin nucleation in many cellular processes including cell shape, cell adhesion and cell migration. Using *Drosophila* macrophages as a genetic tractable in vivo model system we recently identified the Casein Kinase 2 (CK2) as a new conserved putative WRC regulator required for cell shape and directional cell migration. CK2 is a heterotetrameric serine-threonine kinase composed of two catalytic (CK2a) and two regulatory (CK2b) subunits. *ck2a* mutant macrophages show cell shape defects, defective lamellipodia formation as well as defects in cell migration. *In vitro* studies revealed a physical interaction between CK2 and both WAVE and Abi, an important WRC complex subunit suggesting that CK2 might regulate WRC activity by phosphorylation. To further dissect the physiological relevance of phosphorylation of WRC subunits we started to analyze distinct phospho-mutant and phospho-mimicking variants of Abi and WAVE *in vivo*.

A12 | The wave complex regulates epidermal proliferation and morphogenesis

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During epidermal development, cells must coordinate between cell shape dynamics and cell proliferation to establish a functional tissue. How the two processes are coupled is poorly understood. Here, we identify a key role for Abi1, a protein of the wave complex that promotes arp2/3 mediated actin polymerization, in coordinating tissue shape and growth. In utero depletion of Abi1 transcripts in mouse embryos gave rise to defects in the actin cytoskeleton, tissue architecture, and induced hyper-proliferation but had little effect on cell adhesion and differentiation. Depletion of Wave2, an additional wave complex protein, mimicked Abi1 phenotype demonstrating that the defects represent wave complex loss-of-function. However, arp2/3 loss-of-function gave rise to a more severe phenotype that also involved skin paucity and differentiation defects. Seeking a mechanism for this, we demonstrated that Abi1 depletion results in abnormal Wnt signaling and ectopic expression of Sox9, a transcription factor that is not expressed in normal interfollicular epidermis. We further show that forced in utero expression of Sox9 recapitulates Abi1/Wave2 defects. Our study identifies a novel mechanism by which the wave complex orchestrates complex and seemingly distinct processes in a physiologically relevant mammalian system.

A13 | Epithelial-Mesenchymal Transition of the Embryonic Epicardial Cells is Regulated by Extracellular Matrix Protein Agrin

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The epicardium is a mesothelial cell layer covering the heart in vertebrates. During heart development, Wt1 expressing epicardium-derived cells (EPDCs) migrate into the myocardium and differentiate into various cell types, supporting myocardium growth. The epicardium is also an important source of growth factors for myocardium development and maturation. In adult mammals, after myocardial infarction (MI), the dormant epicardium is re-activated and takes part in the regeneration process. Understanding the components of the epicardium and their roles in heart development is essential for understanding the difference in regeneration capabilities of neonatals and adults as well as pursuing methods to unlock the regeneration potential of adult hearts.

Agrin is a proteoglycan protein well known for its function in the establishment of neuromuscle junctions. Recently, the expression of Agrin is identified from the extracellular matrix (ECM) of neonatal hearts. Agrin can stimulate cardiomyocyte proliferation in culture and promote adult heart regeneration after myocardium infarction. However, the role of Agrin in development and the mechanisms that Agrin serves in to promote cardiomyocyte proliferation remain unknown. Thus, a detailed characterisation of Agrin's role in heart development is necessary.

In the E13.5 epicardium, Agrin was detected with immunofluorescence (IF) surrounding $Wt1^+$ cells, colocalising with Integrin alpha4, which is the epicardium-specific integrin subunit. At this stage, the epicardial cell clusters undergoing EMT could be visualized directly using Scanning Electron Microscopy (SEM). These cell clusters are enriched at the apical end and ventricle septum of the heart. Consistent with the SEM observation, cell clusters highly expressing Wt1 and migrating into myocardium were also observed at these positions in tissue sections. By analysing Agrin knockout (KO) mutants at different time points, we found that loss of Agrin results in loss of the $Wt1^+$ cell clusters and fewer $Wt1^+$ cells in the myocardium, suggesting compromised EMT. At E14.5, the Agrin KO hearts exhibit impaired coronary vasculature development. Addition of Agrin in culture medium enhanced Wt1 expression in epicardial cells derived from embryonic heart explants, indicating Agrin may promote or maintain Wt1 expression. Agrin also induced phosphorylated focal adhesion kinase (pFAK) localisation on focal adhesions in human epicardial cells. Our results have shown that Agrin is an important extracellular matrix component of the embryonic epicardium and regulates signal pathways controlling EMT process in embryonic epicardium. Understanding the roles of Agrin in development and regeneration will be key steps for elucidating its therapeutic effects as a heart regeneration medicine.

A14 | RTK/MAPK/ERK- signaling regulates the behavior of the endodermal cells during gastrulation in the sea anemone *Nematostella*

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Gastrulation is a set of conserved morphogenetic movements leading to the formation of germ layers in early embryonic development. Cnidarians, the sister group to all bilaterally symmetrical animals, display a large variety of ways to form two germ layers during gastrulation. In the anthozoan sea anemone *N. vectensis*, gastrulation proceeds via invagination and can be subdivided into several morphogenetic events: i) constriction of the apical ends of the pre-endodermal cells; ii) crawling activity of the pre-endodermal plate cells; iii) inward movements of the blastopore lip. It was shown that inhibition of the MAPK/ERK-pathway results in the failure of the embryos to gastrulate. However, the reason for the disruption of gastrulation in the absence of MAPK/ERK-signalling, and the molecular underpinnings of the observed morphogenetic disorders still remain unclear.

Here we show that upon ERK inhibition by UO126, the embryos are able to differentiate the pre-endodermal plate with bottle-shaped cells. These cells constrict their apical ends and generate leading edges by forming filopodia and lamellipodia on the basal ends. However, unlike in controls, filopodia and lamellipodia spread over the basal surface of the pre-endodermal plate, preventing the crawling activity of the marginal cells. Transmission electron microscopy showed that these filopodia become strongly attached to each other and to the surfaces of neighboring cells by multiple junctions. Moreover, under MAPK/ERK-pathway inhibition, basal junctions between pre-endodermal plate cells do not disappear, as they normally do during gastrulation. Our data suggest that MAPK/ERK-signalling regulates cell morphogenetic movements by influencing the state of intercellular contacts.

In Bilateria, multiple RTK receptors are able to activate signal transduction from the cell surface into the cell nucleus via the MAPK pathway. We treated the embryos with SU5402 (an FGF receptor inhibitor) to check whether FGF signalling was involved in the regulation of gastrulation. After SU5402 treatment, the embryos were not able to gastrulate, and subsequent morphological analysis showed similar abnormalities in embryos upon UO126 treatment, which suggests the involvement of the FGF receptor-mediated signalling in regulating the gastrulation movements. We found eight FGFR/VEGF/PDGF-like receptors in the *Nematostella* genome (including four previously described ones). We showed that two of them, NVE5446 and NVE16312, are strongly expressed in the pre-endodermal plate during gastrulation. RNAi of NVE5446 resulted in a transient delay of gastrulation, whereas knockdown of all other receptors did not affect gastrulation movements.

Recently, the knockdown of the ETS family transcription factor ERG, a possible downstream target of MAPK/ERK-signalling, was shown to lead to gastrulation arrest in *Nematostella*. Using functional analysis, molecular biological, and biochemical methods we have unravelled the hierarchical relationships between MAPK-signalling and ERG.

A15 | Identification of a novel modulator of BMP signaling in a sea anemone

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BMP signaling patterns the dorsal-ventral body axis in Bilateria and the directive axis in the Anthozoa. In cells binding BMPs, phosphorylated SMAD1/5 (pSMAD1/5) translocates to the nucleus and regulates gene expression. However, patterning mechanisms downstream of pSMAD1/5 remain poorly understood in cnidarians. We searched for genes directly regulated by BMP signaling in the sea anemone *Nematostella* by performing ChIP-Seq with an antibody against pSMAD1/5 and compared anthozoan BMP targets with bilaterians ones. Interestingly, among the targets, the fraction of transcription factors and signaling molecules was significantly higher than among randomly chosen genes. We showed that Hox genes and Gbx, which were recently shown to define the identities of the mesenterial chambers partitioning the endoderm, were among the direct targets of BMP signaling. Expectedly, we also identified BMP antagonists *chordin* and *gremlin* as direct BMP signaling targets, however, the expression of BMP genes *BMP2/4*, *BMP5- 8* and *GDF5-like* seems not to be directly regulated by BMP signaling. Among the highly enriched novel targets was a gene coding for an uncharacterized Zn-finger protein with a SWIM domain (ZSWIM). Although clear bilaterian orthologues exist, their function is unknown. In *Nematostella*, ZSWIM encodes a nuclear protein, and its expression is upregulated at the end of the directive axis, where BMP signaling is strongest. ZSWIM knockdown results in the modulation of the shape of the BMP signaling gradient: it flattens in the endoderm at the position where the signaling is strongest, but, simultaneously, the amount of ectodermal nuclear pSMAD1/5 increases. The morphological consequence of this is the formation of fewer endodermal mesenteries on the “strong pSMAD1/5 side” and fewer tentacles in primary polyps. Analysis of marker gene expression upon ZSWIM knockdown and overexpression suggests that ZSWIM facilitates BMP-mediated transcriptional protein, whereas the genes whose expression is activated by BMP signaling are not sensitive to changes in ZSWIM expression levels. Our data will contribute to resolving the topology of the axis-patterning gene regulatory network downstream of BMP signaling in the sea anemone *Nematostella*.

A16 | Regulation of notochord size and shape in mouse development

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The notochord is a transient rod-like structure that extends along the anterior-posterior body axis and lies underneath the neural tube during embryonic development in vertebrates. The notochord secretes multiple proteins, including the morphogen sonic hedgehog (Shh), and has a well established role in patterning the overlying neural tube and somites. During body axis extension, the notochord, somites and neural tube extend coordinately. However, how this coordination is achieved is poorly understood. One reason for this is that the cell behaviours that contribute to notochord morphogenesis are still not fully understood. In particular it is unclear how cell division, convergent extension, notochord cell specification and cell migration contribute to the extension of the notochord. Here, we propose to use live imaging of ex vivo culture of whole mouse embryos and explants to study morphogenetic behaviours of the notochord. Preliminary experiments in mouse and chick show consistent directional movement of cells in the notochord relative to the surrounding tissues. At E9.5 of mouse development (HH15 in chick), the movement cannot be explained by addition of new cells to the notochord. We are currently testing whether the movement is caused by active cell migration or passive displacement of cells. To this end we will collect quantitative data on the cell division and movement patterns in the notochord and aim to reconcile these measurements with the overall shape changes of the notochord over 48h of development. Perturbation experiments will further allow us to test how distinct cell behaviours contribute to notochord extension and diameter, and how they affect the surrounding tissues.

A17 | Crumbs organizes the apical transport machinery by negatively regulating Pten in *Drosophila* larval salivary glands

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Polarized secretion is a vital function of epithelial tissues. Although many components of different intracellular transport machineries are known, it is less understood how these transport machineries are controlled. Here, we show that the polarity protein Crumbs (Crb) is essential for efficient apical secretion of glycoproteins and plasma membrane homeostasis in the salivary glands (SG) of feeding *Drosophila* larvae. Crb is necessary to maintain specific pools of Rab6, Rab11 and Rab30 proteins at the apical domain. This novel function of Crb is independent of its role in epithelial cell polarity. Instead, we show that Crb controls apical secretion by negatively regulating the lipid phosphatase Pten. Downregulation of Pten requires β_H-Spectrin and Myosin V acting downstream of Crb. Hence, loss of Crb leads to disruption of the apical cytocortex and apical secretion. This is accompanied by an increase in $PI(4,5)P_2$ levels and formation of a novel apical membrane compartment enriched in Moesin and $PI(4,5)P_2$. Overall, our results show that Crb regulates the apical membrane composition and morphology by coupling the apical cytocortex organization together with the phosphoinositide metabolism and the membrane traffic machinery. Our findings identify key interactions that might be defective in early stages of different degenerative diseases.

A19 | Disease Modeling in *O.latipes*: Using CRISPR/Cas9 to Generate N-Glycosylation Hypomorphs

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Glycosylation is one of the most common form of co- and posttranslational modifications result in attachment of glycans to proteins or lipids. Protein glycosylation relies on three main types including C-glycosylation, N-glycosylation, and O-mannosylation according to the chemical bond between the sugar residue the amino acid. This complex sugar branching pathway modulates folding and stability of proteins, hence their function. Recessive mutations in enzymes catalyzing the glycosylation of proteins and lipids result in Congenital Disorders of Glycosylation (CDG), which is a rare genetic and metabolic disorder. While the mutations in enzymes requiring assembly and transfer of oligosaccharides classified as CDG-Ix, defects in trimming and processing of oligosaccharide bound to proteins are called as CDG-IIx. Our project focuses on N-glycosylation enzymes, namely Alg2 (alpha-1,3/1,6-mannosyltransferase) and Pmm2 (Phosphomannomutase 2), hence CDG-Ii and GDG-Ia, respectively. Alg2 is an ER membrane bound enzyme catalyzes the addition of the second and the third mannoses to the GlcNAc-dolichol pyrophosphate bound sugar tree, which is one of the very early stages of glycan tree formation in the ER. Pmm2, on the other hand, is upstream of Alg2 enzyme functioning in the cytosol to catalyze the mannose 6-phosphate to mannose 1-phosphate isomerization reaction, which is a precursor to GDP-mannose, the mannose donor for the sugar tree formation.

CDG-Ii is the rarest form of the glycosylation disorder with only one patient reported up to date (Thiel *et al.*, 2003). The patient presented normal at birth but in the first year of life she developed a multisystemic disorder including mental retardation, seizures, hypomyelination, hepatomegaly, coloboma of the iris and coagulation abnormalities. The aim of our project is to study the function of N-glycosylation in terms of neural system development of vertebrates with our model organism Japanese rice fish, medaka. Our hypothesis relies on the fact that the complete knock out of well conserved genes from drosophila to human result in embryonic lethality. To be able to study the function of those enzymes, hypomorphs that have residual enzyme activity are required. Utilizing CRISPR/Cas gene editing together with ssODN (single-stranded oligo donor) occup ying above mentioned patient mutation enabled us to create hypomorphic alleles in medaka model by introducing an early stop codon in the C-terminal end of Alg2. Homozygous mutant fish showed multisystemic diseases including smaller brain, coagulopathy, tubular heart formation, sudden movement of body parts (as in seizures), disturbed swimming behavior and edema around eyes and heart, which is not embryonic lethal. Our data is suggesting that medaka can be used as a model organism to phenocopy one of the well conserved N-glycosylation enzyme disorder, Alg2-CDG (CDG-Ii). Further phenotyping of the fish in molecular level remained to be elucidated.

In the light of results from Alg2-CDG, we would like to expand our research on the defects of cytosolic enzyme Pmm2-CDG, which is the most common form of congenital disorders of glycosylation with 80% of prevalence. To study Pmm2-CDGs we rely on 2 different approaches; creating the most common mutation in Pmm2 patients (R141H and F119L) and utilizing degron system relying on the degradation of GFP labeled proteins. Our results showed that medaka Pmm2 can be endogenously labeled at its C-terminal end and this approach can be combined with deGraFP system to knock down enzyme function. Overall, we showed that Japanese rice fish, medaka, can be used as a model organism to create hypomorphic alleles to mimic patient phenotypes of congenital disorders of glycosylation.

A20 | The endocytic pathway balances membrane organization during subcellular tube formation

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The Drosophila tracheal terminal cells form long subcellular tubes to delivery oxygen to distant tissues. Towards the end of embryogenesis, the size of a terminal cell and its tube increases rapidly, and so, membrane and proteins have to be sorted efficiently. To understand how this is achieved, we analyzed the role of membrane trafficking during tube elongation using light and electron microscopy. By doing live imaging, we observed that ER and Golgi rapidly distribute into the developing cellular extensions prior to the assembly of the membrane-bounded tube within. Growth of the tube and cell membranes is associated with delivery of distinct membrane packets, with apical polarity proteins likely acting as cues for directing transport to the tube. We observed that endosomal components are also enriched towards the growing tip of the cell, suggesting a role for the endocytic pathway in the extension of both outer and inner membranes of the cell. These observations are backed up by electron microscopy data, where we observe an enrichment of endocytic components towards the growing tip of the cell. On transient blockage of endocytosis by inactivation of Dynamin cell growth stops and excess membrane material accumulates in the tube domain. On removal of the Dynamin blockage this excess membrane dissipates accompanied by remodelling of the tube and cell growth. This suggests a role for endocytosis in homoeostasis of the tube and balance in growth of the two membrane domains mediated through transcytosis.

A21 | Signaling transduction during *Drosophila* myoblast fusion

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Skeletal muscles and larval bodywall muscles of *Drosophila* arise by the fusion of mononucleated muscles. The fusion of cells is based on the merger of the plasma membranes into one lipid bilayer. In *Drosophila*, this process requires the recognition and adhesion of two types of myoblasts called founder cells and fusion-competent myoblasts. Myoblast-type specific cell adhesion molecules of the immunoglobulin superfamily (IgSF) interact hetero- and homophilically to from a ring-like cell communication structure at the site of cell–cell contact, which is comparable to other structures like the immunological synapse or podosomes. In the middle of the communication structure branched F-actin accumulates and it has been shown that actin regulatory proteins like Rac, the guanine nucleotide exchange factor Myoblast City, Kette, Scar/WAVE, WIP, WASp and Arp3 are essential for myoblast fusion. Life cell imaging has shown that fusion-competent myoblasts form a dense F-actin focus on cell–cell contact points, whereas in founder cells only a thin F-actin sheath is visible. However, it remains unclear how the actin regulatory proteins are recruited in founder cells and fusion-competent myoblasts to the site of cell–cell contact. Several studies have identified phosphorylated tyrosines in the intracellular domain of the IgSF proteins that might serve as a platform to recruit further downstream factors leading to the recruitment and activation of actin regulatory proteins. In podosomes and the immunological synapse non-receptor tyrosine kinases become sequentially activated to polarize the cells.

Here, we have searched for non-receptor kinases that are involved signaling transduction during myoblast fusion and have generated double mutants to understand the function of these kinases during muscle formation.

A22 | Dynamic pool of Crumbs in the *Drosophila* embryonic epidermis is regulated by the actomyosin cortex and endocytosis

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During morphogenesis epithelial cells undergo dynamic remodelling to form and shape tissues and organs. A key challenge of modern developmental biology is to understand how this large-scale remodelling arises from events taking place on a cellular and molecular level. Apico-basal polarity is crucial to localise components, which drive cell shape changes and neighbour exchange during epithelial rearrangements and its loss can lead to disintegration of the epithelium during development.

How apico-basal polarity is maintained in a dynamically transforming environment of a developing tissue remains elusive. Apical localisation as well as the amount of the evolutionarily conserved *Drosophila* Crumbs protein is critical for the maintenance of apico-basal polarity and epithelial tissue integrity during development. Remarkably, despite the crucial role of Crumbs, the dynamics of its levels at the plasma membrane and its regulation during morphogenesis remain unknown. Applying high resolution live imaging and quantitative fluorescence techniques we studied how Crumbs is regulated in different regimes of morphogenetic activities of the embryonic epidermis. We show that Crumbs dynamics at the plasma membrane correlates with the morphogenetic activity of the embryonic epidermis. Furthermore, taking advantage of *Drosophila* genetics and pharmacological perturbations we provide evidence that the dynamic pool of Crumbs is maintained by endocytic recycling and stabilisation by the actomyosin cortex. In agreement with this, Crumbs dynamics depends on an intact FERM domain-binding motif of its intracellular domain.

Our results thus suggest a mechanism to explain how Crumbs-dependent apico-basal polarity could be maintained and dynamically modulated in a remodelling epithelium in order to preserve epithelial integrity.

A23 | MVBs function to degrade sperm mitochondria after fertilization in *Drosophila*

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Almost all animals contain mitochondria of maternal origin only. However, the exact mechanisms underlying this phenomenon are still unclear, and differ in different organisms. We investigate the fate of paternal mitochondria after fertilization in *Drosophila*. We previously demonstrated that the giant single sperm mitochondrial derivative is rapidly eliminated by mechanisms originated in the egg, in a stereotypical process dubbed paternal mitochondrial destruction/elimination (PMD/PME)[1]. This process is initiated by a network of vesicles, resembling multivesicular bodies (MVBs), and displaying common features of both the endocytic and autophagic pathways. These dual origin vesicles, sometimes also called amphisomes, associate with sperm tail, secrete their intraluminal vesicles into the sperm flagellum and mediate the disintegration of its plasma membrane. Subsequently, the mitochondrion separates from the axoneme and breaks into smaller fragments, which are then sequestered by autophagosomes for degradation in lysosomes. We also provided evidence for the involvement of the ubiquitin pathway and the autophagy receptor p62 in this process. Finally, we showed that the mitophagy-related ubiquitin E3 ligases, Parkin, by itself, has no or minor role in this process. Here, I present new results from our lab indicating that Parkin cooperates with other E3s, such as Mul1, to mediate PMD, and that the source of these E3s is both paternal and maternal. We provide genetic evidence that the abundant inner mitochondrial membrane protein, Prohibitin1 (Phb1), which was suggested to be involved in PMD in some mammals, could be a target for ubiquitination or allow the ubiquitination of proteins on the sperm mitochondrial derivative after fertilization. To identify the repertoire of sperm mitochondrial ubiquitinated proteins involved in this process, we now devised an approach that combines whole fly stable isotope labeling by amino acids (SILAC)[2] together with ubiquitinated protein enrichment followed by mass spectrometry[3]. Furthermore, in order to uncover the egg-derived mechanisms underlying sperm recognition and mitochondrial targeting after fertilization, we devised a combined method to isolate the relevant intra-egg MVBs and subject them to proteomics analysis. Collectively, this work shall shed light on the evolutionary conserved process of PMD in organisms with flagellated sperm, and may provide insight as to why this process is so highly conserved.

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2. Sury, M. D., Chen, J. X. & Selbach, M. The SILAC fly allows for accurate protein quantification in vivo. *Mol.Cell Proteomics.* **9,** 2173–2183 (2010).

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A24 | The extracellular domains of DLL1 and DLL4 mediate differential receptor selectivity in vitro and divergent ligand function in vivo

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DLL1 and DLL4 are two Notch activating ligands in mammals and are essential during embryonic development and in the maintenance of adult tissue homeostasis. Despite high conservation and strong similarities in domain structure we showed that DLL4 is not able to replace DLL1's function during somitogenesis, a process highly dependent on DLL1 function, indicating context-dependent functional diversity of the two Delta ligands.

In this study, we seek to characterize the functional diversity of DLL1 and DLL4, and to define responsible domains using systematic domain exchanges and exchanges of the contact amino acids in biochemical studies, in *in vitro*cell culture analyses, and in *in vivo*mouse models.

Analysis of chimeric molecules during somitogenesis*in vivo*revealed that the extracellular domain determines the different ligand function and that regions beyond the known binding interface are important for full DLL1 functionality. In cell based*in vitro*co-culture assays DLL1 and DLL4 show reciprocal preferences to activate NOTCH1 and NOTCH2 receptors and the N-terminal region up to and including EGF3 encodes the critical range for the observed differences in receptor selectivity.

By exchanging the ligand-specific residues which are in contact with NOTCH1 we observe that the contact interface does not impact the differential receptor selectivity *in vitro*. Furthermore, we demonstrate that a DLL1 ligand with the binding interface of DLL4 is functional *in vivo*, suggesting that regions outside the known direct receptor-binding region in the MNNL and DSL domains are major determinants for the functional difference of the Delta ligands.

A25 | Tissue invasion of Drosophila embryonic macrophages - BMP-activation leads the way

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Immune cells crucially depend on their ability to penetrate dense tissue barriers to reach sites of inflammation, and metastatic cancer cells exploit similar mechanisms.

While mouse studies on the properties of invading cells face the challenges of gene redundancy and difficulties in visualizing movement in the real tissue context, flies offer genetic accessibility and live *in vivo* imaging. Recent studies showed that *Drosophila* macrophages invade a tissue barrier during their colonization of the embryo in a manner similar to mammalian immune cell extravasation from the vasculature, introducing them as a new model. Preliminary data in our lab suggests a need for BMP signalling through the Tkv receptor for this specific invasive step. Moreover, we also identify a subpopulation of macrophages in which BMP signaling occurs as indicated by markers of pathway activation (P-Mad, Dad). Live *in vivo* imaging indicates that the BMP-activated macrophages will eventually become leader cells invading into the germband. To characterize the transcriptional profiles of BMP-activated and the other embryonic macrophages and to identify differentially expressed genes that enable macrophages to penetrate into the germband tissue, we utilized RNA-Sequencing of extracted embryonic macrophages by FACS. Further investigations will focus on the characterization of the identified differentially expressed genes. Moreover, I plan to extend my findings into mammalian systems utilizing mouse immune cells.

These experiments will shed light on the cell autonomous role of BMP signaling in tissue invasion utilizing *Drosophila* embryonic macrophages as a model system and could provide new insights relevant for immune and cancer cell dissemination.

A26 | The roles of BMP and Wnt signalling in dorsal neural tube development

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In vertebrate development, the dorsal neural tube gives rise to a large variety of specialized cell types. On one hand, neural crest cells are generated and subsequently emigrate to form parts of the peripheral nervous system and other structures. At the same time, several molecularly distinct domains of neural progenitors form along the dorsoventral axis, and roof plate cells are specified at the dorsal pole of the neural tube. BMP and Wnt ligands secreted by the roof plate are thought to provide the patterning information for the dorsal neural progenitor subtypes. However, the regulatory logic of how these pathways regulate cell fate decisions as well as proliferation, migration and differentiation is poorly understood. This is partly due to the fact that temporally controlled *in vivo* genetic manipulation of these pathways has been challenging. Moreover, the direct targets of the Wnt and BMP pathways in the dorsal neural tube are unknown. To circumvent these difficulties, we established a novel *in vitro* model for dorsal neural tube development based on directed differentiation of mouse embryonic stem cells. We dynamically administer precise amounts of agonists or inhibitors of BMP and Wnt signalling; this allows us to study the temporal requirement for each pathway for the specification and development of multiple distinct cell types characteristic of the dorsal neural tube. Furthermore, we will use RNA-seq and ChIP-seq to define the molecular signatures associated with distinct dorsal neural tube populations and identify Wnt and BMP target genes. In addition, we will perform *in vivo* lineage tracing to better understand the separation of neural crest, roof plate and progenitor lineages.

A27 | Separate signaling pathways control cortical and basal actomyosin organization in epitheliomuscular cells in *Hydra*

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Formation of actomyosin complexes is essential for cell shape changes and morphogenesis during embryonic development (1). In adult *Hydra*, morphogenesis especially occurs during the asexual budding process where actomyosin interactions control bud detachment (2). To decide whether the basal contractile processes or the cell cortex of the bifunctional ectodermal epitheliomuscle cells are essential for bud detachment, we analyzed the localization of phosphorylated myosin regulatory light chain (MRLC/MLC), which is essential to generate contractile forces (3, 4). The pMLC20 antibody revealed a clear correlation between the subcellular localization of phosphorylated MLC and its function. pMLC20 was detected in the basal contractile processes of ectodermal epitheliomuscular cells, which control body movement. In contrast, at the late, strongly constricting bud base, pMLC20 was detected in the apical and basolateral cortical compartments of a small population of cells. Pharmacological inhibition indicated that MLC phosphorylation occurs by at least two independent pathways during contraction of the basal cell protrusions (movement) or constriction of the bud base (morphogenesis). MLC phosphorylation in the basal cell protrusions is generated by myosin light chain kinase (MLCK). In contrast, apical and cortical reinforcement of MLC phosphorylation in constricting cells at the bud base is stimulated via a Rho/ROCK pathway (2). Our data thus indicate that Rho dependent phosphorylation of MLC is essential for tissue morphogenesis at the late bud base, while MLCK ensures the normal basal contractility and tissue organization/stability of ectodermal epitheliomuscular cells.

(1) Martin and Goldstein, 2014; (2) Holz et al., 2017; (3) Vincente-Manzanares et al., 2009; (4) Karen et al., 2015

A28 | Tools to detect Rho activity and PIP- signaling in *Hydra*

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Signaling to establish cell polarity, to induce cell motility, separation of cell sheets or tissues are hallmarks of embryonic development. In triploblastic animals, tissue separation is mainly controlled by apical constriction of cells. Constriction is mediated by a rearrangement of the actin cytoskeleton and by the establishment of localized cortical actomyosin complexes (1). Rho- GTPases often control cell shape changes (2, 3) and they are involved in *Hydra* bud detachment (4). Since the activation of Rho- GTPases may be regulated via phosphoinositides (PIPs) (4), the question arose, whether the dynamic separation of young *Hydra* polyps from their parent requires, besides Rho signaling, also signaling through PIPs. To answer this question, we used a method to detect activated Rho in whole *Hydra* tissue additional to live imaging of phosphatidylinositol -4,5-bis- or -3,4,5-tris- phosphate (PIP2 and PIP3). The Rho-Activity-Staining-Assay yielded a strong signal in evaginating tentacle buds as well as in regenerating tissue. To detect PIPs, we used existing vertebrate PIP2-GFP and PIP3-GFP sensor constructs, subcloned them in a *Hydra*-specific expression vector and generated transgenic lines for an in vivo evaluation of PIP2 and PIP3 distribution. Both sensors localized differentially revealing, for the first time, a polarized cellular localization of PIP2 and PIP3 in *Hydra vulgaris* epithelial cells. The PIP2 sensor was bound mainly in the apical membrane of epithelial cells, the PIP3 sensor was localized basally. Both sensors responded to morphogenetic signals like budding (PIP2-GFP sensor) indicating an activation of PLCg, or during regeneration (PIP3-GFP sensor) indicating an inactivation of PI3 kinase.

In summary, our data indicate that (i) activated Rho is involved in the evagination process of tissue as well as in its constriction. Moreover, (ii) since a polarized distribution of apical PIP2 and basolateral PIP3 is known from vertebrate and fly cells (5,6), their similar distribution in *Hydra* epithelial cells indicates an ancestral, evolutionarily conserved, mechanism. Signaling via PIPs seems to be directly coupled to cell polarity and cytoskeletal rearrangement in morphogenesis. Our studies aim to further identify the functions of *Hydra* Rho as well as their up- and downstream activating pathways.

(1) Fagotto, 2014; (2) Bora and Shrivastava, 2017; (3) Wheeler and Ridley, 2004; (4) Holz et al., 2017; (5) Saarikangas et al., 2010; (6) Krahn and Wodarz, 2012; (7) Martin-Belmonte et al., 2007

A29 | Irradiation-induced cell migration (ICM) involves EMT and cell migration, the latter of which is regulated by caspases

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Irradiation-induced cell migration (ICM) in *Drosophila* is a recently discovered process in our lab, in which irradiated epithelial cells compromised for caspase activity acquire potent migration and invasion capacities[1]. During this process the cells undergo dramatic morphological and anatomical changes, including loss of apico-basal polarity and cell delamination, extensive cellular matrix (ECM) remodeling, activation of Rho GTPases and formation of cellular protrusions, and eventually intra- and inter-tissue migration and invasion. This dramatic cellular remodeling is reminiscent of many aspects of the epithelial-mesenchymal transition (EMT) process, which is important for organismal development and for cancer cell dissemination. To further characterize these EMT-like aspects, we first carefully followed this cellular transition. We show that ICM involves extensive remodeling of the tissue extracellular matrix manifested by the degradation of the basement membrane (BM). The vast majority of the migrating cells become untethered from the BM and delaminate basally breaking through the degraded BM. We then demonstrate that there are no directional migration paths, rather the cells migrate randomly and are scattered throughout the tissue. At the molecular level, we identified several key regulators of developmental and pathological EMT as mediators of ICM. Importantly, we show that the EMT process during ICM is highly efficient, affecting most of the cells, and that caspases appear to be only involved in the attenuation of the subsequent migration process. Finally, we established a system for conducting an unbiased drug screen *in vivo*, aimed to identify regulators and molecular pathways underlying ICM. Given the high resemblance between ICM and EMT, and the fact that the latter is believed to be involved in metastatic progression of tumor cells, our findings may provide new insights on the role of caspases as tumor suppressor genes.

1. Gorelick-Ashkenazi A, Weiss R, Sapozhnikov L, et al. Caspases maintain tissue integrity by an apoptosis-independent inhibition of cell migration and invasion. *Nat Commun*. 2018;9(1):2806. doi:10.1038/s41467-018-05204-6

A30 | Role of caspases during spermatid terminal differentiation in *Drosophila*

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Programmed cell death (PCD) is a genetically encoded cell suicide mechanism which functions to eliminate superfluous or damaged cells during development and homeostasis of the organism. Apoptosis, the major form of PCD, is executed by a family of cysteine proteases, called caspases, in a cascade of proteolytic events which eventually cleave hundreds of proteins in the cell leading to cell death. However, emerging evidence suggest that activation of caspases does not necessarily lead to cell death, as some cells utilize caspases to promote a variety of non-lethal cellular processes (also called CDPs). Thus far, dozens CDPs have been described in a variety of cell types and organisms, acting to promote cellular processes such as signaling, remodeling, proliferation, differentiation, migration and more. Whereas some progress has been made in understanding how these cells restrict caspase activity and avoid cell death, the functions of caspases during these cellular processes remains largely unknown.

One of the first paradigms of CDPs which has been extensively studied in our lab is the process of spermatid terminal differentiation in *Drosophila*. During this process, called individualization, elongated spermatids activate caspases in a restricted manner in order to eliminate their bulk cytoplasmic contents and separate from one another. In a previous work, we reported a new mitochondrial-based mechanism which limits the rate of caspase activation during spermatids individualization (Aram et al., 2016). Here, to understand how caspases promote spermatid individualization, we started screening through the entire list of known caspase substrates (about 2,000 proteins) for candidate proteins that might be involved in this process. We first compiled a list of potential *Drosophila* caspase substrates based on previous proteomics and other studies of caspase substrates during apoptosis in flies, worms, and mammals. The list was narrowed down to substrates that could be relevant to spermatid individualization, such as proteins involved in cytoskeletal dynamics. Using a collection of RNAi fly lines to target each of these genes in the male germ cells, we then screened for knockdowns that cause male sterility. Among these, we looked for those that grossly exhibited regular spermatid maturation but failed to individualize, and identified several potential candidate genes, which we are now testing for a probable regulation by caspases during the individualization process.

A31 | The Tip60 chromatin remodeling complex is involved in the maintenance of adult midgut precursor cells in the *Drosophila* midgut

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Introduction

Drosophila represents a well-established animal model for studying stem cell biology. Adult midgut precursors (AMPs) constitute a population of cells with stem cell potential in the *Drosophila* larval midgut. During metamorphosis, the larval midgut degenerates and is completely renewed by these cells, giving rise to enteroendocrine cells and enterocytes. AMPs are also the progenitors of adult intestinal stem cells (ISCs), which are required for homeostasis of the adult midgut.

Results

We are investigating the function of the Tip60 chromatin remodeling complex in the larval *Drosophila* intestinal tract. We show that the Tip60 complex, which has been previously assigned a function in stem cell maintenance in the *Drosophila* nervous system, is also involved in the maintenance of AMPs until metamorphosis.

Using a reverse genetic approach by applying the Gal4-UAS system, individual members of the Tip60 complex were knocked down in specific cell types of the midgut and analyzed for the resulting phenotype. The screen revealed that knockdown of several members of the Tip60 complex resulted in similar phenotypes. AMPs were lost along with their niche cells, in a manner independent of Notch signaling and caspase-mediated apoptosis. The catalytic subunits Tip60 and Domino, the homolog of mammalian P400, were further studied with respect to selected signaling pathways involved in cell type specification in the *Drosophila* intestine.

Perspectives

Based on the data so far, we propose a model in which the Tip60 complex is involved in AMP maintenance by preventing premature differentiation. We have recently shown that the complex is essential for maintenance and polarity of larval neuroblasts [1]. Because neuroblasts share many similarities with intestinal stem cells, we are currently investigating the impact of the Tip60 complex in ISCs on gene expression, as well as its impact on cell polarity.

In mammals including humans, the Tip60 complex has been implicated in stem cell maintenance and the development of cancer. This project should advance our current understanding of the role of the Tip60 complex in these processes and thus further our knowledge to be exploited for future therapeutic options.

[1] *Rust K. et al. Myc and the Tip60 chromatin remodeling complex control neuroblast maintenance and polarity in Drosophila. EMBO J* ***16****, e98659 (2018).*

A32 | The role of retinoic acid in the development of zebrafish pelvic fins

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The molecular mechanisms that control the development of paired extremities are broadly conserved among vertebrate species. Consequently, a fundamental knowledge about signalling processes in zebrafish paired fin development might help to understand limb patterning and congenital limb defects in humans. All-*trans*retinoic acid (RA) is a key factor in many developmental processes. The current model for forelimb development in tetrapods proposes an antagonism between RA and fibroblast growth factors (FGFs) along the anteroposterior axis, that mediates the correct positioning of the limb field and establishes a permissive environment for the induction of limb budding. Additionally, RA cooperatively interacts with β-catenin signalling and *Hox* gene activity to control *Tbx5* expression during forelimb development in chicks. However, for hindlimb and pelvic fin development the roles of RA are still controversial. Studies in mice, deficient for the RA synthesising enzymes Raldh2 or Rdh10, indicate that RA is dispensable in hindlimb development. However, based on pharmacological treatments of juvenile fish with the aldh1a-inhibitor 4-Diethylaminobenzaldehyde (DEAB), we could show that RA plays an important role in the development of pelvic fin during the early stages of fin bud formation. It acts in a very limited time frame and is supposed to be involved in fin outgrowth and the formation of the pelvic girdle cartilage. Our current aim is to disrupt RA signalling in zebrafish pelvic fin development in a tissue-specific way using the Gal4-UAS system. Driver lines provide expression of a tamoxifen-inducible *Gal4* (*ERT2-Gal4*) under the control of enhancer sequences of *Pitx1* and *Prrx1*, which are specific for pelvic and/or pectoral fins. For optimization, we use the gene *KalTA4*, a *Gal4* version modified with a Kozak sequence for higher translation efficiency and adjusted for the zebrafish system in terms of codon usage. Our effector lines express genes encoding either a dominant negative retinoic acid receptor (dnRARα2a) or the RA degrading enzyme Cyp26a1 under the control of an upstream activating sequence (UAS). As a proof-of principle, we show that the activation of *dnRARα2a* expression in F3 zygotes of *UAS:dnRARα2a-IRES-eGFP* zebrafish by injecting *KalTA4-ERT2-GI* mRNA, followed by induction with 4-hydroxy-tamoxifen (4-OHT), results in a retinoic-acid deficiency phenotype in zebrafish larvae.

A33 | Cell competition and innate immunity signaling in the *Drosophila* testis niche

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The testis of *Drosophila* is an established model for niche biology. Here, somatic stem cells and germline stem cells surround a cluster of postmitotic cells termed hub. The hub provides the essential niche signals for both stem cell types. Stem cells in the testis are under strong competition for access to the hub, both within and between the two stem cell pools. While winner cells of this competition remain in the niche and retain stem cell status, loser cells are eliminated through differentiation.

We recently found that genes involved in innate immunity signaling are upregulated in the somatic stem cells relative to their differentiated progeny. This includes components of the Imd-signaling cascade, the key NF_{κ}-B transcription factor Rel, and downstream targets genes such as antimicrobial peptides. This suggests that innate immune signaling is active within the somatic stem cell pool. Consistently, we could previously show that the transcription factor Zfh-1, a marker for somatic stem cells, binds to the control regions of Rel and multiple other innate immunity genes. Since innate immunity regulates competition induced cell death in epithelial tissues, I am interested in the connection between innate immunity and cell competition also in the testis. In preliminary experiments I could so far show that the loss of loser cells by differentiation can be partially rescued by inhibiting Rel activity, indicating that innate immunity signaling primes loser cells for elimination from the niche.

Here I will present the current state of my results and will discuss more generally why a niche should promote competition between its resident stem cells.

A34 | Cell focussing: Pattern formation by dancing cells

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Schnabel and co-workers proposed that pattern formation is the result of a sorting process involving extensive cell migrations in the *C. elegans* embryo. These migrations establish stereotypic regions originating from the somatic founder cells at the premorphogenetic stage. This process, termed "cell focussing", is governed by local cell-cell interactions, where "cell addresses" — which are part of their identities — are matched. When cell identities are altered as for example in a *glp-1* (Notch) embryo, cells sort in a spectacular, new pattern corresponding to their new identities (Schnabel *et al*., 2006; Bischoff and Schnabel, 2006). The aim of this study is to extend the original work of Schnabel *et al.* (2006) and elucidate the mechanism by which cell focussing translates perceived addresses into the corresponding positions along the body plan. Towards this aim, we use an advanced assessment of migratory behaviour, genetic analyses and *in silico* tests of hypotheses. The analyses are based on 4D microscopic recordings subsequently analysed using the database SIMI©Biocell.

It appears to be a general notion that cells are more or less stationary and initiate migration only upon recognising a specific signal. In contrast to this, we observe that cells in the *C. elegans* embryo are constantly, independently and randomly moving back and forth at least until the onset of morphogenesis – a process we termed "dance" of cells. This dance of cells resembles the Brownian motion, with cells moving independently of their nearest neighbours. The effective migration of a cell between mitoses appears to be the result of a long term directional bias of this dance. We now show that the loss of components of the CED-10/Rac-WAVE-Arp2/3 pathway, known to regulate actin dynamics, results in a significant reduction of the effective migration towards the terminal positions at the end of the premorphogenetic stage. The general dance movements remain — quite enigmatically — unchanged with cells still dancing back and forth as in wild type embryos. In the strong maternal effect *wve-1* (*ok3308*) mutant, guided migrations are essentially eliminated as cells are just randomly dancing around the positions reached by mitoses only. This reduction of effective migrations coincides with a drastic loss of the normal patterning. *In silico* simulations without any guidance of cells recapitulate both, the reduction of the effective migrations and the patterning defects. We were furthermore able to establish the SAX-3/Robo receptor, previously reported to act in the reorganisation of the actin cytoskeleton during morphogenesis (Bernadskaya *et al*., 2012), as a first candidate for a cell focussing receptor potentially recognizing repulsive cues.

Summarising our new findings in a model, we propose that cell focussing steers the dance of cells without being responsible for the movements per se. Upstream signals perceived by receptors like SAX-3/Robo are integrated via Rac signalling to WAVE/SCAR mediated actin branching and introduce a bias on the random short term movements to eventually generate directed effective migrations.

A35 | Stress-dependent regulation of a liquid droplet component, Rbfox1

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Upon stress, profound post-transcriptional adjustments of gene expression occur in RNP granules, which are formed by liquid phase separation of RNA-binding proteins with low complexity sequence domains (LCDs). While recent studies have provided knowledge about principles of their aggregation, we still know very little about mechanisms that regulate their expression, especially under stress and in living organisms.Previously, we demonstrated that a disease-associated protein, Rbfox1 contains LCDs and aggregates into liquid droplets and amyloid-like fibers and promiscuously joins different nuclear and cytoplasmic RNP granules. We uncovered an elegant mechanism by which Rbfox1 levels are adjusted by a stress-dependent miRNA. *miR-980* acts to buffer Rbfox1 levels, since it can target only the portion of *Rbfox1* transcripts containing extended 3'UTRs. This prevents the reduction of Rbfox1 levels below a certain threshold, which is detrimental for cellular homeostasis. Reduced *miR-980* expression during stress leads to increased Rbfox1 levels, followed by widespread formation of RNP granules and promotes cell survival. Now we found that Rbfox1 can regulate alternative splicing of its own mRNA, generating a variety of *Rbfox1* isoforms, which differ in the presence of mitochondria-localization signal, assortment of LCD domains, and *3'UTR* length. This affects Rbfox1's capacity to assemble various cytoplasmic, nuclear and mitochondrial RNP granules and its availability for miRNA targeting in response to stress. We propose that one of the mechanisms exploited by a cell as a stress response is the establishment of reciprocal self-regulatory circuits, which include miRNAs to balance different levels and isoforms of LCD-containing proteins.

A36 | A centrosome organizing center coordinates early oogenesis in zebrafish

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Oocytes undergo a fascinating differentiation process, simultaneously orchestrating dramatic nuclear events of meiosis, cytoplasmic polarization, and dynamic changes in inter-cellular organization while constructing the germline cyst and the primordial follicle. How do differentiating cells coordinate such simultaneous acquisition of multiple traits is unknown. Through analysis of zebrafish oocyte polarization, we discovered a novel centrosome-based cellular organizer that integrates multiple facets of oocyte differentiation.

Universal to differentiating oocytes is formation of the Balbiani body (Bb), a large aggregate of specific mRNP granules. The zebrafish Bb establishes the oocyte animal-vegetal polarity and is crucial for oocyte and embryonic development. The Bb has been observed for two centuries, but how it forms and positioned was unknown. We previously traced the oocyte symmetry breaking to a nuclear asymmetry at the onset of meiosis called the zygotene chromosomal bouquet. The bouquet is a universal meiotic feature, where telomeres associate with Sun/KASH proteins on the nuclear envelope (NE), connecting to cytoplasmic microtubules that emanate from the centrosome. Microtubule-associated telomeres rotate to facilitate chromosomal homology searches, pairing, and recombination, and cluster to the NE pole that faces the centrosome. We showed that Bb granules first localize to the bouquet centrosome in a microtubule dependent manner, and that centrosome-based microtubules mechanistically couple cytoplasmic Bb granule localization with nuclear bouquet formation. Bb granules then nucleates around the centrosome to assemble the mature compact Bb. The mature Bb contains amyloid b-sheets and granule nucleation requires the prion-like protein Bucky ball, demonstrating prion-like mechanisms in oocyte polarization. The bouquet centrosome functions as a cellular organizer that we termed the Centrosome Organizing Center (COC), coupling meiosis and oocyte patterning.

Here, we show that the COC is involved in broader events in early oogenesis. Oocytes are produced from oogonia, which execute modified mitoses, generating a conserved organization of germ cells connected in a cyst. In the zebrafish cyst, all oocytes execute bouquets symmetry breaking in synchrony. We identified a novel bouquet specific primary cilium that connected to the COC and could synchronize the cyst and mechanically regulate chromosome homology searches and bouquet formation. Earlier, the COC is aligned to the last mitotic division plane, suggesting regulation by mitosis. We uncovered a cellular organizer that integrates mitosis, meiosis, cell polarity, cilia biology and prion-like mechanisms in the early differentiating oocyte. Centrosome functions and regulation in the transition from mitosis to early differentiation are poorly understood in vivo. Our work sheds light on how cells use their centrosomes to coordinate multiple processes into a cooperative differentiation program during post-embryonic development.

A37 | Emergence of retinal stem cells in fish retina

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Proper visual perception is strictly dependent on coordinated differentiation and correct assembly of multiple cell types in the structure of the retina. During the course of retinogenesis differentiation and growth need to be tightly regulated. Although mammalian retinal growth is mostly restricted to embryonic development, in lifelong growing organisms such as fish and amphibians, retinal growth is maintained till adulthood. New cells are continuously added from the most peripheral part of the retina, the ciliary marginal zone (CMZ), the region where the population of post-embryonic retinal stem cells reside. Despite extensive studies of post-embryonic retina stem cells, little is known about how they are being established. Here, using the teleost fish medaka (*Oryzias latipes*) as a model, we aim to elucidate when and how retinal stem cells are specified. Using *in situ* hybridization, immunohistochemistry, BrdU incorporation assays and retinal stem cell progeny tracking we found that retinal stem cells are specified already early in embryonic development, although they are known to contribute to retinal growth mostly in post-embryonic period. Soon after optic cup is being formed discrete population of retinal cells, distinct from common retinal progenitor pool can be recognized. These cells can be distinguished by the expression of retina stem cell-specific genes and different proliferation rate. Further transcriptom analysis combined with knocking out of stem cell-expressed genes might help us to uncover the gene regulatory network behind the retinal stem cell fate decision.

A38 | Regulation of the MAST kinase Drop out in *D. melanogaster*

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MAST (microtubule associated Ser/Thr) kinases belong to the superfamily of the AGC (PKA, PKG and PKC) protein kinases. The human MAST kinase family comprises four homologues, called MAST1-4, while the *Drosophila* genome encodes a single MAST kinase encoded by the gene Drop out (Dop). MAST kinases share an evolutionary conserved domain structure consisting of a DUF1908 (domain of unknown function) domain, a Ser/Thr protein kinase domain and a PDZ (PSD95, Dlg, ZO-1) domain. In humans, MAST kinases are implicated in several diseases including inflammatory bowel disease and breast cancer. Despite their involvement in distinct molecular pathways and in human diseases the regulation of MAST kinase activity is not understood. To investigate regulatory mechanisms that control MAST kinases, *D. melanogaster* offers a perfect model system. Our laboratory created a range of point mutations in the *dop* gene, which affect early embryogenesis and imaginal disc development. The most severe phenotype in *dop* mutant embryos represents a defect in the formation of the polarized blastoderm epithelium. Our previous results suggested that Dop activity might be regulated through its highly conserved protein domains. Genetic analyses showed that the conserved protein domains in Dop are essential for the function of the kinase. We found that the DUF1908 domain is potentially involved in the subcellular localization of the protein, as DUF1908_GFP fusion proteins localized at the furrow canal in early cellularization. The protein level of Dop also seems critical, as overexpression affects the hatching rates of embryos. Interestingly, this effect on survival was even more pronounced, when Dop mutant variants lacking the PDZ or DUF domain were overexpressed. To further investigate the role of the domains, we have begun to identify protein interaction partners of Dop. A yeast-two hybrid screen revealed potential interaction partners of PDZ domain of Dop. A progress of the interaction studies will be presented.

A39 | A highly conserved MFS regulates O-GalNAc glycosylation to optimize *Drosophila* macrophage migration and tissue invasion

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During embryonic development, *Drosophila* macrophages penetrate a tissue barrier, moving between two closely apposed epithelia in a process with many similarities to mammalian invasive migration. We seek to understand the regulation of this process. We have identified a new molecular player required in macrophages – a highly conserved but as yet uncharacterized Major Facilitator Superfamily (MFS) member whose expression is enriched in macrophages before and during this invasion. We find this protein which displays structural similarities to sugar transporters localized in the Golgi and endosomes. Downregulating this protein, which we call Minerva, causes a decrease in GalNAc-type O-glycosylation with reduction in core1 (T antigen) on macrophages. Interestingly T antigen glycosylation is also known to be enriched on invasive cancer cells. Differential O-glycoproteomics analysis reveals that the MFS transporter influences O-GalNAc glycosylation on proteins involved in Decapentaplegic and Notch signaling, protein folding, and glycan modification. The most strongly regulated candidate is a protein sulfhydryl oxidase, Qsox1, which we show is required for macrophage invasion and which in vertebrates has been shown to be associated with cancer metastasis and the regulation of ECM composition. Minerva's function is conserved in vertebrates as the mouse ortholog, MFSD1, can rescue macrophage invasion and the core1 glycosylation defects. We are working to uncover the mechanism for how Minerva regulates glycosylation, and how Minerva's targets influence invasive migration, as well as the role of the highly conserved vertebrate ortholog in immune cell migration.

A40 | Studying a dynamic contractile actomyosin network during *Drosophila* abdominal morphogenesis

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During morphogenesis, various cell behaviours, such as cell migration and apical constriction, need to be coordinated, but it is still elusive how this coordination is achieved. Morphogenesis of the adult abdominal epidermis of *Drosophila* involves the larval epithelial cells (LECs) being replaced by the adult histoblasts. Before undergoing apoptosis, the LECs migrate directedly, constrict apically and delaminate. We studied the mechanisms underlying the transition from migratory to constrictive behaviour. We show that the apicomedial actomyosin network of the LECs undergoes pulsed contractions, which are associated with changes in apical cell area. A change in the polarity of this contractile network underlies the change in LEC behaviour. Migrating cells show planar polarity, protruding at the front while undergoing pulsed contractions in the back. In contrast, the contractile network displays radial polarity during constriction, with contractions being localised centrally. This change in LEC architecture seems to be crucial for the positioning of contractile activity in the cell. Furthermore, we explored properties of the contractile network by manipulating LEC contractility, interfering with the small Rho GTPase Rho1, Rho kinase and Myosin phosphatase. We show that the level of a cell's contractility affects not only pulsatile behaviour, but also cytoskeletal architecture and cell behaviour. Similar to cells during germband extension, only those LECs that have intermediate levels of myosin II activity undergo pulsed constrictions. We also show that LECs with high levels of contractility constrict without undergoing pulsed contractions, which raises questions about the roles the pulsing apicomedial network and the cortical network play in morphogenesis. Overexpression of Rho1 in LECs furthermore revealed that when contractility in cells is increased, pulsing does not lead to rhythmical contractions of the apicomedial network but to cyclic assembly and disassembly of the apicomedial network accompanied by the recruitment of actin to the cell cortex.

A41 | Investigating the function of a novel nuclear protein in tissue penetration of *Drosophila melanogaster* macrophages

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Migrating cells penetrate tissue barriers during development, inflammatory responses and tumor metastasis. *Drosophila* embryonic macrophages migrate out of the head mesoderm to move through a tissue barrier into the germband, displaying molecular parallels to vertebrate immune and cancer cells. We seek to discover novel components underlying macrophage invasion, with the hope that such findings could ultimately lead to the treatment of autoimmunity and cancer.

Through RNAi screening, I identified a conserved nuclear protein of unknown function, called CG9005, which autonomously affects germband penetration, without altering total macrophage number or migration along another non invasive route. Live imaging of macrophages labeled with a nuclear marker showed that macrophages in a CG9005 mutant migrated from their initial position in the delaminated mesoderm up to the germband with similar speed and persistence compared to the control. However, CG9005 mutant macrophages paused at the germband edge for a longer time than the control macrophages before entering. Moreover, I found that the migration speed of the first macrophage as it moves into the germband between the mesoderm and ectoderm is significantly slower in the CG9005 mutant.

Bioinformatic analysis predicts that CG9005 contains nuclear localization signals (NLS), nuclear export signals, and transcriptional activation domains (TAD) as well as DNA binding motifs. Consistent with these predictions, in a macrophage derived cell line, CG9005 localizes in the nucleus, cytoplasmic particles, and at the cell membrane. In embryonic macrophages CG9005 is mainly found in the nucleus. These findings argue that this protein could regulate macrophage migration at the transcriptional level to facilitate the early steps of tissue penetration.

To determine which genes might be transcriptional targets, I performed RNA sequencing on *CG9005* mutant and wild type macrophages. In *CG9005* mutant macrophages, 27 genes were downregulated, while 41 genes were upregulated. I selected potential CG9005 target genes to further characterize based on their having at least a 10-fold change in expression, embryonic expression pattern and potential functions. I found by RNAi knockdown that two genes whose higher expression requires CG9005, a helicase and a glycoxylate reductase, are needed for the germband penetration of macrophages. This argues that these genes could regulate germband migration downstream of the CG9005 protein. Interestingly, the helicase, called Porthos, shows a similar embryonic expression pattern to that observed for CG9005 in embryos. The vertebrate ortholog of this helicase is involved in the biosynthesis of specific proteins. I will test to see if the forced expression of Porthos or glycoxylate reductase can rescue the CG9005 mutant phenotype. I will additionally perform ribosomal profiling to identify Porthos targets that could ultimately regulate macrophage invasive migration.

To further unravel the function of CG9005 in macrophage migration I am testing the effect of deleting CG9005's NLSs, TADs and other motifs. I am trying to rescue the CG9005 mutant with its mammalian orthologs, FAM214A and B, to determine if they conserve CG9005's function. I am also analyzing the nuclear properties of macrophages during germband penetration in the wild type compared to the CG9005 mutant by live imaging.

A42 | Deficient protein O-mannosylation affects signalling pathways in vertebrates

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Post-translational modifications of proteins diversify the means of communication, within and between cells. As such, cell-surface interactions (e.g. cell adhesion) can be influenced by the glycosylation of receptors and cell membrane proteins. However, our understanding on how particular glycan moieties enhance or tweak the function of their target proteins is still very rudimentary.

The aim of this study was to identify critical glycosylation sites, specifically O-mannosylation on signalling pathway proteins and unravel their context-dependent functions. Medaka (*Oryzias latipes*) mutants for the protein O-mannosyltransferases *pomt1* and *pomt2* have been established. These mutants reveal phenotypes that are clearly reminiscent of defective signalling pathways. Preliminary live-imaging indicates that *pomt2* mutants abort somitogenesis, yielding embryos with severe posterior truncations.

Through in-depth live-imaging of mutants, affected signalling pathway proteins will be determined. Combined with glycoproteomics, a clear connection will be drawn between deficient O-mannosylation and the affected protein function. Furthermore, CRISPR/Cas9-derived base editors have been tested in the context of this work and show high efficacy. Ultimately, this approach will validate O-mannosylated amino acids for the full functionality of the glycoprotein and provide access to therapeutic approaches for congenital disorders of glycosylation.

A43 | Progenitor amplification is the rate-limiting step in determining retinal size in medaka

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Teleost fish present a large group of phenotypically variable fish that live in a wide range of different habitats and environmental conditions. To accommodate such diverse needs many features have been adapted to habitat, including the visual system, where retinal size, architecture and cell type composition vary significantly between species. An additional feature of teleosts is their life-long postembryonic growth. Throughout life, organs have to scale in size proportional to increase in body length while simultaneously maintaining functionality. In medaka (*Oryzias latipes*), multipotent retinal stem cells give rise to all retinal cell types, which are integrated into the organ at its periphery. Since function and shape of eyes are closely linked, activity of stem and progenitor cells is tightly coordinated to establish proper cell type composition and number. The medaka retina displays two different growth modes, with increase in circumferential as well as radial size responsible for area and volume expansion. Thickness of the retinal layers, however, does not change over time.

In my project I am using targeted modulation of signaling pathways in retinal stem and progenitor cells to address mechanisms of growth control in the retinal stem cell niche. I found that activation of the insulin-like growth factor (IGF) signaling pathway in retinal stem and progenitor cells is sufficient to stimulate massive proliferation resulting in a phenotype with enlarged eyes. Here, the retina not only grows in radial and circumferential size but also in thickness of the individual layers. Strikingly, such changes cannot be observed when IGF signaling is activated only in retinal stem cells proper. Furthermore, another mitogenic signal expressed in retinal stem and progenitor cells could not recapitulate the phenotype of the large-eyed fish either.

I will present data to show that the increase in cell numbers and retinal size originates from amplification of the progenitor but not stem cell population. Furthermore, I will show my analysis regarding cell cycle length and population size of retinal progenitor cells in large-eyed and wildtype fish. Lastly, behavioral experiments with large-eyed and wildtype fish investigating swim depth preference are currently ongoing.

A44 | Study of human ovarian development & dysgenesis mechanisms in a *Drosophila* model

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The orchestrated signal transduction mechanism and genes underlying ovarian development are poorly understood. XX-Ovarian Dysgenesis (XX-OD) is a rare heterogeneous genetic disorder characterized by underdeveloped, dysfunctional ovaries. Using homozygosity mapping and whole exome sequencing, combined with bioinformatics, we identified a novel homozygous missense mutation in the Nucleoporin107 (Nup107, c.1339G>A, p.D447N) gene as the candidate causative mutation for XX-OD in a consanguineous family with multiple affected females. Nup107 is an essential component of the nuclear pore complex.

Drosophila has proven to be a useful model for human diseases in which orthologous genes exist. To assess the possible role of Nup107 in ovarian development, we created Drosophila flies carrying the specific mutation corresponding to the human mutation found in the family. Strikingly, the transgenic Drosophila females were almost completely sterile with a marked reduction in progeny, morphologically aberrant eggshells and disintegrating egg chambers with increased apoptosis, indicating defective oogenesis. A closer look at the mutant ovaries demonstrated an ovarian dysgenesis like phenotype, where nearly 40% of the female mutant flies had either non-developed or under-developed ovaries. Upon analysis, we found that the larval gonads were fully present in the mutant larvae, indicating that the developmental failure must occur along the way and that the underlying genetic causes are already present at the larval or even embryonic stages. Consistent with this notion, we found that mutant larval gonads contained excess primordial germ cells (PGCs) and lacked intermingled cells (ICs), suggesting problems in the essential soma-germline interactions required for both growth and differentiation. These results suggest a pivotal conserved role for this nuclear pore gene in ovarian development.

Transcriptome analysis of WT and mutant larval and adult ovaries identified several dozen candidate genes whose expression is significantly affected by the mutation. In addition, we discovered a group of genes with unknown functions, all of whom are highly expressed in the larval gonad and not expressed in the adult ovary, suggesting them to be critical developmental genes. Most of our candidate genes have human orthologues, further underscoring their relevance to human ovarian development. To investigate the role of our top candidates in ovarian development, we perform a functional RNAi KD screen using soma (Tj-Gal4) and germline (nos-Gal4) drivers. Our preliminary results are promising and result in severe, tissue-specific ovarian phenotypes.

Our systematic investigation using this XX-OD *Drosophila* model system will elucidate signaling pathways and new players involved in ovarian development and dysgenesis.

A45 | Exploring the functional conservation of a deeply conserved animal microRNA

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MicroRNAs (miRNAs) are a broad class of post-transcriptional repressors that expanded with the increase in animal complexity, and have been proposed to rise cellular diversity. The relative ease with which novel miRNAs and miRNA target sites can be acquired or lost during evolution, also supports the idea that they could facilitate the acquisition of new functions during development. However, the fact that numerous miRNAs are deeply conserved from nematodes to vertebrates also suggests that they have acquired important, shared functions in animal development or physiology.

miR-1 is one of the most conserved microRNAs from *C. elegans* to humans in terms of sequence, specific expression and function in muscle. Moreover, miR-1 is the only known microRNA whose predicted targets show a remarkable level of conservation across evolution. In *C. elegans, Drosophila,* zebrafish, mouse and human, miR-1 has predicted binding sites in the 3'UTRs of multiple subunits of the vacuolar ATPase (V-ATPase) complex[1-3]. However, no experimental evidence supporting this predicted interaction is functionally relevant has been reported, in any model system. On the contrary, only phylogenetically unrelated targets have been implicated in miR-1 function, in the different animal models. Here, we set out to address whether miR-1 has a functional relationship with the V-ATPase complex that could account for its conservation.

In higher organisms, loss of miR-1 causes lethality due to heart problems but, in *C. elegans,* miR-1 deficient animals are viable. Together with the powerful genetic tools available to study miRNA function, this makes this animal a great model to uncover this relationship. We have analyzed the expression pattern of different subunits of the V-ATPase complex in miR-1 mutant animals compared to wild type ones. We have found an upregulation of *vha-1*, *-12* and *-14* in muscle cells after the depletion of the miRNA. In addition, we have identified a number of physiological defects in miR-1 mutant animals: their mitochondrial network integrity is compromised, and they show an impaired proteostasis. In addition, these animals display a defect in pharyngeal pumping and reduced basal level of calcium in pharyngeal muscles. Our results uncover a new role for miR-1 in pharyngeal and skeletal muscle and provide functional readouts that we can use for further genetic studies. In the longer term, by analyzing the link between the V-ATPase complex and miR-1 in other species, we expect to find conserved pathways required for the establishment of muscle identity and function.

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A46 | Epithelial rearrangement dynamics during mouse neural tube development

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Spinal cord formation in amniotes starts from a flat epithelial sheet, the neural plate, which extends and folds at the embryo midline to form a closed neural tube. This morphogenetic transition is accompanied by changes in cell size and shape, and in the rates of cell division and differentiation. Previous studies have suggested that the frequency of epithelial cell rearrangements might also change over time, but the basis for this is unknown. To investigate the temporal dynamics of epithelial rearrangements in the neuroepithelium, we are performing lineage tracing analysis and high resolution imaging at different developmental stages. Consistent with previous results, we observed that clones of related cells appear more spatially fragmented when generated at E8.5 than at E9.5 of development. To understand how processes such as cell proliferation, changes in cell geometry, and mechanical forces affect clonal fragmentation, we are implementing a vertex model of the mouse neural epithelium. We identified model parameters that reproduce closely the cell shapes measured at the apical surface. We are currently performing a screen for parameters that can reproduce the temporal changes in the epithelial organization and clone fragmentation. We will test the model predictions using a combination of mouse genetics and ex vivo embryo culture assays. Overall, the quantitative understanding of epithelial dynamics that we obtain in this study will provide insight into how cell rearrangements may affect pattern formation in the neural tube.

A47 | Balanced Rho activation and inhibition regulates exocytosis by large secretory vesicles

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The small GTPase Rho governs actomyosin-based contractility in a wide variety of cellular settings, by the parallel induction of actin polymerization and of myosin II recruitment and activation. Regulation of Rho function is mediated by guanine exchange factors (GEFs) and GTPase activating proteins (GAPs), which stimulate and inhibit Rho activity, respectively. We have been exploring the dynamics and molecular design of this fundamental circuitry in the context of exocytosis. Specifically, we study secretion of “glue” proteins from the epithelium of the *Drosophila*salivary gland as a model system. Glue protein exocytosis is achieved via uncommonly large secretory vesicles (>5 um in diameter). Rho-mediated assembly and contraction of an actomyosin coat that forms around these vesicles upon their fusion with the apical cell membrane, is critical for release of the glue material into the gland lumen. We have identified RhoGEF2 as the activator of Rho in this setting. RhoGEF2 is recruited to the fused vesicles, and its function is essential for activation of Rho (as monitored with an active Rho sensor) on the vesicle surface, and for vesicle contraction. Interestingly, an actomyosin coat still forms around the vesicles following knockdown of *RhoGEF2*, likely generated by basal levels of Rho-GTP, which diffuse from the apical membrane following fusion. RhoGEF2 recruitment is actin dependent, implying an amplification mechanism for establishing a coat sufficiently robust to enable contraction. This process is counteracted by a dedicated RhoGAP, RhoGAP71E, which is also recruited by actin, and whose inhibitory function is also essential for vesicle contraction. Content release from the giant secretory vesicles thus appears to require a finely timed balance between Rho activation and inhibition. A major challenge is to determine how temporal order is maintained within this circuitry, particularly since actin appears to serve as a shared recruiting element of both activating and inhibiting factors.

A48 | Elevated Wnt-signaling leads to differentiation and restriction of potency of single stem and progenitor cells in vivo

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Wnt-signaling has been extensively studied previously in vitro and by drug treatments. However, it remains unanswered how single cells react to a change of their Wnt-signaling status in vivo. A suitable model for this is Medaka, which harbors postembryonic stem cells in most tissues, facilitating its life-long growth. Therefore, retinal stem cells can be used to investigate the effect of Wnt-signaling on stem and progenitor cells.

To address the response of single cells to change in Wnt- signaling, we established a functional lineage tracing approach, which upon recombination leads to a genetically stable, continuous overactivation of the Wnt-pathway (ubi::>mCherry>eGFP-dominant negative GSK3). Recombination can be spatio-temporallly controlled by expression of Cre-ERT2 with tissue-specific promoters. This allows investigation of the response to Wnt activation in single cells in a wildtypic environment.

We observed a differential response of stem and progenitor cells. Recombination in stem cells leads to a progression in differentiation, where lineages are more often terminating upon the elevation of Wnt-signaling. Early progenitor cells show a similar behavior, with an even higher ratio of terminating clones and a shift in cell type composition of very small clones. Recombination in late progenitor cells leads to a decrease of differentiation potential. Consistent between clones of all origins is the reduction of diameter clone size and the increase of single positive differentiated cells, suggesting increased differentiation and decreased potency upon overactivation of Wnt-signaling.

In conclusion, overactivation of Wnt-signaling in vivo leads to differentiation and restriction of potency of stem and progenitor cells.

A49 | Elucidating the functional role of Hif during brain development

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Eukaryotic cells have developed mechanisms to sense and adapt to hypoxia, a condition where oxygen demand exceeds oxygen supply. The master regulators of the hypoxia response and adaptation in physiological and pathological conditions are the hypoxia inducible factors (Hif). To counteract the negative effects of oxygen deprivation they activate transcription of genes involved in multiple biological processes such as metabolism, erythropoiesis, cell migration or angiogenesis. To understand the role of Hif in angiogenesis we developed a novel transgenic reporter line in zebrafish using a promoter of hypoxia-response elements (HRE) to control expression of UnaG, an oxygen-independent fluorescent protein. Binding of Hif to the hypoxia-response elements activates UnaG expression and therefore allows us to visualize HIF activity in vivo at a cellular level. We validated that this reporter line responds to hypoxia and Hif1a activity. To our surprise we detect strong and specific Hif activity during development independent of physiological hypoxia in the notochord, the heart, brain vasculature and a subset of neurons. Despite the extensive research directed to understand the role of the Hif signaling response to hypoxia in physiological or pathological conditions, little is known about the contribution of Hif signaling to developmental processes. Therefore, we aim to elucidate the functional role of Hif in regulating endothelial development in the zebrafish embryo.

B | Neural Development

B01 | Breaking symmetry: Glial signaling induces lateralization of a *Drosophila* central brain circuit

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Morphological left-right (L/R) asymmetry is a main principle of animal body plans critical for organ function and behavior with defective lateralization leading to severe pathologies in human. Genetic analysis in both vertebrate and invertebrate models has revealed a number of distinct pathways and mechanisms important for establishing L/R asymmetry and for spreading it to tissues and organs. Although morphological and functional lateralization of the central nervous system is established throughout the animal kingdom the underlying developmental mechanisms which specify how and when initial symmetric organization becomes broken are poorly understood. Here we show that glial cells at the central midline are a main organizer of Drosophila brain circuit lateralization.

The central complex (CX) of the adult Drosophila brain is a group of highly interconnected bilateral neuropils critical for multi-modal sensory integration and motor behavior control. We could show that the CX circuit receives specific unilateral synaptic input from a bilateral cluster of ventral projection neurons with a defined L/R connectivity pattern which result in synaptic neuropile only within the right hemisphere. Developmental analysis revealed an initial bilateral symmetric innervation of projection neurons in a continues synaptic layer spanning the midline which undergoes lateralized neuronal remodeling. Target RNA interference of candidate genes identified conserved signaling components of the PCP pathway required not only in projection neurons but also in adjacent midline glial cells to break innervation symmetry. These results provide novel insights into the context-dependent application to translate cellular asymmetry into brain circuit lateralizati

B02 | 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. *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 analyse the potential functional redundancy of the co-expressed *rx1* and *rx2* genes, we established homozygous single and double mutants, of which only the latter show a severe phenotype, emphasizing rx1 and rx2 function in early and late retinogenesis.

B03 | Split-Brain in a fly: Developmental mechanism underlying bi-lateral nervous system organization

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The two brain hemispheres of bilateral-symmetric nervous systems are highly interconnected by diverse commissural neurons. Famous studies by Roger Sperry paved the imporatnce of bilateral connectivity by carrying out callosotomy on epileptic patients to prevent seizures, since then we know the asymmetric nature of brain organization and the importance of interhemispheric connection. However, the molecular mechanisms underlying the coordinated development of neural circuits within and between brain hemispheres are poorly understood. Using the Drosophila olfactory system, in which sensory neurons directly connect the mirror symmetric olfactory brain regions via a contra-lateral branch, we are addressing the cellular and molecular mechanisms underlying the precise coordination of axon branch formation and synaptic partner recognition during olfactory circuit assembly.

Cell adhesion molecule Neuroglian (Nrg) has been shown not to affect ipsi-lateral olfactory receptor neuron (ORN) targeting but result in a specific loss of the contralateral sensory axon branch. We found that in addition to projecting ORNs, *Nrg* is expressed and function in a small cluster of bi-lateral olfactory interneurons which pioneer commisural tracts before sensory axon arrival. Cell specific knock down of *Nrg* via tagreted RNAi in bi-lateral interneurons affects the contra-lateral targeting of ORNs. In a series of cell-type specific loss and gain-of-function studies we could show the developmental interactions between different neuronal types of central and peripheral neurons coordinating the organsation of bilateral symmetric olfactory system.

B04 | $GABA_B$ signaling regulates early development and neurogenesis in the sea anemone *Nematostella vectensis*

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Cnidarians (sea anemone, coral, jellyfish, hydra), dated to 700 million years ago, are among the first multicellular organisms to evolve a nervous system. Although simple and non-centralized, their nervous system shares many components and associated genes with those of higher organisms, including humans. Here, we show that in the basal sea anemone *Nematostella vectensis*, the $GABA_B$ signaling pathway plays important roles in the developmental transitions and specifically in neural development. We identified 8 homologs of $GABA_B$ receptors in *Nematostella*, and demonstrated using sequence and structure analysis that four of these homologs are tentative $GABA_B$-R1 receptors with putative binding sites for GABA and the $GABA_B$ agonist baclofen. Indeed, application of baclofen inhibited the larva metamorphosis program, neurogenesis and ciliogenesis. This effect was reversible and after agonist removal larva development resumed. Using RNA-Seq, we further demonstrate that baclofen treatment resulted in downregulation of numerous neuronal-related genes, whereas soxB1, a negative regulator of neurogenesis in bilaterians, was upregulated. Additionally, we show that larva motility was affected by baclofen and that foxj1, a master regulator of motile cilia, was downregulated.

Our findings suggest an ancestral role for $GABA_B$ signaling that is shared across divergent organisms, and that $GABA_B$–dependent pathways function as negative regulators of neural development and differentiation in cnidarians

B05 | Coordinated cytoskeleton disassembly pathways during dendrite pruning in *Drosophila*

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Pruning, the elimination of unwanted or unspecific synapses or neurites, is an important specificity mechanism during neuronal morphogenesis. Drosophila sensory class IV dendritic arborization (c4da) neurons prune their larval dendrites at the onset of metamorphosis, while their axons stay intact. C4da neuron dendrite pruning is induced by the steroid hormone ecdysone and occurs by a mechanism involving local degeneration and dendrite severing in proximal dendrites. We could recently show that local loss of microtubules in proximal dendrites predetermines severing sites and is induced by the kinase PAR-1 (Herzmann et al., 2017, EMBO J., and Herzmann et al., 2018, Development). How local microtubule loss is coordinated with disruption of other dendrite-stabilizing cytoskeletal components and with eventual dendrite severing is unclear. Here, we show that disruption of dendritic actin filaments is required for dendrite pruning. We show that the phosphatase PP2A is a regulator of dendrite pruning through a role in actin disassembly. PP2A interacts genetically with the actin severing factors cofilin and Mical, and in the absence of PP2A activity, c4da neuron dendrites develop actin aggregates. Our analyses suggest that PP2A acts by limiting the amount of freely available actin to influence actin dynamics. Thus, both regulation of actin levels as well as increased actin severing by the previously identified Mical act together to destabilize dendritic actin during pruning. Interestingly, PP2A inhibition also appears to stabilize dendritic microtubules, indicating that cytoskeletal disassembly pathways are tightly linked during dendrite pruning. Our results provide an integrated view of cytoskeleton disassembly necessary for developmental neurite remodeling.

B06 | Elucidating the multi-functionality of Semaphorin3A/Plexin-A4 signaling

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Proper wiring of the nervous system during development is largely regulated by extracellular cues and their ability to induce diverse cellular responses through activation of receptors on the surface of developing neurons. Semaphorin (Sema)3A signaling thought the Neuropilin-1/Plexin (Plxn)A4 receptor complex had been demonstrated to promote basal dendrite arborization of cortical pyramidal neurons, and to repel CNS and PNS axons both *in vitro* and *in vivo*. How the same ligand-receptor pair induce these distinct cellular responses is largely unknown. We previously suggested that diverse responses elicited by Sema3A signaling could be found at the receptor level, within the cytoplasmic domains of PlxnA4. Here, we generated a new PlxnA4 mouse line where the conserved triplet of basic amino acids KRK, in the cytoplasmic domain of the receptor, were changed to AAA. We found that the KRK-motif is required for Sema3A-induced dendritic elaboration of cortical neurons both *in vivo* and *in vitro*. In contrast, this motif is dispensable for growth cone collapse and axon repulsion of DRG sensory neurons, and the formation of the anterior commissure, which is absent in the PlxnA4 null mutants. Furthermore, we show that the RhoGEF Farp2, which we demonstrated to bind the KRK motif of PlxnA4, is also required for dendritic arborization but not for inhibitory guidance events. Lastly, activation of the downstream target of Farp2, Rac1 GTPase, is required for Sema3A-induced dendrite elaboration but not for growth cone collapse *in vitro*. Collectively, results from our study shed new light on the mechanisms that allow the same ligand-receptor signaling pair to trigger distinct cellular responses in different neuronal cell types, and uncover a signaling pathway that is initiated from a distinct cytoplasmic motif in PlxnA4 and specifically controls dendrite arborization of cortical neurons in response to Sema3A.

B07 | Role of FGF signaling in regulating the stem/differentiation state of hindbrain boundary cells

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Hindbrain boundaries (HBs) serve as compartment borders segregating adjacent rhombomeres in the developing embryo. Recently it was discovered that these boundaries contain an abundant population of neural stem/progenitor cells which support the hindbrain development in the chick embryo. Previous studies have shown that the secreted signal FGF3 and all FGF receptors (FGFRs) are present in the chick hindbrain, mainly in HBs and are essential for the expression of different boundary markers. However, the specific role of FGF and its signaling pathways in HBs remains vague. FGF signals display various roles in the CNS including survival, self-renewal, fate specification, proliferation and differentiation. Depending on the context, FGFs can induce the expression of stem/progenitor cells markers such as Sox2. In other cases, FGFs may promote cell differentiation and migration of neural cells in order to support their development. In this research, we aimed to evaluate the effects of FGF signaling on HBs cells in the chick embryo, in order to determine its role in regulating their stem or differentiation state during development of the hindbrain.

To analyze FGF role in HBs we executed a loss-of function approach using the FGFR inhibitor SU5402 in vivo and in primary hindbrain cell culture. For the *in-vivo* experiments, E2.5 chicken embryos were treated with DMSO (control) or SU5402 (treatment) soaked beads which were inserted into the hindbrain lumen. Immunostaining as well as flow-cytometry analyses were performed to assess the expression of selective stem/differentiation boundary markers that are suspected of being active downstream to FGF. Expression of activated ERK1/2, the ECM boundary marker CSPG and the neural stem cell marker Sox2 was modified following treatment with SU5402. In addition, treatment of hindbrain cultures with SU5402 led to a reduction in neurosphere collapsion and spreading of cell monolayers which reflect differentiated cells. Currently, experiments are conducted to purify HBs cells and examine whether inhibition of FGF signaling affect their cellular/molecular properties compared to the rest of hindbrain cells. Moreover, we are utilizing genetic-based methods to block FGF signaling locally using CRISPER/Cas9 and dominant-negative plasmids.

Collectively, this study will reveal the importance of FGF signaling for the development and function of HBs, and its role in regulating cell fate determination in the embryonic hindbrain.

B08 | Regulation of neural tube growth by the morphogens Shh and BMP

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In the developing vertebrate neural tube a dorsoventral pattern of neural progenitor cell types is determined by opposing morphogen gradients of Sonic hedgehog (Shh) and Bone Morphogenetic Proteins (BMP). These pathways have also been implicated in regulating progenitor proliferation, differentiation and apoptosis as well as neural tube size. However, the precise contribution and spatial-temporal requirement for these pathways in controlling neural tube growth is still poorly understood. To address this question, we are using mouse genetics and *ex vivo* chick and mouse assays to alter Shh and BMP signaling activities in a temporally controlled manner. Our preliminary data indicates that loss of Shh activity does not affect progenitor proliferation, but induces a transient burst of apoptosis across the dorsoventral axis in developing mouse embryos. In chick whole embryo culture, inhibition of either Shh or BMP signaling, results in increased cell death. In this assay, cell survival can be rescued by overactivation of either of the two pathways. This suggests that the two pathways independently control the cell survival machinery or that there is an upstream cross-talk between them. To distinguish between these possibilities, we are combining genetic and genomic approaches. Our aim is to obtain quantitative understanding of how the combined interpretation of Shh and BMP signaling contributes to growth control at the tissue level. These results will provide insight into the role of morphogens in coordinating tissue pattern and size.

B09 | Immunostaining for neuropeptides identifies the neural circuits controlling behavior in *Hydra* described by Dupre and Yuste (2017)

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Using calcium imaging Dupre and Yuste (2017) described four neural circuits in *Hydra* associated with specific behaviors. The neural phenotype of cells constituting these circuits was unknown. Using immunostaining to neuropeptides and transgenic animals we have successfully identified these neural circuits.

Staining with antibodies against three neuropeptides (Hym355/vasopressin, GLWa and Hym176A) revealed neuropeptide-positive nerve cells in the ectoderm in all parts of the animal. GLWa and Hym355/vasopressin were always co-expressed in the same cells; Hym176A was expressed in a different set of nerve cells. Both nerve cell types formed networks throughout the ectoderm. By staining Hym176 transgenic animals we could show that these two networks are distinct. Comparison of the detailed localization of the GLWa and Hym176 networks to the calcium imaging patterns of Dupre and Yuste revealed that the GLWa network corresponds to the RP1 neural circuit and the Hym176 network to the CB neural circuit.

Immunostaining for GLWa also revealed a network of GLWa-positive ganglion cells throughout the endoderm of the body column, but not in the tentacles. Based on their localization, these cells correspond to the RP2 neural circuit described by Dupre and Yuste.

RFa-positive nerve cells were only present in the ectoderm. Based on co-staining experiments, RFa-positive cells in the peduncle correspond to the Hym176A-positive cells and thus are involved in the CB neural circuit. RFa-positive cells in the upper body column below the tentacles probably correspond to the neural circuit controlling nodding behavior.

B10 | PP2A-29B regulates actin disassembly during dendrite pruning in *Drosophila*

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The developmentally regulated degeneration of axons and dendrites without loss of the parent neuron, also known as pruning, is an important specificity mechanism during neuronal circuit formation. In holometabolic insects like *Drosophila melanogaster*, pruning occurs at large scale during metamorphosis in an ecdysone-induced manner. Sensory class IV dendritic arborization (c4da) neurons specifically prune their dendrites at the onset of the pupal phase. During this process, dendrites thin extensively in their proximal regions, where they are eventually severed. Little is known about the mechanisms that lead to local dendrite destruction. Previous studies from our lab have shown that local microtubule disassembly drives dendrite thinning and contributes to severing site localization. Whether regulation of other components of the cytoskeleton also contributes to pruning is not known. Here, we identify the phosphatase PP2A as a pruning regulator that is involved in actin disassembly by interacting with the acting severing protein Mical. Furthermore, loss of PP2A also leads to stabilization of microtubules in pruning dendrites. Our results reveal a novel dendrite destruction pathway and suggest extensive coordination in cytoskeletal disassembly during pruning.

B11 | TRIM29, a E3-ligase involved in neural tube closure and neural crest specification

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Tripartite motif protein 29 (TRIM29) was identified as an AT group D-complementing gene (ATDC) due to its ability to complement the radiosensitivity defect of AT fibroblast cell lines. The TRIM protein family of RING-type E3 ligase is characterized by the presence of a tripartite motif composed of a RING domain, one or two B-box domains and a coiled-coil domain. E3 ubiquitin ligases are enzymes that function as scaffold proteins mediating the interaction between E2 ubiquitin-conjugating enzyme and the substrate. TRIM29 is an untypical member of the family since it lacks the RING domain. TRIM29 has been reported to be overexpressed in several cancers including esophageal, breast, lung, bladder, colorectal and pancreatic cancer. During Xenopus larval development, *trim29* is expressed exclusively in non-neural ectoderm including the region of which neural crest cells emerge. Suppression of trim29 function led to severe neural tube closure defects accompanied by a loss of neural crest specification. Interestingly, those cells that are supposed to contribute to the closure of the neural tube, delaminate from the embryo and rest in the vitelline membrane as shown by live cell imaging. On the other hand, remaining cells in the injected side of trim29 morphants are characterized by a low number of mitotic (pH3) and apoptotic cells (tunel) as well forming a hyperplastic or hypertrophic structure of yet unknown specification. Ongoing work in the lab is focused on the identification of interaction partners and substrates for Trim29 by mass spectrometry-based approaches using embryos and cell culture.

C | Stem cells, organoids and disease

C01 | Mechanism of Floor Plate Induction in the Neuroepithelial Organoids

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Studies in developing embryos have shown the importance of the notochord-derived SHH morphogen for establishment of the ventral signaling center in the neural tube called Floor Plate (FP). However, it remains unclear if additional mechanisms exist, that allow progenitor cells to interact and self-organize to ensure robust patterning. To address this issue, we use the neural tube organoid system in which FP induction occurs in the absence of spatial cues. In this system neuroepithelial cysts derived from mouse embryonic stem cells form localized FP marked by the expression of both *sonic hedgehog (shh)* and *foxa2* genes upon global application of Retinoic Acid (RA). Remarkably, *foxa2* expression is induced prior to *shh* and independently from HH- pathway activity. To study the dynamics of self-organization at early time points, we created FoxA2:venus reporter cell line using the CRISPR/Cas9 system and performed time-lapse live imaging. Shortly after RA pulse, we find $FoxA2^+$ cells scattered throughout the tissue. Life-imaging studies have shown that initially stochastic activation of *foxa2* is followed by re-organization of $FOXA^+$ cells, which over a time-period of 50h gradually coalesce into dense cluster in which *shh* expression is later activated. When multiple $FoxA2^+$ clusters form in the same tissue, we find many cases in which multicluster organoid resolves in a single cluster organoid, whereas, one of the $FoxA2^+$ clusters, often the one with stronger $FoxA2^+$ signal, "wins" over the weaker clusters. Statistical analysis of these events demonstrates preliminary evidence that FoxA2 clusters are mutually repressive. Therefore, induction of the FP in neuroepithelial organoids doesn't require HH-signaling and occurs by self-organisation of $FoxA2^+$ cells in the absence of notochord tissue. To find regulatory pathways involved in this process we employ deep sequencing of total RNA from neural tube organoids. Uncovering of self-organization mechanisms in organoids might help in understanding of mechanisms of dorsoventral patterning of the neural tube in vivo.

C02 | Single cell analysis reveals a novel mechanism controlling neural stem cell plasticity in Alzheimer's disease model of adult zebrafish

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Neural stem cells (NSCs) is thereservoir for new cells and they can be harnessed for stem cell-based regenerative therapies in human brain. In Alzheimer's disease (AD), production of new neurons are suppressed due to hampered proliferative and neurogenic ability of NSCs. Therefore, understanding how the plasticity of NSCs could be induced would be important for designing stem cell-based therapies for AD. Zebrafish has a remarkable ability to regenerate its brain as it can induce NSC plasticity. However, it is still unknown whether the NSC population in zebrafish brain is heterogeneous and different subtypes could respond differently to disease pathology. We recently identified that NSCs enhance their proliferation and neurogenic outcome in an Amyloid-beta42-based (Aβ42) experimental Alzheimer's disease model in zebrafish brain and Interleukin-4 (IL4) is a critical molecule for inducing NSC proliferation in AD conditions (Bhattarai et al., 2016, Cell Rep; 2017a, Neurogenesis; 2017b, JoVE) and this regulation is also observed in human NSCs (Papadimitriou et al., 2018, Dev Cell). However, the mechanisms by which Aβ42 and IL4 affect NSCs remained unknown. Using single cell transcriptomics, we determined distinct subtypes of NSCs and neurons in adult zebrafish telencephalon and identified a novel and IL4-dependent crosstalk mechanism that controls neural stem cell plasticity in Alzheimer's disease conditions in adult fish brain and human 3D cultures. Our results constitute an extensive set of resource in the Alzheimer's disease model of adult zebrafish brain and provide unique insights into how Aβ42 and IL4 affects NSC plasticity and neurogenesis.

C03 | Far advanced network formation in retinal spheroids from chick embryo depends on cholinergic and glutamatergic differentiation

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Purpose: Already in 1984, our reaggregates from E6 embryonic chicken retina provided the first proof-of-principle that a complete retinal tissue reconstruction from dispersed cells is possible. Here we used so-called *rosetted spheroids*, which contain - besides photoreceptor precursor rosettes - areas corresponding to an inner plexiform layer (IPL, *in vitro* called *ipl*). This model allows to analyze cholinergic and glutamatergic effects on *ipl* formation. **Methods:** Such *rosetted spheroids* were cultured in absence or presence of 0.2-0.4 mM L-glutamate and analyzed by immuno- and enzyme histochemistry, RT-PCR, proliferation and apoptosis assays. **Results:** In untreated spheroids *ipl* formation *in vitro* (div) was announced after two days by cells expressing the cholinergic markers acetylcholinesterase (AChE) and choline acetyltransferase (ChAT). Comparable to *in vivo*, ChAT$^+$ *starburst amacrine cells* (SACs) became arranged pairwise within and at the outer *ipl* border, with processes forming two laminar *ipl* subbands. Concomitantly, individual vimentin$^+$ or transitin$^+$ Müller glial cell precursors (MCPs) in *ipl* centers co-expressed ChAT, which possibly functioned as "*ipl founder cells*". In L-glutamate-treated spheroids *ipl*s were disrupted, including loss of SACs and of MCs. Many Pax6$^+$ amacrine cells were lost, while rho4D2$^+$ rod photoreceptors were increased. Cell proliferation was slightly increased, while apoptosis remained unaffected. Glutamate effects were mimicked by NMDA and reversed by co-incubation with the NMDA receptor inhibitor MK-801. **Conclusion:** This study revealed i) a far-advanced differentiation of an IPL in retinal spheroids, as never described in any retinal organoid model before. ii) *ipl* sublamination was initiated by cholinergic precursor cells, which in turn iii) gave rise to both SACs (neurons) and glial MCPs; iv) these two cell types together organized *ipl* formation; and v) this process was counter-acted by NMDA-dependent glutamate actions.

C04 | Genome scale mapping of histone H3.3 turnover rate in mouse embryonic stem cells and during early differentiation

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H3.3 is variant of the H3 histone that has been implicated in the regulation of several epigenetic processes. The H3.3 variant accumulates modifications that are associated to open chromatin and gene activation. Despite of that, studies performed in embryonic stem cells (ESC) show enrichment of the variant H3.3 also in genomic areas that should be silent, such as telomeres and endogenous retroviruses (ERVs). Histone H3.3 turnover is replication independent, but little is known about the role of its dynamic turnover in transcriptional regulation during early development. To characterize the role of H3.3 in the regulation of retroviral sequences in pluripotent cells, we used mouse ESC lines carrying a single copy of doxycycline (Dox) inducible HA-tagged version of H3.3, and monitored the rate of H3.3 incorporation by ChIP at different time points following Dox induction. To follow the changes in H3.3 dynamics following differentiation onset, we monitored the cells before and after retinoic acid (RA) induced differentiation. To study the role of H3.3 in the silencing of exogenous retroviruses, we infected the cells with two different retroviruses and examined both the retroviral expression levels and the H3.3 dynamic at the integrated provirus site. Our results suggest that specific ERVs show fast H3.3 turnover, correlating with known retroviral-silencing chromatin marks, like Trim28 and H3K9me3 binding. Moreover, in many members of this group the turnover rate slows down following differentiation, signifying an important role for H3.3 turnover rate in the retroviral silencing machinery. Additionally, our preliminary data suggest that H3.3 is involved in MLV retroviral silencing and suggest an alternative mechanism for Trim28 binding to the retroviral LTR. This study is expected to provide insights into the basic biology that underlies retroviral silencing.

C05 | Ependymoglia behavior during postembryonic growth of the axolotl telencephalon

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Growth and successful regeneration rely on the presence of proliferating cells which generate the correct differentiated cell types. Post-embryonically generated cells need to integrate into the network of existing cells produced during embryogenesis. We are using the brain of the aquatic salamander axolotl (*Ambystoma mexicanum*) to investigate how the integration of newborn cells during growth and regeneration is accomplished. The axolotl serves as a great model to study such questions since it shows massive growth, increasing its body length up to 20-fold between larval and adult stages. Moreover, it displays extraordinary regenerative abilities as it is able to regenerate large brain lesions.

Currently, we focus on the cellular basis of the growing telencephalon. Proliferating ependymoglia lining the inner ventricle are the source of newly generated neurons which are located towards the outside of the brain. We are examining the long-term self-renewing abilities of ependymoglia, their heterogeneity as well as the neuronal output. I will present our current progress on Cre-loxP-mediated lineage tracing of individual ependymoglia cells and their progeny during postembryonic growth of the axolotl telencephalon.

C06 | Niche signalling in the *Drosophila* testis - cell fate choice or micromanagement?

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Niches are signalling microenvironments that are traditionally thought to assist stem cells in choosing between stemness and differentiation.

However, we could recently show that proliferation rate and differentiation propensity of the somatic cyst stem cells of the *Drosophila* testis can be uncoupled at the level of niche signalling input. We could trace an unbroken link from the niche signals through the stem cell transcription factor Zfh1 to stem cell proliferation. Importantly, none of these steps is sufficient for stemness.

In contrast, we found that, in addition to proliferation, the niche regulates stem cell metabolism at the level of transcription of individual metabolic enzymes, thus "micromanaging" stem cell physiology.

We have since mapped the transcriptomes of niche and somatic stem cells in the fly testis, their differentiated progeny, and tumour cells induced by Zfh1 overexpression that have long been used as proxies for the rarer stem cells.

Here we report that i) the transcriptional profile of the Zfh1 induced tumours differs fundamentally from that of endogenous, Zfh1 positive stem cells, invalidating them as a model and disproving sufficiency of Zfh1 expression for stemness, that ii) the stem cells possess a Warburg-like metabolic state regulated by the niche, and that iii) competition between stem cells in the *Drosophila* testis is directly stoked by the niche through activation of innate immune signalling in the stem cells.

Together our results favour a re-appraisal of stemness not as a cell fate but a physiological state that is continuously and dynamically regulated by the niche.

C07 | T-cell lineage decision is determined by temporal residency in specialized thymic microenvironments

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T-cells belong to the adaptive immune system and can be divided into two fundamentally distinct sublineages, which are defined by their expression of an αβ or a γδ T-cell antigen receptor (TCR). Both T-cell lineages develop from a common lymphoid progenitor derived from the hematopoietic tissue. The choice between αβ and γδ fate is the first lineage decision made by progenitors after they commit to the T-cell fate in the thymus. Based on mainly *in vitro* studies, so far, two models have been proposed to explain this biological process: The “stochastic-selective” model proposes that lineage commitment is stochastic and daughter cells commit to one and exclude the other lineage. The “instructive” model postulates that the strength of pre-TCR and γδTCR signals determine fate-choice. Given that lymphoid progenitors must follow a highly ordered migratory pattern within the thymus to develop into T-cells, the current models do not consider any role for thymic microenvironments in the divergence of αβ and γδ T-cells.

To study a possible role of environmental factors in T-cell lineage decision, we used medaka (*Oryzias latipes*) as *in vivo* model system because it enables us to monitor molecular and cellular activities in all developing T-cells at high spatiotemporal resolution in a fully functional thymus (Bajoghli et al., 2009; Bajoghli et al., 2015; Aghaallaei and Bajoghli, 2018). We found that αβ and γδ T-cells are spatially organized into two distinct thymic microenvironments. Upon entry into the thymus, progenitors have the ability to either stay within the same thymic niche to further develop as γδ T-cells or migrate into the center of the thymus to acquire the αβ T-cell phenotype. Consequently, when the intrathymic cell migration and positioning is genetically altered, developing T-cells are unable to leave the niche and shift towards the γδ versus αβ T-cell phenotype. In line with these results, a shift towards γδ lineage was also observed when the cytokine distribution was manipulated *in vivo* in the thymic environment. Collectively, our findings reveal that T-cell lineage fate is mainly determined by extrinsic factors and is not necessarily specified by TCR signal. Our results establish temporal residency of progenitors in a specialized thymic niche as a decisive factor for αβ and γδ T-cell development and provide insight into mechanisms controlling intrathymic cell positioning and face decisions. Currently, the high-content imaging data with high spatiotemporal resolution is being used to develop computational models to better understand T-cell fate decision in lower vertebrates.

C08 | Efficiency of in vivo Transfection of Primordial Germ Cells in Chickens at two Stages of Embryonic Development

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Transfections of Primordial Germ Cells (PGCs) either directly in embryos or in vitro in culture are the currently favored approaches for gene editing in avian species. Whereas in chickens the long term culture of PGCs is accessible, the direct in vivo transfection of PGCs could be used for various avian species. Different attempts had been reported for direct PGC transfection using either lipofectamin [1] or a cationic polymer (PEI)[2] with Piggy Bac and Tol2 as transposase, respectively. Moreover, the transgene was applied at stage 14 [3] or into non-incubated eggs. Although cocks with transgenic sperm had been reported in both studies, only Tyack et al. [1] were able to generate 5 out of 419 transgenic chicks.

In our study the efficiency of stable integration of Venus-reporter DNA using Sleeping Beauty (SB) as transposase was investigated after injecting 1) in the sub-germinal cavity of laid egg blastoderm, or 2) into blood vessels of stage 13-16 [3]. We observed a significant difference of transfection pattern between the two experiments. Firstly, the overall transfection efficiency 24h after injection varied between 40 and 80% after injections into the blastoderm. In contrast, in experiment 2, stable transfection of more than 90% was received. Secondly, a proportion of 6 to 20% of embryos treated at the blastoderm showed malformations. Similar effects were not recorded at stage 13-16 injections. Although 48 hours after transfection cells with Venus-reporter fluorescence accumulated especially in the germinal crescent of many samples in experiment 1, only few of these cells migrated into the embryos. Only 2 out of 98 embryos at embryonic day 10 (E10) of this series showed Venus-positive cells in the gonads.

In experiment 2, the transfection mix was applied into the blood at stage 13 – 16. We recorded at high vitality of embryos with up to 50% of Venus-positive cells in the gonads at E10. Immuno-histochemical labeling with SSEA1 confirmed a high proportion of stem cells migrating into the gonads. In an experiment with 80 embryos injected at stage 13-16, a total of 24 chicks hatched. From the 11 males, nine were successfully raised to maturity. From the eight cocks that provided semen samples, one cock revealed in five successive semen samples 0.1 to 0.2 % Venus-positive sperm. The Venus protein was localized in the midpiece of sperm.

In conclusion, the in vivo transfection of primordial germ cells in chickens was more efficient using stage 13-16 embryo. The proportion of transfected semen was at the same low level as in the study reported from Tyack et al. [1].

References: [1] Tyack et al. 2013 Transgenic Res 22: 1257-1264; [2] Jordan et al. 2014 J Biotech-nol 173: 86-89; [3] Hamburger & Hamilton 1951 J Morphol 88:49-9

C09 | Unveiling the molecular mechanisms of neural crest migration and formation of dorsal root ganglia using three-dimensional neural tube organoids

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Neural crest cells represent a multipotent cell population that gives rise to numerous cell types in the vertebrate organism, including sensory neurons that form dorsal root ganglia. To date, development of neural crest cell-derived structures is poorly understood. However, studying this *in vivo* is challenging due to several reasons. This is in part due to the fact that in biology same signaling pathways are used to specify different tissue types and thus perturbing one of these in the embryo might lead to pleiotropic effects. The recently emerged field of using organoids as an *in vitro* system to model complex developmental events in a simplified way can overcome these issues. In this work, I will use three-dimensional neural tube organoids to elicit the main factors and signaling pathways directing neural crest cell migration and differentiation into sensory neurons that bundle to form dorsal root ganglia. To address this, I will use a combination of immunohistochemistry, modern synthetic matrices to model an appropriate environment for organoid growth, well-established methods of molecular biology and genetics to perform screens and introduce genetic modifications, as well as advanced imaging techniques. Unveiling the molecular mechanisms of neural crest migration and formation of dorsal root ganglia will shed light on an important developmental biological process.

C10 | Atoh8 a novel regulator of TGF- signaling.

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The basic helix-loop-helix transcription factor Atonal homolog 8 (Atoh8; Math6) plays a crucial role in multiple developmental processes, iron metabolism and tumorigenesis. In the current study, we have generated an Atoh8 - Flag-tag mouse using CRISPR - Cas9 genome editing and evaluated the role of Atoh8 in cellular reprogramming, maintenance of pluripotency and early differentiation. Loss of Atoh8 disrupts mesenchymal-to-epithelial transition during reprogramming and primes pluripotent stem cells into mesendodermal fate. It can be considered as a regulator of reprogramming and pluripotent stem cell fate. Additionally, our results also demonstrate the participation of Atoh8 in SMAD dependent TGF beta signaling. Taken together, we propose Atoh8 as a negative regulator of TGF beta signaling.

C11 | Prediction and control of symmetry breaking in embryoid bodies by environment and signal integration

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In recent years, in vitro 3D models of early mammalian development, such as embryoid bodies (EBs) and gastruloids, have been successful in mimicking various aspects of the early embryo, providing accessible systems for the study of basic rules shaping cell fate and morphology during embryogenesis.
Using a high-throughput 3D live imaging, we study two early differentiation decision points in mESC-derived EBs: the formation of primitive streak and mesendoderm progenitors, and the downstream decision between mesoderm (ME) and definitive endoderm (DE).
Using Brachyury (Bry) as a primitive streak and mesendoderm marker in EBs, we show how mechanical, biochemical and neighboring cell cues affect the positioning of a primitive streak-like locus, determining the AP axis. We find that a Bry-competent layer must be formed in the EB before Bry expression starts. Bry onset locus selection depends on contact points of the EB with its surrounding, and can be maneuvered to occur at a specific locus, a few loci, or in an isotropic peripheral pattern. By spatially separating mechanical and biochemical signal sources, we show these two modalities can be integrated by the EB to generate a single Bry locus.
In contrast, the ME vs DE decision onsets in a spatially stochastic pattern. In both 2D colonies and 3D embryoid bodies, mesendoderm cells (Bry+) precede DE (Sox17+) cells at a preserved temporal gap, where Sox17+ cells emerge from within the Bry+ cell population in a 'salt and pepper' pattern. Soon after, cells undergo self-sorting, creating spatial separation between these germ layers. We study the signals and internal parameters affecting this decision, and its inter-relations with the processes of EMT and MET which these cells undergo, and the resulting cell movements.
The remarkably different strategies demonstrated in these two fate decisions enhance our understanding of basic principles in early development, with potential applications for organ and tissue engineering.

C12 | Subepithelial telocytes constitute the intestinal stem cell niche

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Stem cell niches provide essential signals and growth factors to sustain proliferation and self-renewal of stem cells in continuously self-renewing organs such as the intestine. In searching for the source of cells which provide the signals required for epithelial functional support, we recently identified a novel type of mesenchymal cells, telocytes that form a 3D network with the entire intestinal epithelium, from the crypt base to the tip of the villi. Telocytes are large, flat, cells characterized by extremely long cytoplasmic processes, which are separated from the epithelium by sub-micrometer distances. Intestinal subepithelial telocytes express the surface membrane platelet-derived growth factor receptor α (PDGFRα), while the transcription factor FOXL1 label their nuclei. Notably, telocytes express high levels of a wide range of regulators, both activators and repressors, of key signaling pathways such as the canonical and non-canonical Wnt, Shh, Bmp and Tgfβ. Intriguingly, telocytes compartmentalize transcripts depending upon their position along the crypt-villus axis in correlation to signaling gradients on the epithelium**.** In order to address the question of whether Wnt production by telocytes is important for intestinal stem cell activity, we derived a FOXL1-CreERT2 mouse line which enables temporally controlled gene ablation in telocytes. We then crossed FOXL1-CreERT2 mice with mice carrying a floxed allele of the X-linked Porcn gene, enabling the conditional ablation of all Wnt secretion from subepithelial telocytes. This resulted in a rapid loss of stem cell marker genes such as Olfm4, CD44 and Lgr5, a dramatic reduction in proliferation and no active Wnt signaling within the crypts, causing a complete disruption of the intestinal epithelium. This result clearly demonstrated that telocytes are necessary for Wnt pathway activation and proliferation in intestinal stem and progenitor cells.

D | Single cell approaches

D01 | Ancient animal genome architecture reflects cell type identities

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The level of conservation of ancient metazoan gene order (synteny) is remarkable. Despite this, the functionality of the vast majority of such regions in metazoan genomes remains elusive. Utilizing recently published single cell expression data from several anciently diverging metazoan species, we reveal the level of correspondence between cell types and genomic synteny identifying genomic regions conferring ancient cell type identity.

D02 | Single-cell transcriptome analysis elucidates similarities and differences between the population of intestinal stem cells and their progenitors

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The intestinal epithelium in *Drosophila* consists of a single layer of cells that is constantly renewing to ensure tissue homeostasis. A population of intestinal stem cells (ISCs) gives rise to an immature progenitor, called enteroblast (EB), which further differentiates into an absorptive enterocyte (EC) or secretory enteroendocrine (EE) cell type. To accomplish the different tasks in the digestive process, the intestine consists of distinct regions, which differ morphologically and functionally within both the differentiated epithelium as well as the intestinal stem/progenitor cell population. These regional differences are additionally reflected in the transcriptomic landscape of the intestinal tissue in *Drosophila*. Advances in single cell technologies and computational methods now enables us to elucidate the heterogeneity within the *Drosophila* midgut. We established methodologies for generating single-cell suspensions enriched for ISCs and progenitor cells which were then profiled by single cell RNA-seq. In accordance with published data, we found ISCs and EBs to have a similar transcriptional profile. More in-depth data analysis allowed us to separate ISCs from EBs and consequently a better characterization of both cell types. Novel marker genes were identified and confirmed by fluorescent *in situ* hybridisation (FISH). While regional information was not apparent in the stem/progenitor cell pool, the population of profiled ECs clearly comprises regional heterogeneity. Moreover, the single-cell data uncovered a previously unknown population of differentiated cells with distinctive transcriptional profile. We are currently performing follow-up experiments to decipher their in vivo functional role in tissue homeostasis. Additionally, the provided interactive visualization tool allows users to explore our single-cell transcriptome data for their specific interest.

D03 | Gene expression atlas of a developing tissue by single cell expression correlation analysis

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The wing imaginal disc is a well-studied model system for developmental processes as well as signaling pathways in Drosophila. In itself it is comprised of approximately 50,000 mainly undifferentiated stem-like cells. Many years of research led a collection of well-defined spatially expressed marker genes. However, a comprehensive atlas deciphering spatial gene expression in the wing disc has been missing. Here we used single cell RNA-seq and analyzed the data by a newly developed method, which is based on gene expression correlations rather than cell mappings.

Using this approach an expression map of all genes in the wing disc was created, identifying 824 genes with spatially restricted expression patterns. Known and new clusters of genes with similar expression patterns were identified and validated by RNA in-situ stainings. To prove the functional relevance of the clusters, the previously uncharacterized gene CG5151 that showed a similar expression pattern than wingless was studied in detail. In accordance with its spatial expression pattern, RNAi experiments showed its involvement in Wnt signaling.

This novel approach allows the utilization of complex single cell RNA-seq data for compiling gene expression atlases of undifferentiated tissues.

D04 | Molecular profiling of cells in the sea anemone *Nematostella vectensis*

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The model organism Nematostella vectensis belongs to the phylum Cnidaria, a group of virtually immortal animals who continuously replace cells of all different types over long time spans. The apparent absence of aging, as well as its regenerative capacities, makes Nematostella a compelling model for stem cell research. However, the identity of stem cells in this organism remains unknown. For this reason we perform a time series of whole-organism single-cell RNA sequencing, with the aim to identify stem cell-specific molecular signatures in our datasets. By capturing all cells that are present throughout the full life cycle, we can characterize the molecular profiles of the full diversity of cell types. In addition to this description of the cell type complement, we also use our sc-data to study cell differentiation, since comparing developmental trajectories of diverse cell types can give important insights into population structure, in particular the molecular programs creating different cell types. Molecular characterization of all cell populations, combined with comparative analyses of their developmental trajectories, will reveal how many distinct cell types each progenitor or stem cell population produces. Since Nematostella may be representative of the ancestral cnidarian state, these analyses will also provide important insights into the gene repertoire underlying ancestral cell stemness, and aid in our understanding of the evolutionary origin of stem cells.

E | Biophysics of Development

E01 | Increase in mechanical tension and E-Cadherin mobility facilitate cell extrusion in *Drosophila* epithelia

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A fundamental property of living tissues is to remodel themselves, either during development to acquire their final shape and function or after injury to recover their original form and function. During the development of epithelia, tissue remodelling involves the removal of cells through extrusion, a process that maintains the integrity of the tissue. How extrusion is regulated in the context of a remodelling epithelium remains however poorly understood.

In *Drosophila*, the abdominal epidermis is extensively remodelled during pupal stages. Polyploid larval epidermal cells are replaced by diploid adult progenitor cells termed histoblasts. Histoblasts are organized in groups of cells known as histoblast nests. During replacement, larval cells are removed from the epidermis through extrusion while histoblasts proliferate and the nests expand. Nest expansion is tightly coordinated with larval cell extrusion to maintain tissue integrity. Previous work showed that during early pupal development larval cells mainly extrude at the border to histoblast nests. Interactions between histoblasts and larval cells induce caspase-dependent apoptosis and extrusion of larval cells. Here we show using live imaging and cell tracking that larval cells during late pupal development also extrude away from the histoblast nests at the dorsal midline. Cell extrusion at the dorsal midline also requires caspase activity, but is independent of histoblasts and rather requires interactions with neighboring larval cells. Extrusion of cells at the dorsal midline in late pupal development involves a pulsatile actomyosin network rather than the formation of an F-actin cable as it is the case for cells extruding at the border to histoblast nests. Furthermore, mechanical tension and E-cadherin mobility are increased in larval cells during late pupal development as compared to early pupal development. Compromising membrane trafficking reduces extrusion of larval cells. We propose that increased mechanical tension and E-Cadherin mobility lead to increased tissue fluidity and thereby promote cell extrusion. Our work provides novel insights into the cell and tissue mechanical properties that facilitate extrusion of cells from epithelia.

E02 | Speed regulation of genetic cascades allows for evolvability and robustness in the body plan specification of insects

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During the anterior-posterior fate specification of insects, anterior fates arise in a non-elongating tissue (called the 'blastoderm'), and posterior fates arise in an elongating tissue (called the 'germband'). However, insects differ widely in how much of the AP fates are specified in the blastoderm versus the germband. Here we present a model in which patterning in both the blastoderm and germband of the beetle *Tribolium castaneum* is based on the same flexible mechanism: a gradient that modulates the speed of a genetic cascade of gap genes, resulting in the induction of sequential kinematic waves of gap gene expression. The mechanism is flexible and capable of patterning both elongating and non-elongating tissues, and hence, converting blastodermal to germband fates and vice versa. Using RNAi and heat shock perturbations, the mechanism was dissected and balstodermal fates were shifted to the germband, and germband fates were generated in blastoderm. We also suggest a molecular realization of our speed regulation model, in which a gradient regulates the switching between two enhancers: one enhancer is responsible for sequential gene activation, and the other for freezing temporal rhythms into spatial patterns. This model is supported by imaging enhancer activities in *Drosophila* in live embryos using MS2-MCP system.

E03 | The role of cellular replicative lifespan and stem cell dynamics on corneal epithelium homeostasis and pattern formation

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The anterior outer transparent part of the eye, the cornea, acts as a lens that focuses light into the eye. In addition, it serves as a barrier that protects the eye against external hazards and injury and thus maintaining its integrity and its continuous regeneration is crucial for proper vision in vertebrates. Stem cells reside in niches at the circumference of the cornea, the limbus, which separates the cornea from the conjunctiva replenish and maintain corneal homeostasis. Recent lineage tracing experiments resulted in spike-like patterns that extended from the limbus to the center of the cornea over time. The mechanism that underlies these centripetal dynamics is not fully understood.

We developed a novel mathematical model that capture the stochastic dynamics of epithelial cells and pattern formation in the cornea. Our model capture both short and long interaction range between cells. Moreover, we take into account two opposing models of stem cell dynamics that have been proposed: The Hierarchical model where stem-cells are rare long-lived, slow-dividing cells and the Stochastic model where stem-cells are abundant equipotent cells that divide frequently and their loss is dictated by neutral drift. We show that the replicative life-span of the cells and the spatial correlation between replication and removal from the cornea play a major role in whether homeostasis can be maintained without symmetry breaking signals. We derive the conditions that allow homeostasis that is consistent with biological timescales and mutants dynamics. We show how the conditions for self-organizing homeostasis depend on the stem cell dynamics and provide an experimental prediction to discriminate between the models. The result of this study can be extended to any cellular system in which spatial homeostasis is maintained through cell division.

E04 | Modeling the mechanics of an epithelial sheet deformed by a migrating cell

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Monolayered epithelia play an important role in developing embryos, underlying tissue shape changes such as invagination that occur during gastrulation, as well as providing structural support and a protective cover for inner tissues [1]. These functions are governed by the mechanical properties of the epithelium on macroscopic and cellular levels [2,3]. However, acting as a barrier, epithelia can also interfere with the spreading of immune cells – a process that is also crucial for development [4]. In the *Drosophila* embryo, immune cells migrate along the inner (basal) side of an epithelial layer during their invasion into the germband [4,5]. They exert a force to separate this tissue (ectoderm) from the inner tissue (mesoderm). Signaling events leading to decreased apical surface tension in the ectoderm facilitate cell migration [5]; whereas increased apical tension impedes cell migration. By modeling epithelial deformation in this process and measuring cortex tensions of the ectoderm we derive the apparent stiffness of the sheet and how it depends on apical, lateral and basal tensions, and on the aspect ratios of cells. The results suggest that surrounding cells can exhibit higher resistance to deformation not only by increasing cortical tensions, but also due to their local geometry. Our study aims to understand how a mutual mechanical balance is achieved in tissues to allow for robust mechanical events, including migration of the cells through tissue barriers as, for example, during immune and cancer cell extravasation [6].

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E05 | Regulators of the migration of drosophila testis nascent myotubes

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The migration of testis nascent myotubes (TNM) during fly development is a new, and still poorly understood model system. We established a protocol for live imaging, allowing us to shed light on the process of TNM migration. TNMs are multinuclear predecessors of muscles, which cover the adult testis of the fly. During pupal development, TNMs migrate from the genital imaginal disc onto the early testis in a thin sheet. They seem to lack lamellipodia, but have numerous filopodia. N-Cadherin (CadN), which is located at the tip of cell-cell connecting filopodia, is important for collective behaviour. Upon RNAi based knock down (KD) of CadN, cellsmore often change their relative position inside the migrating sheet. Still, cell-cell adhesion seems not to impair motility on a single cell level. KD of Cdc42 leads to filopodia formation- and migration defects. Some filopodia express focal adhesion markers instead of CadN at their tip. We assume, that TNM migration is based on filopodia dynamics instead of propulsion by a retrograde flow of branched F-actin, like in lamellipodia. A possible motor for propulsion could be actin-myosin contractility or polymerization of bundled F-actin, moving focal adhesion, thus creating force.

E06 | Mechanisms underlying the spatiotemporal organization of BMP-dependent target genes

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Embryonic patterning relies on signaling molecules that orchestrate the expression of developmental genes in time and space. How these distinct gene responses are achieved is an open question. We systematically identified target genes of the signaling molecule BMP in zebrafish and found that these genes exhibit diverse spatiotemporal expression patterns at early gastrula stages. We then used optogenetic manipulation of BMP signaling and NanoString molecular barcoding to examine models explaining these expression differences. Interestingly, most BMP targets can respond to a BMP signaling pulse at early stages when they are normally not expressed, ruling out competence as an explanation for diversity in activation timing. Additionally, differential transcription kinetics among genes responding to strong BMP pulses do not appear to explain spatiotemporal expression differences. To determine whether differences in sensitivity to BMP levels contribute to spatiotemporal diversity, we are currently measuring target gene responses to optogenetically generated high and low levels of BMP signaling. Strikingly, we found that spatial differences in BMP target expression largely collapse when Nodal and FGF signaling are inhibited, implicating combinatorial regulation by BMP, Nodal and FGF as major contributors to the spatial diversity of BMP targets.

F | New Techniques

F02 | A combined, versatile depigmentation and clearing method (DEEP-C) for studying animal nervous systems across scales

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Tissue clearing and deep imaging have emerged as a powerful alternative to histological techniques, as they allow direct access to three-dimensional structures without need for time- and computation-intensive sectioning and reconstruction. Whereas current clearing techniques have been optimized for imaging selected non-pigmented tissues like the mammalian brain, natural pigmentation remains a central obstacle for clearing and deep imaging in many organisms and/or developmental stages. We have developed a combined DEpigmEntation and Clearing method (DEEP-C) that allows full microscopic analysis of animals from organismic to cellular and subcellular scale, providing unprecedented insight into whole nervous systems, eyes, and cell types in in representative models for annelids (*Platynereis dumerilii*), cephalopods (*Euprymna scolopes*), bony fishes (*Danio rerio*) and tetrapods (*Ambystoma mexicanum*). While removing pigments of different chemistry, DEEP-C requires less than 24 hours, and thus can be easily incorporated in existing workflows. Importantly, we find that DEEP-C is compatible with a spectrum of detection techniques ranging from immunohistochemistry, fluorescent reporter proteins, and detection of proliferating cells (EdU) to RNA in situ hybridization. Thus, DEEP-C is a fast and versatile method with broad applicability for studying animal development.

F04 | Broad applicability of a streamlined Ethyl Cinnamate-based clearing procedure (2Eci)

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Turbidity and opaqueness are inherent properties of tissues which limit the capacity to acquire microscopic images through large tissues. Creating a uniform refractive index, known as tissue clearing, overcomes most of these issues. These methods have enabled researchers to image large and complex 3D structures with unprecedented depth and resolution. However, tissue clearing has only been adopted to a limited extent due to a combination of cost, time, safety and complexity of existing methods. Here we describe a radically simple and straightforward clearing method which we call 2Eci (2nd generation Ethyl cinnamate based clearing method). 2Eci can be used to clear a wide range of tissues and organisms, including cerebral organoids, *Drosophila melanogaster*, zebrafish, axolotl, and *Xenopus laevis* in as little as 1-5 days while preserving a broad range of fluorescence proteins including GFP, mCherry, Brainbow, and alexa-fluorophores. Ethyl cinnamate is non-toxic and can easily be used in multi-user microscope facilities on conventional con-focal and spinning disk microscopes. 2Eci will democratize tissue clearing , opening it up to people across developmental biology.

F06 | Efficient single-copy HDR by 5´ modified long dsDNA donors

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CRISPR/Cas9 efficiently induces targeted mutations via non-homologous-end-joining but for genome editing, precise, homology-directed repair (HDR) of endogenous DNA stretches is a prerequisite. To favor HDR, many approaches interfere with the repair machinery or manipulate Cas9 itself. Using medaka we show that the modification of 5' ends of long dsDNA donors amplified by PCR strongly enhances HDR and favors efficient single-copy integration by retaining a monomeric donor conformation thus facilitating successful gene replacement or tagging.

The simplicity and reproducibility of this method will be of relevance for applications requiring precise insertion/replacement of DNA elements. One such application is the inducible acute knock-down of fluorophore-labelled proteins via a deGradFP system, which absolutely requires a precise, seamless single-copy fusion of the fluorophore sequence to the ORF of the gene of interest. With our approach this is promoted in a highly efficient way.

G | Genomic and system approaches of development

G01 | Did genome re-organization drive the formation of new regulatory units in cephalopods?

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Cephalopods are a fascinating clade not only in terms of their morphology and resourceful behaviour, but also in regard to the complex genetic mechanisms underlying these characters which are only now beginning to be investigated. We recently showed that the last common ancestor of octopus and squid underwent large scale genomic rearrangements which led to the loss of bilaterian-specific local gene order (synteny). Genomic reorganisation also led to the emergence of numerous local gene clusters specific to cephalopods, but it remains unknown if these syntenic groups are functional units and if they drove cephalopod-specific morphological novelties. *Euprymna scolopes*, the Hawaiian bobtail squid, is a perfect model to study the mechanisms of genomic reorganisation and regulation in cephalopods. We aim to unlock the history of chromosomal evolution in cephalopods and understand if and how genomic rearrangement led to specific novelties within the clade using the recently published genome, chromosome confirmation capture (Hi-C) and open chromatin assays (ATAC-seq). Preliminary results show that cephalopod-specific gene order correlates with functional units (topologically associated domains). Using this data, we are identifying regulatory differences among syntenic regions conserved across Bilateria and Cephalopoda to understand how genomic changes led to the formation of novel regulatory architecture and functional co-regulatory units.

G02 | Resources and Services at the Vienna *Drosophila* Resource Center (VDRC)

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The Vienna Drosophila Resource Center (VDRC, http://www.vdrc.at), part of the Vienna BioCenter Core Facilities, is a professionally organized, non-profit bioresource center which aims to promote scientific discoveries in *Drosophila*. We primarily maintain unique transgenic *Drosophila melanogaster* stocks and DNA resources and distribute them both locally and worldwide. We additionally aim to acquire, create and develop new VDRC resources and services, according to the emerging new technologies and needs of the international *Drosophila* research community.

As Europe's only *Drosophila* resource center, we currently maintain over 31,000 unique transgenic fly stocks and nearly 14,000 DNA plasmids. The VDRC was established in 2007 and has distributed more than 1.3 million stocks to over 2500 registered users in 52 countries.

Our RNA interference (RNAi) lines, collectively covering 91% of annotated protein coding genes, remain our core focus. In combination with the appropriate GAL4 driver lines, this unique resource enables large scale gene knockdown screens, making it possible to carry out loss-of-function experiments in essentially any tissue or cell at any developmental stage from embryo to adult. To facilitate RNAi screens, we offer the opportunity for researchers to perform their screens directly in our facility.

In addition to creating over 700 new short hairpin (shRNA) lines to complement and extend our GD and KK RNAi libraries, we have diversified to include additional resources: 1) The Vienna Tiles (VT) enhancer-GAL4 driver lines - these are typically used to drive restricted expression of a UAS line (e.g. UAS-RNAi, UAS-GFP). The expression patterns of reporters driven by VT lines have been extensively characterized and annotated throughout embryogenesis and in the adult brain, making it easy to find driver lines in the cells, tissues and developmental stages of interest. 2) The Tagged FlyFos TransgeneOme (fTRG) library. Each line has been engineered to tag a specific protein with a multi-epitope tag at its C-terminus, the majority with 2XTY1-SGFP-V5-preTEV-BLRP-3XFLAG, for use in a variety of downstream applications including live imaging, subcellular localization and interaction proteomics of selected gene products at all stages of *Drosophila* development. Their versatility opens up a wide range of previously unfeasible experiments. 3) Several small collections as part of our "Other Resources" including tagged constructs, mutant alleles and reporters. 4) We will soon incorporate a comprehensive gRNA library targeting all kinases, phosphatases and transcription factors encoded in the *Drosophila* genome for gene knockout using CRISPR technology.

To remain current, we are actively seeking donations of further stocks/resources that are likely to be of broad future interest to add to our public collection as part of our "Community Stock Center" service. We are particularly keen to accept stocks and plasmids generated by local and European scientists. If you have resources which you feel would benefit the wider community and could be exclusively distributed via the VDRC, please contact us at the earliest opportunity to discuss. In addition, we offer a "Private Stock Keeping Service" where we maintain and ship personal stock collections on your behalf for a reasonable fee and for local labs we offer a fly food service.

We are also keen to enter collaborations to create and develop novel resources and make them available to the *Drosophila* research community. Contact us at: office@vdrc.at

G03 | Investigation of genetic causes in a developmental disorder: Oculoauriculovertebral spectrum

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Oculo-Auriculo-Vertebral Spectrum(OAVS) is a genetically and phenotypically heterogeneous disorder which occurs due to a developmental defect in the first and second pharyngeal arches. Main clinical findings consist of microtia, preauricular skin tag, hemifacial microsomia, epibulbar dermoid and vertebral anomalies. Cardiac, genitourinary anomalies and radial defects are also included in the spectrum. These clinical findings are thought to be the result of a disturbance of neural crest cell development; therefore OAVS is considered as a neurocristopathy. There is not a consensus regarding the clinical criteria for diagnosis, but generally the presence of microtia or preauricular tags with hemifacial microsomia is accepted as the minimum criteria. The etiology is heterogeneous since genetic, epigenetic and environmental factors are involved. Copy number variations (CNV) have been described as potentially pathogenic for the disorder and mutations of *MYT1* have been recently identified in some patients. In this study, 23 OAVS patients with diverse clinical findings were studied to identify the genetic etiology of this developmental disorder. All patients fulfilled the minimal diagnostic criteria suggested by Tasse et al, 2005. The mean age of the patients was 7 years and 8 months and 65.2% of the study group was male (Male / Female ratio 15: 8). Middle and inner ear anomalies were also observed among patients. Developmental delay, epilepsy and behavior abnormalities were seen in some patients. Patients were screened for copy number variations using the *Affymetrix CytoScan Optima array Kit* and also were screened for *MYT1* mutations using *BigDye terminator* on an *ABI Prism 3500 genetic analyzer*. Furthermore, two patients underwent whole exome analysis. Using these methodical approaches, three CNVs (one deletion, two duplications) were found on chromosomes 8, 15, 16 and were considered potentially pathogenic. The clinical relevance of the copy number variations is discussed within the framework of incomplete penetrance and monoalelic expression. In addition, the deletion on the 8th chromosome was thought to be associated with clinical findings by altering the genome architecture. In addition, a de novo unbalanced translocation; between X and 4th chromosomes was found in one of the patients and was considered pathogenic. No causative mutation was found in *MYT1*. WES analysis revealed novel heterozygous mutations in *RNF213* and *EFTUD2* that were previously suspected as candidate genes for OAVS in both patients. Duplication of 16p11.3 region, aneuploidies of X chromosome and single point mutations were previously implicated in OAVS molecular etiology. This study provides further genetic heterogeneity to this disorder, confirming the importance of microarray-based studies and whole exome sequencing analysis in patients with a developmental disorder such as OAVS.

G04 | Evolution and Development of Avian Limbs and Digits

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The evolution of wings enable birds dominate the sky for the past 150 milllion years. But many bird species have become flightless independently and evolved a great diversity regarding the limb morphology and digit number, shaped by both developmental constraints and natural selection. Although the evolution trajectories of anatomical features of wings can be inferred from fossils, contradictory evidence start to emerge from molecular studies. More importantly, the evolution of underlying developmental pathways leading to funtional wings can only gain insights from comparing flightless vs. flight species during their early development. To address this quesion, we have been collecting transcriptomes of hindlimbs, forelimbs and digits, subdivided into zeugopod, stylopod and each digit, from ostrich, emu, chicken and turtle across six comparative developmental stages. Preliminary analyses shows the loss of stage-specific expression in emu wings comparing to those of chicken, and the only preserved emu wing digit is likely corresponding to the chicken wing digit 3. Future analyses are expected to reveal the unique developmental programs of wings and digits of emu and ostrich, under a relexed selective scheme, and identify key genes that contribute to the avian wing development after the birds diverged from reptiles.

G05 | A Change of Heart: Medaka as a model for Human Cardio-Vascular Diseases & GWAS

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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 imaging and analysis pipeline, I functionally validate various novel CVD-associated gene hits identified in human GWAS *in vivo* in medaka embryos. Using CRIPSR/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.

H | Regeneration

H01 | Neurogenesis in the zebrafish inner ear: a NeuroD/Nestin-positive progenitor pool as a source of new neurons during growth, homeostasis and regeneration.

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Sensorineural hearing loss affects nearly 5 % of the world's population. It is defined as a permanent and irreversible impaired function of either the sensory hair cells and/or the vestibulocochlear nerve within the inner ear. In contrast to mammals, which lose the ability to generate new sensory hair cells and neuronal cells soon after birth, zebrafish possess a lifelong production and regeneration of sensory hair cells. However, currently it remains unknown whether (i) new sensory neurons are produced in the adult zebrafish statoacoustic ganglion, the sensory ganglion connecting the inner ear to the hindbrain, and furthermore (ii) if they are capable to regenerate and restore function properly after.
In regard to neurogenesis, our results show the presence of a NeuroD/Nestin-positive progenitor pool, which is highly proliferative and gives rise to new neurons at juvenile stages. Although proliferation declines during maturation and has ceased at adulthood, the number of NeuroD/Nestin-positive progenitor cells remains constant at all stages examined. However, lineage tracing shows the very rare but continued production of new neurons also at this later time point. Moreover, lineage tracing reveals the existence of a marker-negative stem cell population that replenishes the Neurod/Nestin-positive progenitor pool at adult stages.
To investigate the regenerative capacity of the adult statoacoustic ganglion, we established a lesion paradigm that models auditory/vestibular neuropathy based on otic capsule injections. Upon lesion, we found that cells of the NeuroD/Nestin-positive progenitor pool reenter the cell cycle and show significantly increased proliferation rates up to 14 days post lesion. At 28 days post lesion, using a BrdU-pulse chase experiment, we confirmed the generation of new neurons and thus, the regenerative capacity of the adult statoacoustic ganglion.
Taken together, we found a NeuroD/Nestin-positive progenitor pool as a source of neurogenesis during growth, homeostasis and regeneration. Understanding adult neurogenesis in the zebrafish statoacoustic ganglion will help in the identification of mechanisms to rekindle neurogenesis in the mammalian inner ear.

H02 | No head regeneration here: Regeneration capacity and stem-cell dynamics of *Theama mediterranea* (Polycladida, Platyhelminthes).

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Most research regarding the regeneration potential in atworms (Platyhelminthes) has been undertaken with planarians (Tricladida), where most species can regenerate a head and no proliferation takes place in the blastema. Only few studies are available for an early-branching group within the Platyhelminthes, the Polycladida. Head regeneration in polyclads is not possible, with a single exception from a study performed more than 100 years ago: Cestoplana was reported to be able to regenerate a head if cut a short distance behind the brain. Here, we show that Cestoplana was misdetermined and most likely was the small interstitial polyclad Theama mediterranea Curini-Galletti et al., 2008. We revisited regeneration capacity and stem-cell dynamics of *T. mediterranea* with live observations and stainings of proliferating and di erentiating cells. In our experiments, after transversal amputation only animals retaining more than half of the brain could fully restore the head including the brain. If completely removed, the brain was never found to regenerate to any extent. Unlike in planarians, but comparable to other free-living atworms, we detected cell proliferation within the posterior regeneration blastema in *T. mediterranea*. Similar to other free-living atworms, proliferation did not occur within, but only outside the di erentiating organ primordia. Our results strongly imply that whole brain regenerationis not possible in polyclads. Proliferation of stem cells within the regeneration blastema is a plesiomorphy in atworms.

Keywords - flatworm, turbellarian, regeneration, neoblast stem cells, *Theama mediterranea*

H03 | A dynamic pattern of auxin sources orchestrates root regeneration

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The root apical meristem (RAM) coordinates root growth throughout the plant life. The RAM structure is maintained by a complex network of transcription factors and phytohormones, the main one being auxin. Remarkably, severe damage to the RAM, such as complete excision of the stem cell niche, triggers rapid regeneration. Auxin is required for this process and auxin response markers are rapidly induced near the excision site. The current model states that auxin distribution in plant tissues is mainly regulated by polar localized PIN-FORMED (PIN) transporters. However, this has not been tested during regeneration, when normal patterns are severely disrupted.

We used genetic and chemical perturbations to test the role of PIN-mediate auxin transport during regeneration. Surprisingly, disruption of auxin transport does not inhibit regeneration, however, local inhibition of auxin biosynthesis resulted in aborted regeneration. High resolution transcriptomics and imaging reveals two sources of auxin production in the regenerating root, acting in temporal succession. Using chemical inhibition and artificial miRNA simultaneously targeting 6 YUCCA auxin biosynthesis enzymes, we blocked auxin synthesis at specific tissues and time points. Inhibition of the early auxin source delays regeneration, while inhibition of the later source blocks root reformation. Active biosynthesis is no longer necessary once tissue patterns have been recovered.

Taken together, we present a novel mechanism for regulating auxin distribution when tissue patterning is disrupted. We propose that the ability to specify new sources of auxin production following damage is one of the cardinal features that enable plant regeneration.

H04 | The dynamics of neural stem cells and neurons in axolotl spinal cord regeneration

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The central nervous system of most vertebrates, such as the spinal cord, has a very limited ability to repair damages. The axolotl is a notable exception, having the amazing ability to regenerate their spinal cords, with full functional and structural recovery. Thus they are ideal as a model to study spinal cord regeneration. Axolotl spinal cord contains neural stem cells (NSCs) which line the central canal and remain pluripotent throughout life. They proliferate after injury to reform the ependymal tube and replenish lost neurons, and are the key to successful regeneration. However, the proliferation of NSCs must be accompanied by directional cues, for the spinal cord to regenerate in the right direction and reform the tube structure. Neurons might provide the necessary cue, as axons were observed to associate closely with the ependymal tube during regeneration.

Using 3D imaging with optical clearing, we show that axons and NSCs in the regenerating spinal cord are in close proximity to each other, with axons extending in front of the leading NSCs. By labelling of NSCs and neurons genetically and performing live imaging, we show that axons and leading NSCs in the regenerating spinal cord are dynamic and exhibit probing behavior, often with processes of the leading NSCs conforming to a nearby axon. Next, by using laser to ablate axons and neurotrophic cues to redirect them, we aim to demonstrate the interaction between axons and NSCs, and whether axons provide the directional cue for proliferating NSCs in axolotl spinal cord regeneration.

H05 | Development of a high-content in vivo screening platform using automated laser-assisted photoablation in an acute kidney injury model in zebrafish

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Acute kidney injury (AKI) manifests with sudden (hours to days) deterioration of kidney function, which may be reversible, if timely recognized. It develops in 10% of all pediatric patients of the intensive care unit. With the increasing severity of the underlying disease, prevalence of AKI can reach up to 80%. The research on regenerative substances in the context of AKI has been hindered by the limitations in currently available methodologies for performing high-throughput kidney screening and the lack of automation of kidney damage procedures, which would allow for larger-scale investigations. Zebrafish is a well-established model organism used in drug screening research. The identification of regenerative substances for AKI based on zebrafish could thus pave the way for new therapeutic approaches in humans. A recently established model of unilateral, targeted kidney damage uses light-induced ablation (photoablation). This one-sided AKI model allows the survival of the larva and thus offers the possibility to investigate regeneration processes. In order to find disease-modulating and regeneration-promoting substances in an image-based high throughput screen, an automated platform to model kidney injury and subsequent regeneration is needed.

We aim to develop a high-content screening (HCS) pipeline with integrated automated photo manipulation for the automated unilateral ablation of the zebrafish pronephros to ultimately identify AKI drugs in a living model system. We additionally aim to understand the molecular mechanisms behind the dedifferentiation of injured renal tubular epithelial cells (RTECs) and the reactivation of pathways common during early renal development. To do so, we have to identify and optimize parameters for kidney injury and validate the damaged area. We have applied different laser settings to induce kidney injury in well-defined regions in zebrafish. Photoablation with 405 nm laser resulted in apoptosis within zebrafish larval kidney. When 355 nm and 375 nm lasers were used, damage of somites and neighboring organs (i.e. liver and pancreas) was observed. Our findings revealed the 405 nm laser (continuous wave laser) to be best suited for automatic photoablation of zebrafish larvae in order to induce AKI. By deploying histological and apoptosis analyses, we could identify the best parameters for 405 nm laser to avoid “off-target” damages in surrounding tissues.

A synergetic combination of zebrafish, high-content screening and targeted photoablation is a feasible and promising approach to overcome existing hurdles, especially in AKI drug discovery with high translational value and foster novel analysis assays. Ultimately, the developed pipeline will be compatible with various other tissues or model systems such as *in vitro* cultures, 3D cell culture applications and other whole organisms.

H06 | Testing regeneration potential of mouse skin fibroblasts

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Limb regeneration as seen in axolotls involves the recruitment of fibroblastic connective tissue cells to the amputation site. These cells form limb bud progenitor-like cells that faithfully regenerate the appropriate skeletal segments. While mammals such as mice have abundant limb connective cells, a coordinated regeneration response is not observed after limb amputation. It is currently not known if this deficit is related to inhibitory signals in the environment, or an intrinsic limitation in potential of the connective tissue cells. To assay the intrinsic potential of limb connective tissue cells, specifically mouse skin fibroblasts, we have developed ultrasound guided transplantation into the mouse limb bud. We have developed several output assays including the diversity of differentiated cells, and proximal-distal position to assess the development potential of the transplanted cells.

H07 | Testing the regenerative potential of limb blastema cells in post-metamorphic *Xenopus laevis*

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In regenerative species, such as axolotls, limb regeneration is achieved by the formation of blastema, a population of proliferating cells that accumulates at the amputation plane. A post-metamorphic *Xenopus laevis* also forms a blastema after limb amputation, but instead of forming a fully patterned limb, it only produces a hypomorphic cone structure mainly composed of cartilage and dermis. It remains unclear whether *Xenopus* blastema cells are intrinsically incompetent of forming a complex limb pattern, or the post-metamorphic environment prevents the blastema cells from doing so. In this project, we are investigating the cell intrinsic potential of *Xenopus* limb blastema cells. To do so, we evaluate their abilities to produce multiple tissue types and multiple cartilage segments after being transplanted into embryonic limb buds, where the environment is permissive for limb patterning. Preliminary transplantation results show cell intrinsic differences between *Xenopus* blastema cells and embryonic limb bud cells in their bone/cartilage-forming potential. The transplanted blastema cells contribute to dermis and soft tissues, but not the cartilage or bone of host limbs, while transplanted limb bud cells could contribute to all different tissue types. Remarkably, blastema cells could contribute to cartilage when transplanted to another blastema, indicating a change of contribution to the limb structures in different environments. To further characterize the contribution of blastema cells to different cell types, we will fractionate blastema cells by genetic labeling and perform transplantation and lineage tracing experiments. With both transplantation method and transgenic tool established, we will be able to elucidate the cause of the *Xenopus* regeneration phenotype at both cellular and molecular levels.

H08 | Skin and muscle connective tissue cells in bone fracture healing in axolotl

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While small bone fractures can heal without scar formation, large and complicated bone fractures fail to regenerate, leading to bone non-unions. To develop an effective treatment, a good understating of the cellular and molecular mechanisms of fracture callus formation and bone healing is necessary. Critical size bone defect (CSD) is an injury paradigm in which ≥ 30% of the bone length is removed, leading to bone non-unions. In axolotls (Ambystoma mexicanum) bone CSD cannot heal as in mammals but amputated limbs fully regenerate, including extensive growth of bone. In axolotl regenerating limb, a transient progenitor cell mass, the blastema is formed, giving rise to the tissues of regenerate. Recently, it was shown that periskeletal cells build the proximal bone next to the injury, while soft connective tissue (SCT) cells build the distal region of the bone. Inability of axolotls to heal the CSD can be potentially caused by diminished migration ability of SCT cells, leading to insufficient bone callus formation and fracture non-union. Here we aim to understand if SCT cells contribute to CSD healing in the axolotl. For this purpose, we have developed an axolotl femur fracture model comparable to mammalian models. An external upper hind limb polyolefin fixators or titan/PEEK plates were used to ensure femur alignment upon osteotomy and constant distance between the bone fragments. In small fracture samples, we observed callus formation and fracture bridging at 3-6 weeks, and woven bone formation at 12 weeks post-surgery. In older axolotls, bone healing process took significantly longer than in younger ones. In CSD samples, fracture bridging was not observed at 12 weeks post-surgery, despite the accumulation of blastema marker-positive cells in the fracture gap. To trace SCT cells, full-thickness skin or muscle bundles from axolotls with SCT cell-specific fluorescent labelling using Prrx1 (limb bud and blastema-specific) and Col1a2 (cartilage progenitor marker) promoters, was transplanted to wildtype animals. Then, femur small fractures or CSD were created. After 3 weeks, the fluorescently labelled cells were observed in the fracture region. Further investigation of SCT cell proliferation and differentiation into cartilage/bone in fractures is necessary. This will shed light on the potential differences in SCT capacity to contribute to bone healing in limb regeneration, in small and critical-size bone fracture, offering processes which could be targeted by therapies.

H09 | A cellular profiling approach to dissect the regulation of regeneration in a marine annelid

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The marine annelid *Platynereis dumerilii* is capable of rapid regeneration upon amputation of its caudal trunk, including its central nervous system. This process appears to recapitulate segmental growth during regular development. Posterior regenerates include cells expressing members of the germ line multipotency programme (GMP), suggesting that regenerative growth depends on proliferation and differentiation of dedicated stem cells. In addition, regeneration depends on the presence of a brain hormone whose levels orchestrate the balance between somatic growth and germ line expansion. Despite these general conceptual insights, however, it remains unclear if GMP cells are a homogeneous population, or represent subsets of cells with different potency. Likewise, it remains enigmatic by which molecular mechanisms the brain hormone is able to modulate the regenerative process.

In order to resolve these questions, we are pioneering research into individual cell types and cell states occurring during posterior regeneration of *Platynereis*. One of our central efforts was the establishment of a protocol for dissociation of regenerates into single cells. By a series of systematic trials, we have devised a robust procedure that allows the recovery of individual regenerate cells, with viability exceeding 90%, thus providing compatibility with single cell RNA profiling approaches. Quantitative PCR on cDNA derived from these cells indicates that they comprise both GMP cells and differentiated cell signatures. We have begun to extend these efforts by generating systematic cellular profiles of both undifferentiated and differentiated cells in the regenerate, and to intersect these profiles with germ layer- and cell-type-specific profiles established from complementary cell labeling approaches.

Our aim is to use these signatures to derive a set of distinct markers for both stem cell states and differentiated cells, and to delineate differentiation trajectories between these. These signatures and trajectories will provide a highly valuable reference to assess molecular and mechanistic differences between regeneration and regular growth, and to determine the precise molecular effects of the brain hormone in these processes.

H10 | Single cell sequencing reveals diversity of newborn neurons in the adult zebrafish brain

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Zebrafish display widespread and pronounced adult neurogenesis, which is fundamental for their amazing regeneration capability after injury of the central nervous system. However, the cellular identity and the biological properties of adult newborn progenitors and neurons are elusive for most brain areas.

Here, we used short-term lineage tracing of radial glia progeny to prospectively isolate newborn neurons from the her4.1^{+}radial glia lineage in the homeostatic adult forebrain. Transcriptome analysis of radial glia, newborn neurons and mature neurons using single cell sequencing identified distinct transcriptional profiles including novel markers for each population. Specifically, we detected 2 separate lineages of newborn neurons, which showed clear diversity of cell fate commitment and anatomic location. Further analyses identified a clear neurogenic commitment in proliferating radial glia and indicated that the majority of newborn neurons becomes committed to a glutamatergic projection neuron fate.

Thus, our results support understanding of adult neurogenesis in teleost fish through defining molecular markers to distinguish newborn from mature neurons and identifying novel functional populations of newborn neurons in the adult zebrafish brain.

I | Polarity and axis formation

I01 | The secreted tyrosine kinase PKDCC and the Wnt pathway during gastrulation in the rabbit embryo

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Gastrulation is an evolutionarily conserved principle in the development of all animal species: morphologically with regard to the development of the body axis and three germ layers, and molecularly with regard to the similarity of signaling pathways involved. In amphibians and amniotes, the Wnt pathway is well known for its functional division into a canonical and a non-canonical pathway (the latter also known as planar cell polarity pathway). The canonical pathway regulates the transcription of known mesoderm markers (such as the T-Box genes) by accumulation of ß-Catenin. The planar cell polarity pathway regulates convergent extension movements by modulation of the cytoskeleton and is thus important for cell movement and axis formation. Recently, it was shown in the amphibian model organism Xenopus that PKDCC, a secreted tyrosine kinase also referred to as Vlk or ADTK, regulates both pathways: PKDCC mRNA is expressed in a dorsal region of the embryo where Wnt expression is exceptionally down-regulated. Remarkably, PKDCC is suggested to inhibit the Wnt pathways independently of its kinase activity. If PKDCC were to have a similar role in mammals as well, it would also be expected to be expressed in a reciprocal manner to Wnt. This study, therefore, compares at the cellular level the expression pattern of PKDCC with that of Wnt3, of the inhibitory signaling molecules Cer1 and Dkk1, and of the T-Box gene Brachyury. As a model organism the rabbit embryo was chosen due to its mammotypical topography during gastrulation: It has a flat embryonic disc, a feature also shared by avian embryos during gastrulation. PKDCC expression appears in the rabbit hypoblast simultaneously with the anterior marginal crescent, which is the first sign of anterior-posterior polarity; subsequently it develops into a strong expression in the anterior streak domain and thereby marks the anterior border of the Wnt expression domain. Furthermore, PKDCC is weakly and continuously expressed in the centre of the so-called anterior gastrula plate. After primitive streak formation PKDCC is coexpressed together with the Wnt inhibitor Cer1 (but not with Dkk1) and with Wnt3 inthe epiblast of the anterior part of the primitive streak. Apart from confirming the molecular subdivision of the flat embryonic disc of the early rabbit embryo PKDCC expression in the hypoblast of the anterior gastrula plate can now be seen as a suggestion of its evolutionary conserved inhibitory function in the Wnt pathway. Functional experiments in the rabbit may now be designed to confirm this unexpected role of PKDCC in the Wnt pathway.

I02 | An early chick embryo culture device for studying molecular and morphological left-right patterning

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Left-right asymmetry of visceral organs is a well known phenomenon in externally symmetric bilateria and ciliary flow is considered to be a common mechanism in many vertebrate species to establish molecular asymmetry of the left-right body axis during gastrulation. In the chick embryo, however, there are no motile cilia in Hensen’s node and there must be other mechanisms for breaking the initial symmetry of the embryo. Many widely used culture techniques for studying the formation of the anterior-posterior body axis in the early chick embryo lead to morphological left-right defects in a high percentage of control embryos. This may be due to the position of the embryo within the culture dish or due to the variability in the tension on the vitelline membrane, which plays a pivotal role in morphogenesis but is difficult to adjust. For an easy control of this tension we developed a culture method using a special device. All cultured control embryos showed normal signs of left-right asymmetry from (1) left-sided nodal expression in the paraxial and lateral plate mesoderm to (2) right-sided heart looping as our method also allowed us to study advanced stages of development in which blood circulation and folding of the embryo are established. Experimentally, we used our culture technique to study the role of mechanical stress in left-right patterning by microsurgery as well as the role of putative left-right signaling pathways by pharmacological inhibition. Hedgehog inhibition after cyclopamine administration, for example, abolished the left-sided nodal domain and led to randomized of heart looping but left the asymmetry of Hensen's node intact.

I03 | Three pathways regulate spindle directions in three dimensions: a New function for FGF in the *C. elegans* embryo

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Inductions depend on cell-cell contacts and hence on cell positions (Hutter und Schnabel, 1994, Hutter und Schnabel, 1995). Therefore, the proper cleavage directions of blastomeres are essential for the early embryonic development of *C. elegans*. Theoretically, two forces are sufficient to specify a cleavage axis on a sphere. However, depending on the direction on the sphere, the required forces are subjected to the sine or cosine, which thus may require a very fine tuning. To date the Wnt pathway (Thorpe *et al.*, 1997, Rocheleau *et al.,* 1997, Walston *et al.*, 2004) and LAT-1, a G protein-coupled receptor (Langenhan *et al.*, 2009), were identified as two regulators of the cleavage directions of the four AB derived blastomeres in the 8-cell stage embryo. However, we expected that three forces in a three-dimensional space should procure more precision and robustness in the control of spindle directions. Indeed, we were able to identify a third pathway, the Fibroblast Growth Factor (FGF) pathway, also controlling spindle alignment.

So far FGF is known to regulate sex myoblast migration and several other important processes, but not cell cleavages (DeVore *et al.*, 1995, Fleming *et al.*, 2005, Huang und Stern, 2004, Szewczyk und Jacobson, 2003). In the wild type one AB derived blastomere divides perpendicularly to the other three. After a reduction of the EGL-15 activity, the only known FGF receptor in the worm, all four AB blastomeres divide nearly parallel to each other, very much tilted to the anterior direction. This differs from the orientation in Wnt mutants, where all four blastomeres cleave almost perpendicularly to the a-p axis. As expected, reducing CLR-1 activity, a tyrosine phosphatase, alters the cell division orientation antagonistically to an *egl-15* mutant. The phenotype of the *clr-1* and *egl-15* double mutant indicates a role of CLR-1 as a negative regulator of the FGF pathway. This was already described for other diverse processes (Kokel *et al.*, 1998). It is now an interesting question if other components of the FGF pathway, for example ligands, are also involved in specifying the early cell division orientation. We also wonder if this function is conserved in other animals.

To investigate the individual contribution of the three pathways on spindle direction, we use a new bioinformatics approach for this type of problem. The cell division coordinates of wild type and mutant embryos are plotted in a 3D coordinate system. Their distributions are investigated by a variation of the Principal Component Analysis. The Principal Component Analysis is a statistical method to simplify a multi-dimensional data set into fewer dimensions. Using PCA we are trying to find the direction of the maximum variance of the data in order to identify the direction regulated by a gene. We speculate that the differences of the main variance axes between wild type and mutant embryos indicate the direction regulated by a specific pathway.

Our data indicate that the spindle directions of the four AB derived blastomeres are regulated individually and differentially by the three polarity pathways. This hints that the spindle direction may be coupled strictly to the blastomere identity.

I04 | Toddler signaling is essential for cell polarization during gastrulation

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Gastrulation is essential for embryogenesis, as the specification and assembly of germ-layers provides the blue-print for the body architecture. Although the main regulators of germ layer specification are largely known, the molecular mechanisms controlling tissue assembly remain poorly understood. We have recently identified the peptide Toddler/Apela/Elabela as a key regulator of mesendodermal cell migration, yet the molecular and cellular basis of how Toddler signaling drives gastrulation movements is still unclear.

In vivo cell transplantation assays combined with high-resolution live-cell imaging enabled us to delineate and quantitate the cellular behavior of mesendodermal cells. Internalizing cells in wildtype embryos are characterized by directional, actin-rich protrusions that allow efficient cell migration. In contrast, internalizing cells in *toddler*$^{-/-}$ mutant embryos fail to polarize, and instead form either excessive blebs or randomly distributed filopodia, both of which lead to non-directional and inefficient cell movement. In addition to this in-depth cellular phenotypic characterization, we have established a high-throughput cell tracking method to investigate cell migratory defects and differences between cellular subpopulations on a global scale.

Having identified the cellular and migratory defects in the absence of Toddler, we will now be able to investigate the cell autonomy of Toddler signaling, possible ligand-receptor interactions and the distinct role of Toddler signaling in endodermal vs. mesodermal cell migration. In summary, our integrative study will elucidate the Toddler-mediated molecular and cellular processes controlling cell migration, and broaden our understanding of the regulation of global gastrulation movements.

J | Germline and early cleavages

J01 | The MAST Kinase Drop out controls Dynein-mediated transport and polarised membrane growth in *Drosophila* cellularisation

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Microtubule-associated Ser/Thr (MAST) protein kinases have been implicated in several human diseases including breast cancer, neurodegenerative diseases and inflammatory bowel disease, but the cell biological functions of this kinase family are poorly understood. We identified a single *Drosophila* MAST kinase homolog encoded by the *drop out* (*dop*) gene. Mutations in *dop* affect Dynein-dependent transport in early embryos including transport of polarity proteins and lipid droplets. By creating the first null mutants in *dop*, we demonstrate that Dop is essential for polarised membrane growth during cellularisation. In the fly embryo, cellularisation is the concerted cytokinesis during which a syncytial embryo is transformed into a monolayered blastoderm epithelium by polarised growth of plasma membranes. This polarised membrane growth is dependent on Golgi-derived vesicles and Rab11-dependent endosomal transport. Towards a better understanding of Dop's function in membrane growth, we investigated endomembrane transport in *dop* mutants. Apical transport of Golgi vesicles and movement of Rab11 vesicles in the periphery of the recycling endosome (RE) were reduced in *dop* mutants. Furthermore, the distribution of the Rab11 effector Nuclear Fallout (Nuf), the fly homolog of human FIP3/Arfophilin was abnormal in *dop* mutants. Since FIP3 is a known binding partner of Dynein-light-intermediate chain (Dlic), our results suggest that Dop regulates the transport of endosomal vesicles by controlling the interaction of Dlic with the Rab11 effector Nuf. In a quantitative proteomic approach, we identified *bona fide* substrates of Dop kinase and found a 4.8-fold reduction in phosphorylation of the conserved serine 401 residue of Dlic in *dop* mutants. We performed in vitro phosphorylation of Dlic and mapped phosphorylation sites that are dependent on Dop kinase. To test the function of S401 phosphorylation in the context of the *dop* phenotype, we generated a mutant form of Dlic that mimics S401 phosphorylation (S401D) and show that it is able to suppress the membrane growth defect when expressed in *dop* mutant embryos. These results indicate that Dop is a novel regulator of cytoplasmic Dynein, which controls vesicle transport during polarised plasma membrane growth in cellularisation.

J02 | The TRIM32-related ubiquitin ligase, GRIF-1, reprograms primordial germ cells to ensure germ cell immortality

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During an initial phase of transcriptional repression, embryonic germ cell development primarily depends on a maternally donated gene expression program that consist of RNA-regulatory proteins and their mRNA targets. However, to put the zygotic genome in charge, these germline-intrinsic factors of germ cell fate and development must be eventually terminated. While numerous works have addressed how the expression of mRNA regulators is controlled during post-embryonic germ cell development, little is known about how their expression is terminated to reprogram primordial germ cells (PGCs) for zygotic gene expression. In studying the cytoplasmic polyA polymerase (cytoPAP), GLD-2, a prime example of a maternally donated mRNA regulator whose expression ceases upon birth of both PGCs in *C. elegans*, we identified GRIF-1, a TRIM32-related putative ring finger domain-containing ubiquitin ligase. Our work demonstrates that GRIF-1 protein is exclusively expressed in embryonic germ cell progenitors; just prior to GLD-2's disappearance in PGCs, and its removal causes a prolonged expression of GLD-2. In co-immunoprecipitation experiments using embryonic extracts, we found that GRIF-1 associates with GLD-2 cytoPAP in an RNA-independent manner. Moreover, we identified in yeast two-hybrid binding tests the germline-specific intrinsically disordered domain of GLD-2 as important for a direct interaction with GRIF-1. Consistent with a regulatory role of this N-terminal GRIF-1-interaction domain in embryogenesis, its deletion affected neither GLD-2 expression nor GLD-2's enzymatic function as a cytoPAP in the adult; however, it led to an extended expression of GLD-2 in PGCs. Also, a timed ectopic misexpression of GRIF-1 during gametogenesis in the adult led to a reduction of GLD-2 protein level. Importantly, upon compromising *grif-1's* functions by RNAi or generating CRISPR-induced loss-of-function mutations, we found that these embryos produced animals with a mortal germline phenotype: despite maintaining their identity and proliferative capacity across several generations, germ cells eventually lose their proliferation capacity and undergo cell death in a manner that is partially dependent on apoptosis during postembryonic development. Taken together, we report that GRIF-1 is a maternally expressed, germ cell-intrinsic turnover factor that removes GLD-2 cytoPAP, a known stabilizer and translational activator of mRNAs. These findings suggest that GRIF-1-mediated protein turnover increases germ cell fitness across generations presumably by cleanly reprogramming the maternal to a zygotic gene expression system in PGCs, revealing a new role for tripartite-motif proteins.

K | Organogenesis

K01 | Fgf8 mRNA and protein relay for a long-range FGF8 concentration gradient

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Morphogen gradients provide positional information for cell fate determination and are important for spatial patterning in many developmental systems of multicellular organisms. In addition to classical diffusion mechanisms, mRNA gradients represent a novel mechanism for morphogen gradient formation in a growing tissue. Whether and how these two mechanisms integrate in shaping morphogen gradients remains unclear. Here we show that the FGF8 gradient along the presomitic mesoderm of chick embryos is established by cooperation between freely diffusible FGF8 protein and graded *fgf8* mRNA expression. Free diffusion of FGF8 is limited to a short distance but is required for establishing its long concentration gradient by maintaining *Fgf8* expression. Our results suggest that a short-range diffusible morphogen can be extended into a long-range concentration gradient by a protein-mRNA-relay mechanism

K02 | The transcription factor Osr1 marks embryonic progenitors of brown adipose tissue (BAT) and adult adipose stem/precursor cells (ASPCs), and is essential for BAT formation

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Unlike white adipose tissue (WAT), which is mainly responsible for energy storage in form of triacylglycerides, the major role of brown adipose tissue (BAT) is heat production and thus energy expenditure. BAT has been identified in several mammalian species, especially rodents. Brown fat depots are most prominent during early postnatal development, however in mice, BAT persists throughout life. While human infants have BAT depots, it was long thought that these disappear and are absent in adults. Only recently it was recognized that adult humans have depots of brown adipose tissue that can be stimulated to produce heat by e.g. cold exposure. This has raised considerable hope for therapeutic application in common metabolic diseases as diabetes or obesity. White and brown adipose depots have mostly distinct developmental origins. While white adipose tissue was traced to lateral plate mesoderm, BAT is thought to arise from $Pax7^+$, $Pax3^+$, $Myf5^+$ myogenic progenitor cells of the paraxial mesoderm / somitic dermomyotome that transfate to BAT cells. We recently showed that the zinc-finger transcription factor Osr1 marks a mesenchymal fibro-adipogenic progenitor population in mouse embryos giving rise to e.g. muscle connective tissue and subcutaneous white adipose tissue. Here we show, using Osr1-CreERt2 knock-in allele-mediated *in vivo* genetic lineage tracing that at embryonic stage E11.5 Osr1 marks progenitors of brown adipose tissue. Long-term lineage tracing of embryonic $Osr1^+$ cells resulted in robust labeling of adipocytes as well as interstitial $Sca1^+$ ASPCs in several BAT depots. Notably, at E11.5, which is considered critical for the myogenic-to-brown adipogenic fate switch, a small set of $Pax7^+$ dermomyotomal cells co-express Osr1. Concomitantly, the first $PPARg^+$ adiopgenic cells were found in close proximity to the $Osr1^+$/$Pax7^+$ cells, suggesting that Osr1 may identify $Pax7^+$ cells undergoing myogenic-to-brown adipogenic fate transition. Concordantly, lineage tracing of $Osr1^+$ progenitors in an Osr1-deficient background revealed a myogenic potential of $Osr1^+$ cells that was not present in heterozygous embryos, indicating that Osr1 function may be required for the myogenic-to-brown adipogenic fate switch. In line with this, Osr1 deficient embryos showed a severe reduction of pre-adipogenic $PPARg^+$ cells in developing BAT depots. This altogether indicates that Osr1 is part of the machinery defining the brown adipogenic fate in the myogenic progenitor pool, and indicates that adult BAT-resident ASPCs arise from this lineage.

K03 | Epigenetic regulation of chondrocyte differentiation

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Endochondral bones are formed as a cartilage template, which is subsequently replaced by bone tissue. During this process, the differentiation from proliferating into hypertrophic chondrocytes, which takes place at a precisely defined morphological border, is tightly regulated by a complex network of transcription factors and epigenetic modifiers.

To receive insight into the epigenetic alterations that are linked to the switch in cell fate, we analyzed distinct epigenetic histone marks that are linked to gene transcription and repression. To investigate pure cell populations, we established a flow cytometry protocol allowing the isolation of YFP expressing proliferating (Col2-YFP) and hypertrophic (COL10-YFP) chondrocytes from mouse embryos.

The epigenetic profile of the two cell types was analyzed by ChIP-Seq using a panel of chromatin markers including H3K9ac and H3K27ac for active promoters, H3K27ac for enhancers, H3K36me3 for expressed genes, H3K4me3 to detect genes primed for expression and H3K9me3 and H3K27me3 for repressed regions. In parallel, gene expression was assessed by RNA-Seq of microdissected chondrocytes. Bioinformatic analyses using ChromHMM and pareto analysis identified similar numbers of expressed genes in both chondrocyte populations. Occurance of activating histone marks correlates with high levels of gene expression observed in RNA-Seq expression. Interestingly, we detected an increase in repressive histone marks, H3K27me3 and H3K9me3, in hypertrophic cells. Moreover, genes carrying a combination of activating and repressive marks occur more frequently in hypertrophic cells, and are enriched on genes that are turned off in hypertrophic cells. The addition of repressive marks seems thus to provide a novel mechanism for fast changes in expression levels in closely related cell types.

K04 | Caveolin 1a is required for muscular and neuronal integrity in *Xenopus laevis*

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Caveolin 1 is a central mediator of endocytosis, by driving the formation of Caveolae –specialized invaginations of the plasma membrane. Loss of Caveolin 1 function in mice causes distinct neurological phenotypes leading to impaired motor control, however, the underlying cellular and molecular mechanisms are still unknown. In this study we find that Morpholino-mediated knockdown of Caveolin 1α in *Xenopus laevis* results in a striking swimming defect, characterized by paralysis of the morphants. Muscle differentiation and maturation are unaffected by Caveolin 1 knockdown. Phalloidin staining as well as transmission electron microscopy of muscle cells revealed aberrant sarcomeric structures and disorganized actin fibers. Interestingly, Caveolin 1 is expressed in the notochord and neurons but not in muscle cells, supporting the hypothesis that muscular abnormalities are a consequence of neuronal defects. Consistently, we show that axonal outgrowth of motor neurons and cranial nerves is disrupted by Caveolin 1 knockdown. We are currently in the process of elucidating the molecular mechanism how caveolins affect axonal outgrowth and subsequently muscle patterning. Taken together, these results suggest a previously unrecognized function of Caveolin 1α in neurogenesis in respect to axonal pathfinding of both motorneurons and cranial nerves establishing an important link to muscle development in *Xenopus laevis.*

K05 | Outgrowth of zebrafish gill filaments is regulated by an interplay between the RA- and BMP-signalling pathways.

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Fish gills play important physiological roles, including oxygen uptake, osmo- and pH-regulation as well as maintaining the nitrogen equilibrium. Considering their significance, it comes as a surprise that the developmental mechanisms shaping the gills are relatively unknown. In zebrafish (*Danio rerio*) the development of the branchial arches has been shown to be dependent on various signalling pathways, including the retinoic acid (RA) and the BMP signalling pathway throughout 5 days post fertilisation (dpf). Each of the first four branchial arches buds several pairs of gill filaments that grow into the opercular cavity. The buds of emerging gill filaments consist of blood vessels, mesenchymal cells and an epithelial cover. In order to understand the molecular processes involved in the budding of gill filaments, we have started to follow the cell types present in gill filaments as well as to unravel the expression patterns of distinct genes therein. We show that emerging gill filaments primarily consist of a looped blood vessel that encloses a mesenchymal core that expresses the RA-producing retinaldehyde-dehydrogenase Aldh1a2. Furthermore, we found that *bmp2b* is expressed in the periphery of the outgrowing filaments. These findings prompted us to investigate the roles of RA- and BMP-signalling during gill filament development. Here, we demonstrate that both reduced endogenous RA-signalling and overexpression of *bmp2b* lead to a significant reduction in outgrowth of gill filaments. Moreover, we discovered that overexpression of *bmp2b* suppresses the expression of *aldh1a2* within the gill arches, leading to the assumption that gill filament budding might be dependent on RA-signalling, which in turn is regulated by the BMP-signalling pathway. We will discuss these findings and integrate them into a model of early gill filament development.

K06 | Dynamic cell motility in pancreatic islet morphogenesis

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During pancreas development, endocrine progenitors emerge as single cells and progressively coalesce to form mature islets. The mechanisms of islet formation remain poorly understood due to the deep internal location of the pancreas in most vertebrate model organisms. Zebrafish is an in vivo system well suited for studies of islet cell assembly due to its transparency and the accessible location of the larval pancreas, which enables live imaging of dynamic processes. We have generated novel transgenic lines with fluorescent proteins expressed in islet cell populations, to provide tools for highlighting cell morphology during islet formation. High resolution time-lapse imaging of nascent endocrine cells during islet assembly revealed fine dynamic filopodia acting as exploratory structures. We further established that PI3K and G-protein coupled receptor (GPCR) signaling direct this actin-based cell motility and that perturbation of these cellular dynamics disrupted endocrine cell coalescence.

To further study islet morphogenesis, we are implementing cell-type specific, inducible gene expression using the Gal4ER/UAS system. Combined with our previously established induction approach, in which islet morphogenesis consistently progresses during larval stages within a relatively short time frame, we can detect Gal4ER-dependent fluorescent marker expression in induced secondary islet cells after tamoxifen treatment. We are using this system to identify additional signals and receptors essential for cell-cell recognition and adhesion, and to elucidate the molecular machinery driving islet compaction.

K07 | Dividing the early metanephric field - the role of *Tbx18* in ureter specification

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The collecting duct system of the kidney and the straight tube of the mature ureter derive from a common embryonic primordium, the ureteric bud. Differential development along its proximal-distal axis is not an autonomous program of this epithelial tissue but is triggered by the surrounding mesenchyme.[1] We have previously shown that the T-box transcription factor gene *Tbx18* is specifically expressed in the undifferentiated mesenchyme of the distal ureter and marks this lineage in the mouse.[2] Loss of *Tbx18* results in formation of a severely shortened and widened ureter that lacks differentiated mesenchymal and epithelial cell types.[3] Here, we show that ectopic expression of *Tbx18* in the posterior trunk mesenchyme including the entire metanephric field of mouse embryos results in kidney agenesis due to a failure of ureter bud formation after the early loss of the nephrogenic cord by apoptosis. Specific misexpression of *Tbx18* in either of the two mesenchymal sublineages of the metanephric kidney, the nephrogenic and the stromal mesenchyme, leads to severe kidney anomalies. Expression of an activator version of Tbx18 in the ureteric mesenchyme recapitulates the phenotypic changes obtained by the loss of *Tbx18* in the urogenital system. Together, these findings argue that Tbx18 specifies the ureteric mesenchyme by repression of key factors of the metanephric stromal and nephrogenic programs. We used microarray analysis of isolated wildtype and *Tbx18*-deficient ureteric mesenchyme to identify these Tbx18-regulated genes. We present the validation and characterization of candidate factors the deregulation of which may account for the dramatic defects in ureter development observed in *Tbx18*-deficient and Tbx18-misexpressing mice.

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K08 | *Drosophila* Twist - a myogenic switch?

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During the development of the mesoderm in *Drosophila melanogaster* the bHLH transcription factor *twist* has been identified as a key player. Previous research has provided strong indication that *twist* has different functions during successive stages of mesoderm and muscle development. First, before and during gastrulation, *twist* is expressed uniformly in a ventral strip of cells and specifies these cells to become mesoderm. Second, in the elongated germ band stage, *twist* is expressed in a segmentally-modulated pattern and was reported to act as a switch to induce different mesodermal fates, namely somatic versus visceral muscles, depending on its segmental levels. Third, persistent *twist* expression is present in undifferentiated adult muscle precursors (AMPs) and is thought to be necessary for their maintenance and/or developmental dormancy during larval stages, and particularly for adult muscle development during metamorphosis. Whereas the early *twist* function has been clarified by analyzing *twist* null mutants, studying its specific role during somatic muscle development and in adult muscle precursors has been more difficult as *twist* is already essential for mesoderm formation and gastrulation.

In order to investigate the functions of *twist* post-gastrulation, particularly during its segmental modulation and in AMPs, we generated genetic situations in which normal *twist* activity was only present until gastrulation due to the absence of the enhancer regions driving *twist* post-gastrulation. We demonstrate that in these mutant backgrounds Twist protein is only present until stage 10 but is absent subsequently when it normally would show striped expression, and is also absent in AMPs both in embryos and on imaginal discs.

Unexpectedly, these mutant embryos and their larvae are able to develop normal somatic muscles, visceral muscles and a heart even in the absence of segmentally modulated *twist* expression. These results are in partial disagreement with the previous reports and led us to reassess the contribution of *twist* to somatic myogenesis. Furthermore, we show that adult muscle precursors are present in these mutant embryos even though they lack Twist, and AMPs are also present in abdominal segments and on imaginal discs in mutant larvae. These mutants survive until late pupal stages but do not eclose as adults. Interestingly, the pharate adults completely lack the indirect flight musculature except for the remaining larval muscle templates. In the abdomen the muscle pattern partially develops but features fewer and smaller muscles.

Our results indicate that *twist* is not essential during the specification of somatic versus visceral cell fates. However, *twist* is essential in AMPs of imaginal wing discs during pupal stages for the development of the adult indirect flight musculature. We will present our analyses of this role of *twist* in more detail and show that it includes the activation of several downstream genes in the developing AMPs.

K09 | Same same, but different - the anterior lateral line

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Organs are the functional units of multicellular organisms and as such their integrity needs to be maintained. Loss of organ integrity can lead to malfunctions and disease. Organ malfunctions can often be traced back to abnormalities during organogenesis. Therefore, organogenesis needs to be tightly regulated to guarantee proper organ function.

The lateral line, a sensory system of fish and amphibia, is used as a simple model to study organogenesis. It consists of small sensory organs called neuromasts that are distributed across the entire surface of the body and it is divided into the posterior lateral line (pLL) and the anterior lateral line (aLL). The pLL is formed by neuromasts on the trunk and the aLL is formed by neuromasts on the head.

We use 4D imaging to study mechanisms of organogenesis in the lateral line of medaka (*Oryzias latipes*), a small teleost fish. Previously, our lab has shown that the pLL of medaka is formed by a single migrating primordium in a Cxcr4 and Cxcr7 dependent manner. At the end of embryogenesis medaka displays two parallel lines of neuromasts along the trunk. The number and position of neuromasts in the pLL is variable and in most cases differs between left and right of the same fish. So far, the basis of this variability is unknown.

In contrast to this, aLL neuromast number and position is highly stereotypic and, in most cases, left and right of the same fish are roughly symmetric. Our results suggest that aLL neuromasts are derived from both primordia and sensory ridges, specialized tissue regions that give rise to sensory organs. Moreover, analysis from mutants with heavily perturbed pLL, but relatively unaffected aLL indicates that aLL and pLL patterns are built differently.

K10 | Twist affects lineage reprogramming and transdifferentiation of syncytial alary muscles during *Drosophila* metamorphosis

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Lineage restriction and plasticity are very important concepts in multicellular organisms and have attracted a lot of attention particularly since it was shown that differentiated fibroblasts can be reprogrammed into pluripotent stem cells. Recent work in our group has revealed a unique muscular reprogramming and transdifferentiation process during natural development in *Drosophila melanogaster*. During metamorphosis three of seven pairs of syncytial alary muscles (AM) that connect the embryonic heart to inner organs and the exoskeleton dedifferentiate and fragment into mononucleated myoblasts (alary muscle derived cells, AMDCs). These AMDCs are reprogrammed and give rise to a new syncytial muscle structure, the so-called ventral longitudinal musculature (VLM), a triangular structure composed of twelve muscle fibers that cover the ventral side of the heart and separate it from the body cavity. This reprogramming process crucially depends on the AM identity gene *optomotor-blind-related-gene-1* (*org-1*; *Drosophila Tbx1*), which during embryogenesis is expressed in all seven pairs of AMs, during early metamorphosis exclusively in the three anterior AM pairs, and eventually in the VLM derived from these. By utilizing *org-1* expression as a lineage marker, this naturally occurring process serves as a good experimental system to investigate the molecular mechanisms underlying *in vivo* muscle lineage reprogramming, as well as a model for transdifferentiation in general.

Many regulatory factors that play an important role during embryonic development have recurring and often comparable roles during metamorphosis. Therefore, in a search for genes involved in our lineage reprogramming model, we screened for potential functions of such regulatory factors in transdifferentiation. One prominent example for a gene we identified in this screen encodes the bHLH transcription factor Twist. During early embryonic development in *Drosophila* the function of Twist is crucial for gastrulation and mesoderm formation. Later, its expression is restricted to the adult muscle precursors (AMPs), muscle stem cells that will give rise to the adult somatic musculature. However, herein we show that *twist* expression and the activity of a *twist*-reporter is also persisting in the embryonic and larval AMs after its early pan-mesodermal expression. Moreover, we can detect *twist* reporter activity during AM to VLM transdifferentiation via confocal fluorescent live imaging. After induction of AM fragmentation, the reporter remains active in the resulting AMDCs and during the following reprogramming process. Induction of AM-specific CRISPR/Cas9 or RNAi mediated knockdown of *twist* during transdifferentiation results in perturbation of proper VLM formation, providing evidence towards an important function of Twist during AM lineage reprogramming.

In sum, these results indicate that Twist fulfills a crucial role in AM to VLM lineage reprogramming, which calls for further research to understand the underlying mechanisms.

K11 | Tracheal apical extracellular matrix maturation in Drosophila melanogaster is mediated by evolutionary conserved serine proteases

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Organ development and function is dependent on the assembly and remodeling of extracellular matrix (ECM) structures. Extracellular and membrane-associated proteases are emerging key regulators of ECM dynamics. In particular, members of the type II transmembrane serine protease (TTSP) family play a central role by initiating proteolytic cascades. While deregulation of TTSPs is linked to many pathogenic conditions and developmental defects, fundamental mechanisms by which they regulate ECM assembly, remodeling and degradation remain elusive.

Here, we identify the *Drosophila* proteases Notopleural (Np) and Tracheal-prostasin (Tpr) as functional homologs of the vertebrate TTSP matriptase and serine protease prostasin, respectively. Vertebrate matriptase regulates ECM dynamics and maintenance of the epithelial barrier function in diverse tissue. Prostasin is activated by matriptase via proteolytic cleavage and regulates epithelial homeostasis and development. Accordingly we report, that the *Drosophila* TTSP Np mediates apical ECM differentiation during tracheal system development, regulates maintenance of the transepithelial barrier function, and activates Tpr by proteolytic cleavage. Conclusively, we show that loss of *Np* and *tpr* in *Drosophila* is rescued by expression of human matriptase and human prostasin, respectively. Furthermore, we identify zona-pellucida-domain (ZPD) proteins of the *Drosophila* apical ECM as targets of Np and Tpr. Both mediate degradation of the ZPD protein Dumpy during tracheal apical ECM maturation and Np cleaves the ZPD of the tracheal ECM protein Piopio *in vitro*. Similar to Np, human matriptase cleaves the ZPD of Piopio and activates *Drosophila* Tpr by proteolytic cleavage *in vitro*, indicating a conserved substrate specificity of Np and matriptase.

While the flexible, collagen-based ECM of vertebrates differs in many aspects from the rigid, chitin-based apical ECM of insects, ZPD proteins are a common feature and found in virtually all animals. Our data therefore suggest a novel fundamental mechanism of ECM regulation, by which degradation and remodeling of ZPD proteins is mediated by the conserved matriptase–prostasin proteolytic cascade.

K12 | Matrix metalloproteases 2 and 9 are fundamental for neural crest and skeletal development in the mouse embryo

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Matrix metalloproteases (MMPs) are a large family of enzymes known for their ability to degrade the extracellular matrix (ECM) and basal lamina of diverse cell types. MMPs are implicated in many physiological processes which involve remodeling of the ECM such as embryo implantation, cell migration, angiogenesis, and bone growth via their involvement in cell proliferation, differentiation and motility. Imbalance in MMP's activity causes pathological conditions such as metastasis, arthritis, multiple sclerosis and Alzheimer's disease. Neural crest cells (NCCs) are a unique cell population in the embryo that initially resides at the dorsal part of the neural tube, whereas later they detach and migrate throughout the embryo and differentiate into several derivatives including peripheral neurons and craniofacial bone and cartilage cells. We have previously found that the gelatinases' subfamily of MMPs, MMP2 and MMP9, regulate NCC onset of migration in the chick embryo. Yet, the role of these MMPs in mammalian NCC development is not known. In this study, we set to determine the individual and mutual roles of MMP2 and/or MMP9 in mouse NCCs, in early stages of migration and final stages of cranial skeletal formation.

First, we utilized *ex-vivo* explants in which hindbrains from E8.5 WT mouse embryos were treated with single or double inhibitors of MMP2/MMP9. Upon all treatments, NCC migration was significantly inhibited. Next, we obtained MMP2/MMP9 single knockout (sKO) mice to examine NCC migration in comparison to WT, and found typical migration in all cases. This finding suggested that one gelatinase may compensate for the loss of the other in the genetic model, and was supported by finding increased levels of MMP9 transcripts in MMP2 sKO embryos. To further elucidate this possibility, explants from sKO embryos were treated with the reciprocal MMP inhibitor, revealing decreased NCC migration. These findings suggest that both MMP2 and MMP9 are involved in mouse NCC migration process as in the absence of one MMP, NCC migration is maintained but when both are lacking, migration is impaired.

To better reveal the single or combined activity of these MMPs, we generated double KO mice (MMP2-/-MMP9-/-). Initial analysis of these mice demonstrated fertility problems, small litter size, dystocia, mortality of newborns and lower gain of weight. Furthermore, various skeletal developmental defects were observed including severe craniofacial deformities such as malformed jaws and skull sutures, significant shortened long bones, lower body mass density, and abnormal growth plate morphology. Current analyses are conducted to reveal effects on NCC development and ECM properties in this genetic model in order to elucidate for the first time the mutual role of MMP2 and MMP9 in the development of mammalian neural crest and skeletal cells.

K13 | The zinc finger transcription factor DBcl11/CG9650 is required for proper somatic and cardiac muscle development in Drosophila

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The formation of syncytial muscle fibres from single myoblasts and the utilization of sarcomeres as contractile units are central muscle properties conserved in most invertebrate and vertebrate species. Therefore, *Drosophila* as an established model organism provides a powerful tool to investigate the details of how muscles develop and are maintained. Here, we focus on the characterization of a novel gene as a potential new regulator of myogenesis in *Drosophila*.

Drosophila Bcl11 (*DBcl11* or *CG9650*) encodes a still uncharacterized putative zinc finger transcription factor that was identified in our lab as the gene that is mirrored by the widely used 1151-GAL4 enhancer trap line. 1151-GAL4 is specifically active in adult muscle precursors, dormant larval stem cells set-aside during embryonic myogenesis that will eventually give rise to the adult somatic musculature. Human *BCL11A* and *B* are required for normal lymphocyte and brain development, and *BCL11A* was found to be a transcriptional repressor regulating the switch from fetal to adult haemoglobin.

In *Drosophila* embryos, *DBcl11* mRNA and protein expression levels peak during specification and fate determination in all types of developing myogenic tissues followed by a decrease with the onset of differentiation and morphogenesis. *DBcl11* expression in parts of the nervous system is persistently strong.

Mutant *DBcl11* embryos show missing muscle fibres or fibres with an abnormal morphology in several groups of somatic muscles. Additionally, the embryonic heart structure is disrupted in *DBcl11* null embryos indicating a second role in embryonic cardiogenesis. We will present these phenotypes using both a splice mutant allele and new null alleles for *DBcl11* generated by CRISPR/Cas9-mediated deletion of the third exon that carries the zinc finger DNA binding motifs.

To examine the role of *DBcl11* during adult muscle development and to circumvent the lethality of the *DBcl11* null mutants, we are performing CRISPR/Cas9-mediated muscle tissue-specific knockdown of *DBcl11* and will present the obtained phenotypes as well.

Taken together, our results provide the first in-depth characterization of *DBcl11/CG9650* expression and function in myogenic tissues in the embryo and furthermore introduce *DBcl11* as a potential novel regulator of muscle development in *Drosophila*.

K14 | The Hox transcription factor Ubx stabilizes lineage commitment by suppressing cellular plasticity

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K15 | Org-1 drives direct muscle lineage reprogramming through negative regulation of Hippo signalling

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Lineage reprogramming has become an increased focus of recent research since it was demonstrated that the expression of lineage restricted transcription factors can be used *in vitro* for direct reprogramming. Recently, we described that the ventral longitudinal musculature (VLM) of *Drosophila* arises *in vivo* by Org-1 (*Drosophila* Tbx1) dependent direct lineage reprogramming from alary muscles (AM).At the onset of the transdifferentiation process the syncytial AMs initiate dedifferentiation and fragmentation into mononucleate alary muscle derived cells (AMDC). These are reprogrammed into VLM founder cells and initiate redifferentiation into the syncytial VLM.

We demonstrate that the induction of AM lineage reprogramming crucially depends on the activity of Yorkie (Yki) and Scalloped (Sd), the *Drosophila* orthologs of the vertebrate YAP/TAZ and TEAD proteins respectively, that is triggered by AM lineage specific inactivation of the Hippo (Hpo) pathway by *Drosophila* Tbx1 and its targets. The inactivation of atypical Protein Kinase C (aPKC) signalling in the AMs leads to the inactivation of the Hpo kinase and therefore to the nuclear translocation of the Yki/Sd transcriptional activator complex, inducing dedifferentiation and fragmentation of the syncytial AMs. We show that in parallel to these processes the induction of *Drosophila* Jun-amino-terminal kinase (Basket/dJNK) signalling as well as the function of the *Drosophila* orthologs of the vertebrate c-Jun and c-Fos proteins, Jun related antigen (Jra) and Kayak (Kay), represent additional prerequisites for the induction of AM dedifferentiation and fragmentation. We propose that the transcriptional effectors of the Hpo (Yki/Sd) and dJNK (dJun/dFos) signalling pathways are acting co-operative to induce the upregulation of *Myc* and *P-element induced wimpy testis* (*piwi)*, both crucially required for lineage reprogramming but fulfilling different functions. Whereas the direct lineage conversion of AMs into VLMs requires Myc function for the dedifferentiation and fragmentation of the AMs into the ADMCs, Piwi is crucially required for the cell fate switch in the generated AMDCs probably into a VLM muscle progenitor like state.

Further work has shown that YAP/TAZ, JNK/SAPK, c-Myc as well as mammalian homologues of Piwi are closely connected to mechanisms of direct reprogramming of mammalian cells. We provide genetic data that connects the transcriptional effectors of Hippo (Sd/Yki) and dJNK (dJun/dFos) with Myc and Piwi mediated function to a muscle lineage reprogramming process, associating their functions to mechanisms of lineage plasticity in *Drosophila* and proposing an evolutionary conserved role of these factors in cellular reprogramming. The further dissection of this naturally occurring *in vivo* direct lineage reprogramming process will uncover additional factors mediating muscle cell plasticity that have not been identified yet and may shed light on general mechanisms of lineage plasticity and cellular reprogramming.

L | EvoDevo

L01 | Characterization of putative stem cells in *Nematostella vectensis*

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Stem cell research in cnidarians has, until now, been focused on the genus *Hydra*, as they possess an intricate population of so called interstitial or i-cells, which function as dedicated stem cells. However, i-cells have so far only been found in Hydrozoa and may therefore not represent the ancestral condition. The anthozoan *Nematostella vectensis* is, like hydrozoans, highly regenerative, reproduces asexually as well as sexually and shows no signs of senescence, suggesting a stem cell and tissue homeostasis system. As a means to identify putative stem cells in *Nematostella*, we cloned >20 homologs of genes that have a conserved role in vertebrates and *Hydra* and conducted an *in situ* hybridization (ISH) screen. In 10 cases, we generated transgenic lines for *in vivo* lineage tracing. Through this approach, we were able to identify a population of putative stem cells in *Nematostella*. Future prospects of this project include the generation of additional transgenic lines as well as the characterization of labelled cell populations through the generation of cell type-specific transcriptomes. Results generated from these experiments will help us understand how stemness is established, regulated and maintained in Cnidaria, as well as allow insight into the evolutionary history of this integral cell type.

L02 | The role of brachyury in „mesoderm“ determination in metazoans

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All animals, except sponges can be categorized as triploblastic or diploblastic attributed to number of germ layers formed during their embryonic development. Unlike bilateria, its sister group cnidaria lacks a third germ layer, the mesoderm. Vertebrate homologs of the *brachyury* gene play a crucial role during early development, specifically in gastrulation and the elongation of the posterior body axis as well as the formation of the mesoderm. Yet, *brachyury* was present in the ancestor of fungi and animals. In order to gain insights into the origins of the mesoderm and the evolutionary transition from diploblasty to triploblasty, we investigated targets of the cnidarian *Nematostella vectensis* and echinoderm *Strongylocentrotus purpuratus brachyury* orthologs using ChIP-seq and RNA-Seq. By comparing our results to those of similar experiments performed in vertebrates *Xenopus tropicalis* and *Mus musculus*, we were able to identify vertebrate-specific targets of *brachyury* which may be crucial in the original formation of the mesoderm. Interestingly, the single common target of *brachyury* between vertebrates and invertebrates is *brachyury* itself, suggesting conserved self-regulation. With our binding motif analysis across the species, we found a significant difference between bilaterian and non-bilaterian species. We found considerable number of *brachyury* binding sites within very close vicinity of SOX binding sites. Earlier studies in vertebrates suggest that the *brachyury* and *sox2* act antagonistically to control the fate of neuromesodermal progenitors. Our analysis suggest a possibility of competitive binding of transcription factors within diploblastic cnidarians, while as we do not see any such possibility in triploblastic deuterostomes.

L03 | Functional diversification of interleukin-1 during vertebrate evolution

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Cytokines and chemokines orchestrate the innate immune response by mediating intercellular communication between many different cell types and are known to undergo rapid evolution. One notable example is the evolution of interleukin-1 (IL-1) cytokine, which first emerged in the genome of cartilaginous fish and then expanded into two gene copies in reptiles. Mammalian IL- 1α and IL-1β are involved in distinct immunological processes and can be activated by either cysteine proteases or Caspase-1, respectively. Although lower vertebrates possess only one copy of this gene, the current paradigm is that it resembles mammalian IL-1β. However, supportive experimental evidences are still missing.

To address this issue, we performed comprehensive *in silico*, *in vitro* and *in vivo* analyses. Protein sequence analysis indicated that cleavage sites for both enzymes are missing in cartilaginous fish, while teleost possess only a putative cleavage site for cysteine proteases.A first Caspase-1 cleavage site emerged in one of the duplicated IL-1 genes in some amphibians. We used an *in vitro* assay to test whether cysteine proteases or Caspase-1 are able to process the IL-1 proteins from amphibians, reptiles, chicken, teleosts and cartilaginous fishes. In agreement with *in silico* data, teleost IL-1 was cleaved by cysteine proteases but not Caspase-1. To further characterize the cleavage and functional diversification of IL-1 in teleosts, we created a medaka (*Oryzias latipes*) transgenic reporter. Medaka IL-1 is cleaved in a Ca^{2+}dependent manner, which is inhibited by exposure to MDL-28170, an inhibitor for some cysteine proteases of the calpain and cathepsin family. Thus, the mechanism underlying the cleavage of teleost IL-1 resembles the mammalian IL- 1α and not IL-1β.

In summary, our results provide essential information for the understanding of IL-1 functional diversification and proteolytic cleavage during the evolution and highlight that the catalytic pathway of cysteine proteases is the ancient mechanism in regulating IL-1 protein activity.

L04 | Release from yolk sac is required for extraembryonic envelope formation in the scuttle fly *Megaselia abdita*

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The formation of an extraembryonic envelope is a common element of early vertebrate and invertebrate development that provides a physical barrier and cover around the yolk or the developing embryo. At the cellular level, the formation of these extraembryonic envelopes is associated with a process called tissue-spreading, which typically involves a collective change in cell shape from columnar (long and tall) to squamous (short and wide), the dissociation from an underlying substrate, and, occasionally, cell proliferation and intercalation. Because of a stepwise reduction and eventual loss of extraembryonic envelope formation, flies provide a particularly rewarding framework to address the evolution of extraembryonic tissue spreading and its consequences for early development. To identify unknown mechanisms required for extraembryonic tissue spreading in insects, we investigated early development in the scuttle fly *Megaselia abdita*. We reveal the dynamics of extraembryonic envelope formation in long-term, whole-embryo time-lapse recordings, and show that extraembryonic tissue spreading requires the release from mechanical coupling to the underlying yolk sac. We find mechanical coupling prolonged and serosa spreading impaired after knockdown of *M. abdita Matrix metalloprotease 1* and conclude that tissue-tissue interactions provide a critical functional element to constrain spreading epithelia.

L05 | The evolution of the microRNA pathway and its essential role in cnidarian development

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Over the past decade microRNAs (miRNAs) have been shown to play pivotal roles as post-transcriptional regulators of gene expression in developmental processes in plants and animals. miRNAs can be found in a wide range of animals, yet their functions were studied almost exclusively in members of the Bilateria such as insects, nematodes and vertebrates. Thus, studying their function in non-bilaterian phyla as Cnidaria (sea anemones, corals, hydras and jellyfish) is crucial for understanding the evolution of miRNAs in animals and can provide important insights into their roles in the ancient ancestor of Cnidaria and Bilateria that lived more than 600 million years ago. Our previous results indicated that miRNAs in Cnidaria frequently have a nearly perfect match to their messenger RNA targets, resulting in target cleavage (slicing). Slicing is common to plant miRNAs, but very rare in Bilateria. Perfect binding of a miRNA to its target such as during slicing also frequently leads to the degradation of the small RNA, unless it is protected by a methyl group at its 3′ end. Indeed, we find that miRNAs are frequently methylated in Cnidaria and that their stability depends on the HEN1 methyltransferase. Loss of this enzyme results in a significant decrease in miRNA levels and defective development of the cnidarian model *Nematostella vectensis*. Interestingly, knockdowns of major miRNA biogenesis factors and effectors such as Dicer1 or the two members of the Argonaute (AGO) family, result in abnormal development reminiscent of the HEN1 knockdown. Thus, we conclude that like in both plants and bilaterian animals, cnidarian development depends on a functional miRNA pathway. We also reveal that the two AGO proteins of *Nematostella* carry vastly different small RNA cargos, composed of structurally distinct microRNAs and siRNAs and that they specialize in the regulation of different genes. This duplication of AGO happened in the last common ancestor of corals and sea anemones that lived roughly 500 million years ago. Duplication of AGO-encoding genes followed by specialization in different classes of small RNAs and regulating different processes is a recurrent theme in animal evolution and occurred independently in cnidarians, arthropods and nematodes.

L06 | Supernumerary segments forming in a short germ insect through repair of a disrupted initial pattern suggest autonomous patterning capabilities downstream of the segmentation clock

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While long germ Drosophila and short germ Tribolium both employ double-segmental pair-rule stripes during segment formation, the pair-rule patterns in Drosophila are primarily formed through individual regulation by gapgenes, while in Tribolium strong evidence points to a pair-rule based segmentation clock as dominating patterning principle. However, other short germ insects do not obviously use the pair-rule principle and it is not clear how their segmental patterns are generated. One possibility would be a segmentation clock with segmental periodicity, as in more basal arthropods which employ the Notch pathway similarly as during vertebrate somitogenesis.

Here we propose that some insects may employ a different mechanism, based on lateral inhibition acting at the level of segment-polarity genes. This gene class has been shown even in Drosophila to possess some pattern repair capabilities, which likely serves to stabilize the segment pattern during morphogenic movements. Such improvement of the segment-polarity stripes over developmental time also has been observed in several loss of function situations in the flour beetle Tribolium. Most evident is this mechanism, however, in experiments where Tc-hb was transiently overexpressed in early germ band stages. A transient peak of hb activity initiates an ordered sequence of gap gene activities (Boos et al. submitted: doi.org/10.1101/321786), but results in severely disrupted patterns of pair-rule and segment-polarity stripes. The latter initially form an almost randomly arranged pattern of distinct dots of expression. In the course of embryogenesis, however, these dots combine and rearrange to form much more complete stripes, and at the end of embryogenesis such embryos may develop the normal number of segments, or somewhat fewer segments than wild type, or several (up to 5) supernumerary abdominal segments.

This pattern repair principle may involve similar genetic interactions as has been described for Drosophila, i.e. mutual stabilization of adjacent heterologous stripes, likely supplemented by long-range inhibition between segmental domains through diffusing growth factors. Such a pattern stabilizing principle could serve in other insects as the primary mechanism of segmentation by inducing a series of posteriorly added segment-polarity stripes at intervals not defined by temporal periodicity but by the spatial diffusion range of factors such as Hh and Wg (Wnt).

L07 | The evolution of animal muscle cell types: Insights from the diploblast *Nematostella vectensis*

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Metazoan muscle cells share a general core contractile apparatus (CoCA) of actin, myosin and others. Within Bilateria (including insects, annelids, vertebrates and others), three different types of myocytes are present, i.e. gut smooth, cardiac striated and somatic striated myocytes. However, recent comparative work in model bilaterians showed that gut and cardiac myocytes show a similar slow, involuntary contraction and share a common core regulatory complex (CoRC including *GATA, Nkx, Fox*), suggesting their common origin from ancestral smooth, slow-contracting myocytes. In contrast, the myogenic TFs of voluntary, fast-contracting somatic striated myocytes belong to the bHLH class. While the evolution of bilaterian myocytes has been studied in detail, the situation in other metazoan groups such as cnidarians is unclear, impeding the reconstruction of muscle cell type evolution as a whole.

The anthozoan cnidarian *Nematostella vectensis* is equipped with smooth muscles made of general CoCA proteins and can be classified as slow-contracting circular body muscles and as fast-contracting longitudinal muscles. However, putative myogenic factors (CoRC) and function are unknown to date. To this end, we seek to identify the CoRC in cnidarian muscle cells, in order to unravel the evolution of muscle cell types in Metazoa.

Here, we present an in-depth characterization of the longitudinal retractor muscle of *N. vectensis* using single cell and bulk transcriptomics from FAC sorted transgenic animals, validated by in situ hybridization. We identified myogenic factors conserved with the CoRCs of both bilaterian slow and fast-contracting myocytes. However, we also identified several conserved transcription factors that have not previously been implicated in muscle development in bilaterians.

Using transgenics, transcriptome analysis and TEM we show evidence of neuromuscular junctions (NMJs) in the retractor muscles expressing Acetylcholine (ACh) and GABA receptors, but not Glutamate receptors. This is indicative of a voluntary stimulation of the smooth, fast-contracting retractor muscle similar to vertebrate somatic striated myocytes. Thus, in conclusion the smooth and fast-contracting retractor muscle of *N. vectensis* displays a CoRC that is partially shared with both striated and smooth muscles from bilaterians. We hypothesize that the eumetazoan muscle cell ancestor was smooth, had fast-acting properties and was innervated at a NMJ using ACh and GABA receptors.

L08 | Ancestral character of primate gastrulation.

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The notochord defines the anterior-posterior axis in chordates and its material derives from the dorsal organizer tissue which in lower vertebrates is represented by the dorsal lip of the blastopore. Birds and mammals develop the primitive node and a straight primitive streak instead of the dorsal blastopore lip and the lateral and ventral blastopore lips, respectively. Reptiles represent an intermediate state with a bona fide a blastopore and the so-called primitive or blastoporal plate immediately posterior to it. At the cellular level, involution generates the midline mesoderm within the dorsal lip of the reptilian blastopore, whereby internalized cells keep their epithelial polarity. In contrast, ingression of so-called bottle cells through the basement membrane is believed to be the main mechanism of mesoderm formation in the primitive plate and this is also described for the primitive node and streak in birds and mammals. High-resolution histological analysis of some new human and non-human primate embryos of the species Callithrix jacchus suggests both the presence of a blastopore equivalent and of bona fide involution within the notochord forming domain. We therefore propose that the structure commonly described as the node in human and non-human primates displays typical features of the reptilian blastopore and discuss our findings in framework of cellular mechanisms responsible for different types of gastrulation and the evolution of amniote gastrulation.

L09 | Evolution of Life Cycles in Polyclad Flatworms (Platyhelminthes)

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The majority of animal phyla are marine invertebrates of which many have complex life cycles that often include an intermediary stage in form of a ciliated, planktonic larva. The question how such biphasic life-cycles evolved and if ciliated larvae with an overall similar body plan represent an ancient metazoan feature is fundamental for our understanding of how life evolved on this planet. Polyclads, a member of the free-living flatworms (Platyhelminthes), provide a suitable system to study life-cycle evolution because they exhibit both developmental types: 1) indirect development, which features a ciliated, multilobular larval stage and 2) direct development, which gives rise to a juvenile similar to the adult form. All polyclads, regardless if they exhibit direct or indirect development, start embryogenesis with a highly conserved cleavage pattern called spiral quartet cleavage. This allows to identify and study homologous blastomeres in early cleaving polyclad embryos and follow them during development, a crucial advantage for detailed comparative developmental studies at a cellular level. It is the goal of this project to identify and map the earliest changes in developmental lineages of polyclads leading to a larva or adult form respectively. This is achieved by using state-of-the-art imaging techniques (single plane illumination microscopy or SPIM) which generates sophisticated live-imaging recordings and 3d-recosntructions of polyclad development.

L10 | Comparative studies of expression kinetics reveal developmental constraints and plasticity

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Developmental programs are capable of generating similar morphologies within the species, despite genetic variations and within broad environmental conditions. This raises the question: How is embryo morphology maintained through genetic and environmental variations? Here we present comparative studies of gene expression kinetics between closely related echinoderm species. Our studies reveal how correct temporal scaling exposes the striking conservation of developmental gene expression between morphologically similar species. This conservation is lower at larger evolutionary distances. We used different clustering approaches to address various comparative questions and identify the conservation and divergence of large gene sets. We discovered an unexpected contribution of the housekeeping genes to the interspecies correlations that distorts the hourglass pattern generated by developmental genes. Overall, we demonstrate how comparative studies of gene expression kinetics can provide novel insights into the developmental constraints and plasticity that shape animal body plans.

L11 | Uncovering the role of taxon-restricted genes in the neurons of the freshwater polyp *Hydra*.

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In every animal species, including human, highly-conserved genes make up the functional core of their genomes, essential for their development. Yet an equally large portion of each genome is represented by so called taxon-restricted genes (TRGs). They show no homology to other genes outside that species or phylum. Therefore, their functions can hardly be predicted *in silico* and remain mainly unclear. Recent studies, however, provide evidence for an important evolutionary role of the TRGs in emergence of novelties, such as new cell types (*e.g*. Khalturin et al., 2009). Recently, we uncovered (Klimovich et al., in prep.) that the transcriptomes of the nerve cells in the freshwater polyp *Hydra* are enriched in TRGs: over 80% of genes differentially expressed in the neurons are TRGs, compared to only 13% in the stem cells. Even more remarkably, the diversification of seven cell types of neurons in *Hydra* is driven mainly by TRGs. Here, we sake to systematically analyze the complexity and function of the TRGs in *Hydra* neurons. By combining genomic, transcriptomic and proteomic dataset analysis, *in silico* peptide analysis and machine learning with *in vitro* assays, we reveal the role of the neuron-specific TRGs in *Hydra*. In particular, we examine their involvement in modulating the tissue homeostasis and morphogenesis, neuronal signal transmission, and host-microbe interactions. Our study reveals the previously under-appreciated role of the TRGs in generating the cell-type diversity. Furthermore, it provides new insights into the functional complexity of the genomes in animal kingdom and highlights the role of non-conserved species-specific genes in gene-environment interaction and evolution.

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L12 | A new gene buds out and takes over an essential role in tall blastoderm formation in higher flies

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Novel genes in organismal evolution play important roles in adaptation and specification in developmental processes. They are created by various modes (1) duplication and divergence of an entire gene (2) rearrangement of pre-excisting genetic elements or (3) de novo ie. out of previously non coding sequences. Our research focuses on morphogenetic differences in early fly development between evolutionary distant fly species. By sequence analysis of available fly genomes, we could identify five novel genes involved in the process of cellularization but only found in higher flies (cyclorrhapha). Blast and synteny analysis of one of these novel genes, *slam*, proposes a new mechanism of novel gene formation: budding out an exon of a gene build of several exons. We assume that this exon adapted to take over an essential role in the process of cellularization in cyclorrhaphan flies. In *D. melanogaster* it is known to recruit myosin to the invaginating membrane and regulate the actomyosin network to ensure the fully emergence of the furrow canal and thus the formation of a tall columnar blastoderm. In lower flies, which are missing *slam*, the blastoderm is thinner and consist of a small cuboidal cells. We find that early expression of *D. melanogaster slam* in *C. riparius* is sufficient to invoke a tall blastoderm, suggesting that slam act as switch from cuboidal to columnar blastoderm. We propose *slam* was quickly incorporated into cellular blastoderm formation because their ability to recruit actin and myosin to the furrow canal provided a beneficial add-on to an ancient developmental program.

L13 | Ancestral complexity and function of the nervous system: Insights from single-cell transcriptomics in *Hydra*

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Cnidaria is the oldest extant group of metazoans where the nervous system emerged. Therefore, members of this phylum are particularly informative for tracing the evolution, ancestral complexity and function of the nervous systems. In the freshwater polyp *Hydra*, the nervous system is made up of two cell types – sensory and ganglion neurons, organized in a diffuse nerve net. In spite of this simplicity, this ancient nerve system generates diverse and complex behaviors. Molecular profiling of the *Hydra* nervous system by single-cell transcriptomics revealed that it is composed of at least 7 discrete neuron classes, that correspond to spatially restricted cell populations. Further, functional analysis allowed assigning different elements of behaviour (such as control of body contractions periodicity or regulation of feeding reflex) to distinct neuronal subpopulations. Our data indicate that cnidarians use a complex toolbox of transmitters, receptors, and ion channels to generate complex behaviors with a simple nerve net.

Unexpectedly, the neurons express a rich repertoire of immune receptors and signaling pathways, and secrete a variety of putative antimicrobial peptides. Further analysis of some neuropeptides by transgenesis demonstrated that they shape the microbiome on the body surface of *Hydra*. Behavioral assays uncovered that the symbiotic bacteria influence the activity of the *Hydra* nervous system by likely directly interfering with neuronal receptors. In bilaterian animals, the host-associated microbiota is also in a permanent dialog with the host enteric and central nervous systems. Together these findings point to a universal role of the nervous system in mediating host-microbe interactions throughout the Metazoa and suggest that it emerged to orchestrate multiple functions involved in maintaining the metaorganism integrity.

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L14 | A new gene family of short collagens in the development and regeneration of the sea anemone *Nematostella vectensis*

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The collagen triple helix repeat containing protein (*Cthrc1*) gene was identified at first as being induced upon injury to arteries in rats and was later detected in multiple human cancers where it was found to increase cell migration, invasion and EMT. The functions and molecular modes of operation of this gene are still preliminary and knowledge about its phylogeny was very limited. We have recently performed a transcriptional screen in order to explore whole-body regeneration in the sea anemone *Nematostella vectensis* (Nv), a cnidarian model animal. In this screen we compared the oral ("head") to aboral ("tail") profiles of gene expression during this process, and discovered a gene family of high similarity to mammalian *Cthrc1*, most members of which demonstrated high differential oral-aboral expression along the regeneration process. Here we report the characterization of this eight members gene family in Nv in terms of sequences, structure and genomics and explore the general phylogeny in metazoans which was hitherto unknown. The expression patterns of the different family members along the developmental stages, in adult polyps and during the time course of regeneration were also analyzed. Upon regeneration, these genes show a highly dynamic and unique mode of temporal and spatial expression, suggesting a putative role in the re-construction of the missing body parts. Our new data on the *Cthrc* genes in *Nematostella* as well as our phylogenetic studies in metazoans may shed light on the general logic of their operation and on the evolution of this gene family, which may thus enhance the limited knowledge on this little explored but of potential great medical importance gene in man.

L15 | Tinkering with development: the Lateral line as a model to study pattern formation and evolution

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How are different patterns built and molded during evolution? We try to address this fundamental Evo/Devo question by making use of the fantastic diversity of lateral line patterns that exist in teleost fish. The basis of which is still poorly understood. By preforming long-term *in-vivo* imaging in Medaka fish during development we were able to show that there are fundamental differences between the embryonic lateral line patterns of Medaka and Zebrafish. Molecularly, we show that the same chemokine genes that initially drive primordium migration are re-utilized to subsequently form secondary organs. We make use of existing mutants (DA) and newly generated CRISPR knock-outs (*Keratin15*) to tinker with the embryonic posterior lateral line pattern in Medaka. This allows us to begin unraveling the guiding principles behind pattern formation. Additionally, we have characterized the embryonic and adult lateral line patterns of 27 closely related *Oryzias* species. We report that the patterns underwent a rapid radiation and diversification. Using inter-species transplantations we attempt to answer whether extrinsic (speed of development, tissue tension, physical constraint) or intrinsic cues (within the primordium) are regulating the diversity of lateral line patterns we observe in the different species.

L16 | Extrinsic and intrinsic factors regulate body size in *Hydra* by conserved signaling pathways

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How multicellular organisms assess and control their size is a fundamental question in biology, yet the molecular and genetic mechanisms that control organ or organism size remain largely unresolved. The freshwater polyp *Hydra*, member of the phylogenetically ancient phylum Cnidaria, demonstrates a remarkable capacity to adapt its body size to changing environmental conditions. Uncovering the molecular mechanisms controlling this remarkable form of phenotypic plasticity promises to provide insight into the process of size regulation shared across the animal kingdom. Using a number of wild type and genetically modified *Hydra* strains cultured at different temperatures combined with a transcriptomic approach we uncovered insulin like peptide receptor (INSR) and forkhead box protein O (FoxO) as important genetic drivers of size determination in *Hydra*. Our analyses also identified developmental regulators including TGF-β and Wnt as additional major players in size determination. Environmental and genetic factors thus appear to directly affect conserved developmental mechanisms in which cell number but not cell size turns out to be the strongest determinant of body size. These findings highlight the value of an evolutionary informative model organism when attempting to decipher the basic mechanisms on how size is regulated at the whole organism level.

L17 | Towards understanding an ultimately simple metaorganism: Impact of symbiotic microbes on developmental processes of *Hydra*

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Animal developmental programs occur within the context of coevolved associations with microbes. As one of the prime model systems for evolutionary developmental biologists, hydra allows easy access to combined genetic, cell biological, molecular and computational approaches for studying host-symbiont interactions. The ongoing project aims to determine what portion of *Hydra vulgaris* AEP's developmental processes are affected by interactions with the microbial partners and how these interactions are integrated into its well characterized network of developmental regulators and signal transduction pathways. Study approach includes functional characterization of already identified candidate genes differentially expressed in response to bacteria using microarray analysis as well as an unbiased and systematic identification of bacteria-responsive genes using RNA-seq technology comparing libraries from germ free polyps to control conventionalized polyps sampled at different developmental stages and under different environmental conditions. This project will identify host genes which are dependent on the presence of symbiotic bacteria and play an important role in its developmental processes, reveal the specific bacterial components involved in controlling gene expression in the host, and provide first insight into the genetic basis of the relationship between host and microbes in the *Hydra* metaorganism.

L18 | Echinoderms adapted the VEGF-driven vascularization program to generate calcite skeletons

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Sea urchin calcite spicules and vertebrates blood vessels are quite distinct in their function yet both have a tubular structure and both are controlled by the vascular endothelial growth factor (VEGF) signaling. VEGF pathway regulates tubular structure formation across the animal kingdom, while its control of biomineralization seems to be unique to the echinoderm phylum. The molecular mechanisms that VEGF activates during sea urchin skeletogenesis and whether there are other parallels between these to those that drive vertebrates' vascularization were largely unknown. Here we reveal remarkable similarities between sea urchin skeletogenesis and vertebrates' vascularization, in the upstream gene regulatory network, the downstream effector genes and the cellular processes that VEGF signaling regulates. We show that human VEGF is capable of inducing ectopic spicule branching in the sea urchin embryo, indicating the conservation of VEGF-VEGFR recognition between the two organisms. Five of the seven upstream skeletogenic transcription factors have vertebrates' homolog expressed in endothelial cells that regulate different aspects of vascularization. VEGF signaling activates a complex network of regulatory, biomineralization and vascularization related genes, among them, *angiopoetin, notch1* and *rhogap24l/2*. Two human homologs of Rhopag24l/2 are the most enriched Rhogaps in human endothelial cells and both overexpression and down-regulation of sea urchin rhogap24l/2 expression leads to ectopic spicule branching. We propose that sea urchin skeletogenesis and vertebrates' vascularization diverged from a common ancestral vascularization program specifically adapted for biomineralization in the echinoderm phylum.

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