https://amphiclub2022.sciencesconf.org/

European Amphibian Club 2022

1-4 Jul 2022
GHENT
Belgium

Friday, July 1, 2022

TIME	EVENT
14:00 - 18:00	REGISTRATION
18:00 - 19:00	Refreshment and food
19:00 - 19:10	Welcome words - Kris Vleminckx/Eric Bellefroid
19:10 - 19:40	Mechanical and Chemical signals interplay during neural crest migration - Roberto Mayor
19:40 - 21:10	Ciliated epithelia and cytoskeleton - Peter Walentek
19:40 - 20:00	› Mechanics and development of the mucociliary epithelium - *Jakub Sedzinski - The Novo Nordisk Foundation Center for Stem Cell Biology*
20:00 - 20:20	› Oxygen-producing microalgae in the brain of Xenopus tadpoles rescue neuronal activity - *Hans straka, Ludwig-Maximilians-Universität München*
20:20 - 20:40	› In vivo time-lapse imaging of olfactory sensory neuron birth, differentiation, and axogenesis - *Ivan Manzini, Department of Animal Physiology and Molecular Biomedicine, Justus-Liebig-University Gießen, Germany, Center for Mind, Brain and Behavior (CMBB), Justus-Liebig-University Gießen, Germany*
20:40 - 20:55	› dmrt2 and myf5 link early somitogenesis to left-right axis determination in Xenopus laevis - *Melanie Tingler, University of Hohenheim*
20:55 - 21:10	› Mucus, ciliation, stemness - Spdef does it all - *Maximilian Haas, Renal Division, Department of Medicine, University Freiburg Medical Center, Freiburg, Spemann Graduate School of Biology and Medicine, Albert Ludwigs University Freiburg, Freiburg, IMITATE - Institute for Disease Modeling and Targeted Medicine*
21:10 - 23:00	Happy Hour

Saturday, July 2, 2022

TIME	EVENT
07:30 - 09:00	Breakfast
09:00 - 09:30	The role of memories in axolotl regeneration - Elly Tanaka
09:30 - 10:30	Stem cells and regeneration - Elly Tanaka
09:30 - 09:50	› Re-generating the principles of appendage regeneration in the single-cell era - *Can Aztekin*
09:50 - 10:10	› Uncovering the cells and genes responsible for limb regeneration - *Nicolas Leigh, Lund Stem Cell Center, Lund University, Lund*
10:10 - 10:30	› Modeling spinal cord regeneration in the axolotl - *Osvaldo Chara, University of Nottingham-School of Biosciences*
10:30 - 11:00	Coffee break
11:00 - 12:05	Stem cells and regeneration - Elly Tanaka
11:00 - 11:20	› The Axolotl limb skeleton through growth and regeneration - *Tatiana Sandoval-Guzman, Center for Molecular and Cellular Bioengineering [TU Dresden, Germany]*
11:20 - 11:35	› Redox control of stemness: insights from neural stem cells of the Xenopus retina - *Morgane Locker, Institut des Neurosciences Paris-Saclay*
11:35 - 11:50	› Xenopus tropicalis Immature Sertoli Cells show potential in promoting muscle regeneration in tadpoles - *Qing Zhao# , Charles University, Faculty of Science, Vinicna 7, 128 44, Prague 2, Czech Republic*
11:50 - 12:05	› Hmmr modulates Wnt signaling to drive mesenchymal-to-epithelial transition in the developing forebrain - *Fee Wielath, Universität Hohenheim Zoologie, University of Hohenheim, Institute of Biology, Dept. Zoology*
12:05 - 12:35	Disease modeling and translational research - Enrique Amaya

TIME	EVENT
12:05 - 12:20	› Phenotyping embryonic development and disease using deep learning and mesoSPIM light-sheet microscopy - *thomas naert, Institute of Anatomy, University of Zurich , Zurich 8057, Switzerland ; Swiss National Centre of Competence in Research (NCCR) Kidney Control of Homeostasis (Kidney.CH) , Zurich 8057*
12:20 - 12:35	› The ribosomal proteins Bop1 and Rpl5 affect Xenopus laevis development - *Corinna Gärtner, Institute of Biochemistry and Molecular Biology, Ulm University*
12:35 - 14:00	Lunch
14:00 - 15:35	Disease modeling and translational research - Enrique Amaya
14:00 - 14:20	› A functional Operon imparts laterality at the ciliated LR organizer - *Bruno REVERSADE, A*Star*
14:20 - 14:40	› SOX transcription factors direct tissue-specific WNT responsive transcription independent of TCFs - *Aaron Zorn, Center for Stem Cell and Organoid Medicine, Divisions of Developmental Biology, Cincinnati Children's Hospital, and the Department of Pediatrics, College of Medicine University of Cincinnati*
14:40 - 15:00	› Cross-talk between thyroid hormone and glucocorticoid signalling - *Laurent Sachs, Physiologie moléculaire et adaptation*
15:00 - 15:20	› CHARGEd with neural crest defects - using Xenopus to characterize neurocristopathies - *Annette Borchers, Faculty of Biology, Molecular Embryology, Philipps-Universität Marburg*
15:20 - 15:35	› TBC1D32/Bromi mutations are associated with retinitis pigmentosa: a study combining clinical ophthalmology, iPS-based disease modeling and Xenopus functional approaches - *Caroline Borday - Paris-Saclay Institute of Neuroscience, CERTO-Retina France*
15:35 - 15:55	Coffee break
15:55 - 16:40	Workshop : Using Xenbase - Christina Zorn
16:40 - 18:40	Cellular and molecular biology - Jerome Jullien
16:40 - 16:55	› European Xenopus Resource Centre, modelling rare monogenic human disease in Xenopus: recent progress in the behavioural analysis of tadpole models of Neurodevelopmental disorders. - *Annie Godwin - European Xenopus Resource Centre, Portsmouth*
16:55 - 17:15	› The PKA-PP2A dynamics keep oocytes arrested in meiosis I - *Aude Dupré - Institut de Biologie Paris Seine*
17:15 - 17:30	› Let's make a quantum leap: *Xenopus laevis* advantages to shed light on axonal circRNAs - *Linda Masante - Centre for Integrative Biology (CIBIO), University of Trento*
17:30 - 17:45	› Role of foxn1 in innate T cells driven immune tolerance of X. laevis tadpoles. - *Dionysia Dimitrakopoulou - University of Rochester Medical Center*
17:45 - 18:00	› Hnf1b renal expression directed by a distal enhancer responsive to Pax8 - *Muriel Umbhauer - IBPS - UMR7622*
18:00 - 18:15	› The Rho GEF Trio is a major regulator of neural crest cell migration and dynamically localized at microtubules in cranial NC cells - *Stefanie Gossen, DFG Research Training Group, GRK 2213, Phillipps University Marburg, Department of Biology, Molecular Embryology, Philipps University Marburg*
18:20 - 19:00	Fast Talks
18:20 - 18:23	› Impact of glyphosate-based herbicide on early embryonic development of the amphibian Xenopus laevis - *Hannah Flach, Institute of Biochemistry and Molecular Biology, Ulm University*
18:23 - 18:26	› Using Xenopus laevis tadpoles to study basic principles underlying vertebrate motor control - *wen-chang li, school of psychology and neuroscience, the university of st andrews*
18:29 - 18:32	› Modelling USH2A-associated retinal disease in Xenopus tropicalis to investigate the pathogenicity of human missense variants implicated in inherited blindness - *Marjolein Carron, Developmental Biology Unit, Department of Biomedical Molecular Biology, Ghent University, Centre for Medical Genetics, Ghent University and Ghent University Hospital, Ghent*
18:32 - 18:35	› Investigating EZH2 as a druggable mediator of immune cell exclusion in desmoid tumors - *Marthe Boelens, Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium, Cancer Research Institute Ghent (GRIG), Ghent*
18:35 - 18:38	› Ptk7 is dynamically localized at NC cell-cell contact sites and interacts with the RhoGEF Trio - *Katharina Till - Philipps Universität Marburg*
19:00 - 23:00	POSTER_SESSION

Sunday, July 3, 2022

TIME	EVENT
07:30 - 09:00	Breakfast
09:00 - 09:30	The drivers and blockers of retinal regeneration - Muriel Perron
09:30 - 10:40	Cellular and molecular biology - Jerome Jullien
09:30 - 09:50	› Subcellular translation in neurons: when mRNAs meet endosomes - *Jean-Michel Cioni, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan*
09:50 - 10:10	› Trim29: Ubiquitin signaling in ectodermal development - *Thomas Hollemann, University of Halle-Wittenberg*
10:10 - 10:25	› A non-transcriptional function of Yap regulates the DNA replication program - *Odile Bronchain, Institut des Neurosciences Paris-Saclay*
10:25 - 10:40	› Otic Neurogenesis in Xenopus: Proliferation, Differentiation, and the Role of Eya1 - *Gerhard Schlosser, National University of Ireland Galway*
10:40 - 11:10	Coffee break
11:10 - 12:30	Disease modeling and translational research - Enrique Amaya
11:10 - 11:30	› Development and Evolution of Tetrapod Motor Circuits - *Lora Sweeney - Institute of Science and Technology [Austria]*
11:30 - 11:45	› Xenopus: An in vivo model for studying skin response to UVB irradiation - *Joudi El Mir - University of Bordeaux*
11:45 - 12:00	› Novel penetrant and short latency models for liposarcoma and wilms tumor using CRISPR multiplexing in Xenopus tropicalis - *Dieter Tulkens - Cancer Research Institute Ghent (CRIG), Ghent, Belgium, Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium*
12:00 - 12:15	› Using CRISPR/Cas9 in Xenopus tropicalis to investigate cis-regulatory element variants in developmental eye anomalies : identification of an Otx2-binding site in a novel putative enhancer of the mab21l2 gene - *Munevver Burcu Cicekdal - Department of Biomedical Molecular Biology, Ghent University, Center for Medical Genetics and Department of Biomolecular Medicine, Ghent University and, Ghent University Hospital*
12:30 - 14:00	Lunch
14:00 - 15:30	Signaling and morphogenesis - Thomas Hollemann
14:00 - 14:20	› Receptor-mediated endocytosis orchestrates anterior neural tube closure - *Kerstin Feistel, University of Hohenheim, Institute of Biology, Dept. Zoology*
14:20 - 14:40	› microRNAs: novel transport route and action in axons development - *Marie-Laure Baudet, University of Trento [Trento]*
14:40 - 15:00	› Dr. Strangefoot (aka, Xenopus) or: How did I Learn to Stop Worrying and Love Metabolism - *Enrique Amaya, University of Manchester [Manchester]*
15:00 - 15:15	› R-spondin 2 as a BMP receptor 1A antagonist in the Spemann Organizer function to regulate Xenopus axial patterning - *Hyeyoon Lee, DKFZ*
15:15 - 15:30	› ENTPD5: a new player during kidney formation - *karine masse, Institut des Maladies Neurodégénératives*
15:30 - 16:00	Coffee break
16:00 - 16:45	Workshop : Resources and Husbandry - Matt Guille
16:45 - 17:25	Signaling and morphogenesis - Thomas Hollemann
16:45 - 17:05	› A new regulator of the cerebellar granular neuron stem/progenitors niche size and behavior: A study in amphibian - *Béatrice Durand - Sorbonne Université, CNRS UMR7622, IBPS Developmental Biology Laboratory, Campus Pierre et Marie Curie*
17:05 - 17:20	› Temporal integration of Notch signaling in mucociliary epithelia cell fate specification - *Magdalena Brislinger Engelhardt - CIBSS - Center for Integrative Biological Signalling Studies, Albert-Ludwigs-University Freiburg, Spemann Graduate School of Biology and Medicine, Albert Ludwigs University Freiburg, Freiburg, Renal Division, Department of Medicine, University Freiburg Medical Center, Freiburg*

TIME	EVENT
17:25 - 18:30	BUSINESS MEETING AND PRIZES
19:30 - 21:30	Dinner
21:30 - 23:00	Party

Monday, July 4, 2022

TIME	EVENT

List of sponsors

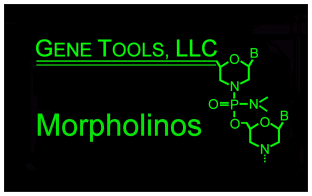
Molecular Instruments
Molecular Instruments® (MI) designs and synthesizes kits for multiplexed, quantitative, high-resolution bioimaging.

Seeing beyond

ZEISS
an internationally leading technology enterprise operating in the fields of optics and optoelectronics.

CIBSS
CIBSS is a Cluster of Excellence (EXC-2189) funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) and faculty-bridging Central Research Institute of the University of Freiburg

GfE
The Society of Developmental Biology (Gesellschaft für Entwicklungsbiologie e.V. or GfE), founded in 1975, comprises scientists from German-speaking countries who address developmental questions in the areas of genetics, molecular biology, medicine, zoology, and botany.



Gene Tools

Gene Tools makes Morpholino antisense oligonucleotides.

Nephrogenetics (NephGen)

"Collaborative Research Center 1453 - Nephrogenetics (NephGen)"

Mechanical and Chemical signals interplay during neural crest migration

Roberto Mayor [*†] [1]

[1] UCL – United Kingdom

[*]Speaker
[†]Corresponding author: r.mayor@ucl.ac.uk

The role of memories in axolotl regeneration

Elly Tanaka *† [1]

[1] IMP – Research Institute of Molecular Pathology Vienna – Austria

*Speaker
†Corresponding author: elly.tanaka@imp.ac.at

The drivers and blockers of retinal regeneration

Muriel Perron *† [1]

[1] Institut des Neurosciences Paris-Saclay – Université Paris-Saclay, CNRS – France

*Speaker
†Corresponding author: muriel.perron@universite-paris-saclay.fr

Mechanics and development of the mucociliary epithelium

Jakub Sedzinski [*†] [1]

[1] The Novo Nordisk Foundation Center for Stem Cell Biology – Denmark

[*]Speaker
[†]Corresponding author: jakub.sedzinski@sund.ku.dk

Oxygen-producing microalgae in the brain of Xenopus tadpoles rescue neuronal activity

Hans Straka [*†] [1]

[1] Ludwig-Maximilians-Universität München – Germany

[*]Speaker
[†]Corresponding author: straka@biologie.uni-muenchen.de

In vivo time-lapse imaging of olfactory sensory neuron birth, differentiation, and axogenesis

Ivan Manzini *† [1,2]

[1] Department of Animal Physiology and Molecular Biomedicine, Justus-Liebig-University GieÃŸen, Germany – Germany
[2] Center for Mind, Brain and Behavior (CMBB), Justus-Liebig-University GieÃŸen, Germany – Germany

*Speaker
†Corresponding author: ivan.manzini@physzool.bio.uni-giessen.de

Re-generating the principles of appendage regeneration in the single-cell era

Can Aztekin [*†] [1]

[1] Swiss Federal Institute of Technology Lausanne (école Polytechnique Fédérale de Lausanne), EPFL – Switzerland

[*]Speaker
[†]Corresponding author: can.aztekin@epfl.ch

Uncovering the cells and genes responsible for limb regeneration

Nicolas Leigh *† [1]

[1] Lund Stem Cell Center, Lund University, Lund – Sweden

*Speaker
†Corresponding author: nicholas.leigh@med.lu.se

Modeling spinal cord regeneration in the axolotl

Osvaldo Chara [*†] [1]

[1] University of Nottingham-School of Biosciences – United Kingdom

[*]Speaker
[†]Corresponding author: osvaldo.chara@nottingham.ac.uk

The Axolotl limb skeleton through growth and regeneration

Tatiana Sandoval-Guzman *† [1]

[1] Center for Molecular and Cellular Bioengineering [TU Dresden, Germany] – Germany

*Speaker
†Corresponding author: tatiana.sandoval_guzman@tu-dresden.de

A functional Operon imparts laterality at the ciliated LR organizer

Bruno Reversade *† [1]

[1] A*Star – Singapore

*Speaker
†Corresponding author: bruno@reversade.com

SOX transcription factors direct tissue-specific WNT responsive transcription independent of TCFs

Aaron Zorn *† [1], Shreyasi Mukherjee , Scott Rankin

[1] Center for Stem Cell and Organoid Medicine, Divisions of Developmental Biology, Cincinnati Children's Hospital, and the Department of Pediatrics, College of Medicine University of Cincinnati – Cincinnati OH 45229,, United States

WNT/ß-catenin signaling controls gene expression in many biological contexts from development to stem cell homeostasis with dysregulation causing cancer. How ß-catenin is recruited to distinct enhancers to activate context-specific transcription is unclear, given that almost all WNT/ß-catenin-responsive transcription is thought to be mediated by HMG-box TCF/LEF transcription factors (TFs) with identical DNA-binding specificities. With time-resolved multi-omics analyses, we show that SOX TFs can direct lineage-specific WNT-responsive transcription in *Xenopus* embryos and during the directed differentiation of human pluripotent stem cells (hPSCs) into definitive endoderm and neuromesodermal progenitors. We demonstrate that SOX17 and SOX2 are required to recruit ß-catenin to lineage-specific WNT-responsive enhancers, many of which are not occupied by TCFs. Moreover, we show that ectopic overexpression of SOX17 in cells lacking all four TCF/LEFs is sufficient to direct ß-catenin recruitment to chromatin. At enhancers co-occupied by SOX and TCF TFs, we find that they compete for the recruitment of a limited pool of ß-catenin to lineage-specific enhancers, thus influencing target gene activation. Functional genomics, biochemistry, and proteomics indicate that at TCF-independent enhancers, SOX TFs establish a permissive chromatin landscape and recruit a WNT-enhanceosome complex to regulate tissue-specific SOX/ß-catenin-dependent transcription upon Wnt activation. Given that SOX TFs are expressed in most cell types, these results have broad mechanistic implications for the specificity of WNT responses across developmental and disease contexts.

Keywords: transcription WNT SOX Endoderm stem cells genomics

*Speaker

†Corresponding author: aaron.zorn@cchmc.org

Cross-talk between thyroid hormone and glucocorticoid signalling

Laurent Sachs *† [1]

[1] Physiologie moléculaire et adaptation – Museum National d'Histoire Naturelle, Centre National de la Recherche Scientifique : UMR7221 – France

*Speaker
†Corresponding author: laurent.sachs@mnhn.fr

CHARGEd with neural crest defects - using Xenopus to characterize neurocristopathies

Janina Schwenty-Lara [1], Hanna Berger [1], Pauli Silke [2], Annette Borchers [*1]

[1] Faculty of Biology, Molecular Embryology, Philipps-Universität Marburg – Germany
[2] Institute of Human Genetics, University Medical Center Göttingen, Göttingen, Germany – Germany

Congenital malformation syndromes are frequently accompanied by craniofacial defects, ear malformations and heart defects suggesting that neural crest development may be affected in these patients. Although trio exome sequencing of the patients and their parents can identify potential de novo mutations responsible for the respective phenotypes, research using animal model systems is required to determine if neural crest development is affected. In recent years, we have used *Xenopus* to determine if human malformation syndromes belong to the so called neurocristopathies, pathologies caused by defects in neural crest development, and to characterize which steps of neural crest development are affected. Here, we will take a closer look at Kabuki-syndrome, an autosomal dominant disorder with high similarities to CHARGE syndrome, characterized by a typical facial gestalt in combination with short stature, intellectual disability, skeletal findings and additional features like cardiac and urogenital malformations, cleft palate, hearing loss and ophthalmological anomalies. The major cause of Kabuki syndrome are mutations in KMT2D, a gene encoding a histone H3 lysine 4 (H3K4) methyltransferase belonging to the group of chromatin modifiers. Here, we present data showing that major features of Kabuki syndrome can be recapitulated in *Xenopus* and that Kmt2d is required for neural crest formation and migration. Time permitting, we will also present first evidence that a novel syndrome caused by mutations in FBRSL1 belongs to the neurocristopathies.

Keywords: neurocristopathies, neural crest development, Kabuki, syndrome, Kmt2d, neural crest migration

[*]Speaker

The PKA-PP2A dynamics keep oocytes arrested in meiosis I

Aude Dupré *† [1]

[1] Institut de Biologie Paris Seine – Sorbonne Université, Centre National de la Recherche Scientifique : UMR7622 – France

*Speaker
†Corresponding author: aude-isabelle.dupre@upmc.fr

Subcellular translation in neurons: when mRNAs meet endosomes

Jean-Michel Cioni [*†] [1]

[1] Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan – Italy

[*]Speaker
[†]Corresponding author: cioni.jeanmichel@hsr.it

Trim29: Ubiquitin signaling in ectodermal development

Thomas Hollemann [*†] [1]

[1] University of Halle-Wittenberg (Institute for Physiological Biochemistry) – Germany

[*]Speaker
[†]Corresponding author: thomas.hollemann@medizin.uni-halle.de

Development and Evolution of Tetrapod Motor Circuits

Lora Sweeney *† [1]

[1] Institute of Science and Technology [Austria] – Austria

*Speaker
†Corresponding author: lora.sweeney@ist.ac.at

Receptor-mediated endocytosis orchestrates anterior neural tube closure

Kerstin Feistel *† [1]

[1] University of Hohenheim, Institute of Biology, Dept. Zoology – Germany

*Speaker
†Corresponding author: k.feistel@uni-hohenheim.de

microRNAs: novel transport route and action in axons development

Marie-Laure Baudet *† [1]

[1] University of Trento [Trento] – Italy

-

*Speaker
†Corresponding author: marielaure.baudet@unitn.it

Dr. Strangefoot (aka, Xenopus) or: How did I Learn to Stop Worrying and Love Metabolism

Enrique Amaya *† [1]

[1] University of Manchester [Manchester] – United Kingdom

*Speaker
†Corresponding author: enrique.amaya@manchester.ac.uk

A new regulator of the cerebellar granular neuron stem/progenitors niche size and behavior: A study in amphibian

Béatrice Durand *† [1]

[1] Sorbonne Université, CNRS UMR7622, IBPS Developmental Biology Laboratory, Campus Pierre et Marie Curie – Sorbonne Université UPMC Paris VI – France

*Speaker
†Corresponding author: beatrice.durand@sorbonne-universite.fr

Mucus, ciliation, stemness - Spdef does it all

Maximilian Haas *† [1,2,3], Magdalena Brislinger-Engelhardt [1,2,4,5], Peter Walentek‡ [5,6,7,8]

[1] Renal Division, Department of Medicine, University Freiburg Medical Center, Freiburg – Germany
[2] Spemann Graduate School of Biology and Medicine, Albert Ludwigs University Freiburg, Freiburg – Germany
[3] IMITATE - Institute for Disease Modeling and Targeted Medicine – Germany
[4] Center for Systems Biological Analysis, Albert Ludwigs University Freiburg, Freiburg – Germany
[5] CIBSS - Center for Integrative Biological Signalling Studies, Albert-Ludwigs-University Freiburg – Germany
[6] University Freiburg Medical Center, Department of Internal Medicine IV – Germany
[7] ZBSA - Center for Biological Systems Analysis, Freiburg – Germany
[8] SGBM - Spemann Graduate School of Biology and Medicine, Freiburg – Germany

Mucociliary epithelia line the conducting airways of terrestrial vertebrates as well as the epidermis of aquatic species and serve as a first line of defence against pathogens via mucociliary clearance. The embryonic *Xenopus* epidermis is a stratified mucociliary epithelium consisting of a deep layer, which harbours the basal stem cells (BCs), and an outer layer initially consisting of mucus-secretory outer/goblet cells, into which the BC-derived cell types intercalate after specification. A key molecular pathway regulating cell type specification in this tissue is Wnt signalling, for which we report a novel role in specification of outer cells. We conducted both expression and functional screens for Wnt pathway components, which identified Wnt11b as the main Wnt ligand in mucociliary epidermal development, regulating goblet cells as well as BCs and their derivatives. In particular, tightly regulated Wnt levels are crucial for goblet cell specification, as both increase and decrease lead to severe defects. Moreover, we find that Spdef, a Wnt-responsive Ets transcription factor known to be essential for specification of mucus-secretory cells, has an unexpected function in mucociliary epidermal development. While Spdef loss-of-function expectedly leads to defective Goblet cells, we also observe defects in multiciliated cells and BCs, indicating that Spdef positively regulates expression of the BC stemness regulator ΔN-*tp63*. Spdef gain-of-function, on the other hand, induces ΔN-*tp63* in both layers, suppressing specification of all cell types, including goblet cells. These surprising findings shed new light on the role of Spdef and on the molecular biology of chronic airway diseases such as goblet and basal cell hyperplasia, which might both be reducible to abnormal Spdef levels in different Wnt signalling environments.

Keywords: Wnt, Spdef, mucociliary, basal cell

*Speaker
†Corresponding author: maximilian.haas@medizin.uni-freiburg.de
‡Corresponding author: peter.walentek@medizin.uni-freiburg.de

The tadpole foregut as new model to study mucociliary epithelia in Xenopus

Mona Hansen * [1,2], Julia Mor Galvez [1,3], Peter Walentek [1,2,4,5]

[1] Renal Division, Department of Medicine, University Freiburg Medical Center, Freiburg – Germany
[2] IMITATE - Institute for Disease Modeling and Targeted Medicine, Freiburg – Germany
[3] Present address: Universitat de Barcelona, Barcelona – Spain
[4] Centre for Integrative Biological Signalling Studies [Freiburg] – Germany
[5] NephGen a Collaborative Research Center 1453 Nephrogenetics, Freiburg – Germany

The amphibian *Xenopus laevis* is widely used as a model organism for mucociliary epithelia. Multiple organs in mammals are lined with this type of epithelium, which serves as a first line defense against pathogens by mucociliary clearance. Mucociliary epithelia are composed of multiciliated cells (MCCs), Ionocytes (ISCs), and multiple secretory cell types. Next to the by fare most studied *Xenopus* embryonic epidermis, another accessible mucociliary epithelium exists in the tadpole foregut, which allows comparative studies as well as the investigation of developmental aspects not possible to study in the epidermis. For example, MCCs are known to be most abundant in the esophagus as well as in the posterior portion of the stomach, but they are not found to be evenly distributed and separated from each other by secretory cells as in the epidermis. The specification of secretory and multiciliated cells depends on Notch signaling in the *Xenopus* embryonic epidermis, as well as in the mammalian airway. While Notch signaling in the *Xenopus* epidermis is the main signaling pathway directing cell fate decisions in mucociliary epithelial maintenance, development and disease, additional data on the *Xenopus* foregut mucociliary epithelium are missing. Therefore, it remains elusive how Notch signaling contributes to the generation of different cell type compositions along the anterior-posterior axis of the gut in *Xenopus*. The foregut epithelium resembles noteworthy characteristics of mammalian airway epithelia due to the endodermal origin, the similar MCC spacing, the pseudostratified epithelium architecture and the variable cell type composition along the anterior-posterior axis. This work aims to find the precise time point of mucociliary development onset in the gut, specific manipulation approaches to target the foregut, and to elucidate how differential pattern formation is achieved along the anterior-posterior axis.

Keywords: foregut, multiciliated cells, Notch signaling

*Speaker

Tra2b – a new paradigm in coordinated post-transcriptional control of ciliogenesis

Charlotte Softley * [1,2], Magdalena Engelhardt [1,2,3], Darwin Dichmann [3], Richard Harland [3], Peter Walentek [1,2]

[1] University Hospital Freiburg – Germany
[2] University of Freiburg – Germany
[3] Department of Molecular and Cell Biology, University of California, Berkeley – United States

While transcriptional control of cilia formation and function is a topic of great interest in the scientific community, little is known about the post-transcriptional regulation of these processes. We study the activity of a key alternative splicing factor Transformer 2b (Tra2b), required for normal development in mice, flies and Xenopus. Elucidated targets include a wide variety of cilia-related transcripts, affecting ciliogenesis, basal body polarity and multiplication-actin organization. These targets show differential effects of splice and knockdown manipulations where alternative isoforms were created, confirming the differing roles of splice variants and the coordinated effects on cilia of the Tra2b targets. This represents a new paradigm in cilia regulation and function: that Tra2b regulates coordinated alternative splicing in a functionally coherent set of transcripts, all contributing to a common cilia-related function. Gaining insights on this novel mode of regulation in ciliated cells could lead to the identification of a variety of therapeutic targets for genetic cilia-related diseases, and elucidate how splicing is functionally coordinated to regulate cell type- and tissue-specific cell biology. It is therefore critical that we learn more about these mechanisms, how they are implemented in the cell and specifically, how defects are induced at the molecular level during basal body assembly, cilia formation and cell function.

Keywords: Alternative splicing, Tra2b, ciliopathies, ciliogenesis

*Speaker

dmrt2 and myf5 link early somitogenesis to left-right axis determination in Xenopus laevis

Melanie Tingler * [1], Kerstin Feistel [2], Axel Schweickert† [3], Amelie Brugger

[1] University of Hohenheim – Department of Zoology, Garbenstrasse 30 BIO II, 70599 Stuttgart, Germany
[2] Universität Hohenheim – Schloß Hohenheim 1, 70599 Stuttgart, Germany
[3] University of Hohenheim, Institute for Zoology – Germany

The vertebrate left-right axis is specified during neurulation by events occurring in a transient ciliated epithelium termed left-right organizer (LRO), which is made up of two distinct cell types. In the axial midline central LRO (cLRO) cells project motile monocilia and generate a leftward fluid flow, reflecting the mechanism of symmetry breakage. This directional fluid flow is perceived by lateral positioned sensory LRO (sLRO) cells, which harbor non-motile cilia. In sLRO cells on the left side, flow-induced signaling triggers post-transcriptional repression of the multi-pathway antagonist *dand5*. Subsequently, the co-expressed Tgf-β growth factor Nodal1 is released from Dand5 mediated repression to induce left-sided gene expression. Interestingly, *Xenopus* sLRO cells have somitic fate, suggesting a connection between LR determination and somitogenesis. Here we show that doublesex and mab3-related transcription factor 2 (Dmrt2), known to be involved in vertebrate somitogenesis, is required for LRO ciliogenesis and sLRO specification. In *dmrt2* morphants, misexpression of the myogenic transcription factors *tbx6* and *myf5* at early gastrula stages preceded the misspecification of sLRO cells at neurula stages. *myf5* morphant tadpoles also showed LR defects due to a failure of sLRO development. Gain of *myf5* function reintroduced sLRO cells in *dmrt2* morphants, demonstrating that paraxial patterning and somitogenesis are functionally linked to LR axis formation in *Xenopus*.

Keywords: laterality determination, left, right organizer, dmrt2, myf5, leftward fluid flow, cilia, paraxial patterning

*Speaker
†Corresponding author: axel.schweickert@uni-hohenheim.de

CP110, a surprising new regulator of cilium disassembly

Alexia Tasca * [1,2], Sarah Bowden [1,2,3,4], Mona Hansen [1,2], Magdalena Engelhardt [1,2,4,5], Maximilian Haas [1,2,5], Charlotte Softley [1,6], Peter Walentek† [1,4,5,6,7,8]

[1] Renal Division, Department of Medicine, University Freiburg Medical Center, Freiburg – Germany
[2] IMITATE - Institute for Disease Modeling and Targeted Medicine, Freiburg – Germany
[3] International Max Planck Research School for Organismal Biology – Germany
[4] CIBSS - Centre for Integrative Biological Signaling Studies, Albert Ludwigs University Freiburg – Germany
[5] Spemann Graduate School of Biology and Medicine, Albert Ludwigs University Freiburg – Germany
[6] NephGen a Collaborative Research Center 1453 Nephrogenetics, Freiburg – Germany
[7] IMITATE - Institute for Disease Modeling and Targeted Medicine, Freiburg, Germany – Germany
[8] IMPRS - International Max Planck Research School for Immunobiology, Epigenetics, and Metabolism, Max Planck Institute of Immunobiology and Epigenetics, Freiburg – Germany

Cilia are hair-like microtubule-based structures that project from cells. Ciliated cells can be monociliated or multiciliated and can form either motile or primary cilia. Primary cilia are transient structures and are lost *via* a process called cilia disassembly in serum-induced quiescent cells. Motile cilia can also be retracted via a similar mechanism in trans-differentiating multiciliated cells. During disassembly, the ciliary tip is excised via an actin dependent mechanism, and cilia length reduction is accompanied by the activation of cilia disassembly factors, *e.g.* polo-like kinase 1 (PLK1) to induce the de-acetylation and de-polymerization of axonemal microtubules.
The centrosomal protein CP110 was shown to interact with microtubule depolymerizers, actin promoting factors, and to localize to the ciliary tip of ciliated cells. We hypothesize that CP110 might control cilia length by regulating cilia tip excision and axonemal microtubule depolymerization, two events occurring during cilia disassembly.
Here, we are using two different models to study how CP110 contributes to cilia disassembly: (1) primary cilia disassembly in human RPE1 cells and (2) multiciliated cells of the *Xenopus* epidermis. To gain more mechanistic insights about CP110 function at the ciliary tip and its accumulation at the ciliary base, we are now developing opto-genetical tools to be able to manipulate protein levels of CP110 without affecting ciliogenesis. This will allow us to address how CP110 is regulating cilia disassembly.

Keywords: CP110, cilia disassembly, multiciliated cells, Xenopus

*Speaker
†Corresponding author: peter.walentek@medizin.uni-freiburg.de

Timing of swim and limb motor neuron generation in the Xenopus spinal cord

Clara Becker * [1], Florina Toma [1], Stavros Papadopoulos [1], Shadi Gharagozlou [1], Julia Bicher [1], Johanna Sonntag [1], Iurii Ignatev [1], Lora Sweeney† [1]

[1] Institute of Science and Technology [Austria] – Austria

The transition from aquatic to terrestrial habitats during evolution was accompanied by movement pattern diversification. In aquatic vertebrates, alternating segmental activation of axial muscles leads to undulatory swimming. In tetrapods, variations in limb muscle contraction generates a range of gaits and movements. Whether such limb movements evolved by modifying axial motor circuits or addition of new circuits is unclear.
Using *Xenopus laevis*, we will exploit the transition from axial to limb movement during metamorphosis to determine whether swim and limb motor circuits share a lineage and what drives changes in cell type number and composition. Preliminary data shows this motor pattern change in frog occurs concurrently with an expansion in motor neuron (MN) number and subtypes. This increase begins as larval tadpoles transition to free swimming. Around NF st40-42, MNs increase in number from 5 to 15 per 16 μm section, and a limb-innervating lateral motor column and thoracic hypaxial and preganglionic subtypes are added. Building on these findings, we aim to delineate the clonal relationship of motor circuits, identify the developmental programs that drive MN diversification, and determine their contribution to axial versus limb motor activity.
To assess the mode and continuity of MN subtype generation during this larval to free-swimming transition, we use pulse/chase experiments to tile embryonic (NF st15-17) and pre-metamorphic development (NF st35-43). By EdU pulse/chase labeling, we mark proliferating spinal cord progenitors and the MN types generated from them. Our preliminary analysis indicates MNs are generated continuously with low quantity throughout pre-metamorphic development, with stage-dependent variability in MN subtype generation. By extending the chase until metamorphic stages (stage 54/limb circuit) we determine the long-term fate of early-born MNs in larval swim circuits. First results indicate neurons born from the NF st35 pulse are present in comparable numbers at metamorphic stages.
Experimental goals include to 1) characterize timing of MN addition, 2) identify lineage relationships between swim and limb motor circuits, 3) evaluate how lineage impacts MN expansion and diversification during metamorphosis. In short, our study of frog metamorphosis will reveal the developmental and functional basis of two motor circuits in one organism.

Keywords: Spinal cord, motor neurons, progenitor cells, pulse, chase, EdU

*Speaker
†Corresponding author: lora.sweeney@ist.ac.at

Redox control of stemness: insights from neural stem cells of the Xenopus retina

Alicia Donval [1], Cinthia Hernandez Puente [1], Muriel Perron [1], Morgane Locker [*†] [2]

[1] Institut des Neurosciences Paris-Saclay – Université Paris-Saclay, CNRS – France
[2] Institut des Neurosciences Paris-Saclay – Université Paris-Saclay – France

Reactive oxygen species (ROS) are both harmful molecules sustaining the pathogenesis of several diseases and essential modulators of cell behaviours. In particular, a growing wealth of data suggest that ROS-dependent signalling pathways might be critical in conferring embryonic or adult stem cells their specific properties. However, how stem cells control ROS production and scavenging, and how ROS in turn contribute to stemness remain poorly understood. Using the *Xenopus* retina as a model system, we first investigated the redox status of retinal stem cells (RSCs). We unexpectedly discovered that they exhibit peculiar redox characteristics with higher ROS levels compared to progenitors and retinal neurons. In addition, we found that RSCs express a set of specific antioxidant genes under the transcriptional control of the Hippo pathway. We next addressed the question of ROS functional involvement in these cells. Our data show that pharmacological or genetic inhibition of NADPH oxidase (NOX)-dependent ROS production triggers RSC quiescence. We further identify Wnt signalling as a candidate to relay NOX activity in RSCs. Altogether, these results highlight that RSCs exhibit distinctive mechanisms to maintain their redox homeostasis, while exploiting NOX-signalling to maintain their proliferative state.

Keywords: Retinal stem cells, Reactive oxygen species, Proliferation, Hippo/YAP pathway, NADPH oxidase

[*]Speaker
[†]Corresponding author: morgane.locker@universite-paris-saclay.fr

Link between Müller cell proliferation and inflammation in Xenopus laevis upon retinal injury.

Karine Parain * [1], Léa Siron [1], Yann Audic [2], Christian Cozma [1], Alicia Donval [1], Muriel Perron† [1]

[1] Institut des Neurosciences Paris-Saclay – Centre National de la Recherche Scientifique (UMR9197), Université Paris-Saclay – France

[2] Institut de Génétique et Développement de Rennes – Universite de Rennes 1, Centre National de la Recherche Scientifique : UMR6290, Structure Fédérative de Recherche en Biologie et Santé de Rennes – France

While teleost or amphibian Müller glial cells efficiently sustain retinal regeneration, mammalian ones are unable to do so. *Xenopus* is a particularly valuable model to explore mechanisms that either sustain or constrain the regenerative potential of Müller glia. Indeed, we unexpectedly discovered, in a CRISPR/Cas9-mediated model of retinitis pigmentosa, that Müller cell dependent retinal regeneration is hampered in young *Xenopus* tadpoles, while being efficient in old premetamorphic ones. We found a remarkable correlation between these different Müller cell capacities and the status of microglia, the resident immune cells of the retina. In support of the importance of neuroinflammation, we discovered that Müller cells can exit quiescence, even at refractory stage, in a model of degeneration that triggers severe neuroinflammation. Finally, our preliminary functional data using pro- and anti-inflammatory drugs demonstrate the role of the inflammatory response on the regulation of Müller cell proliferation in *Xenopus laevis* upon retinal injury. Together, this work suggests that the differential responses of Müller glia cells to injury in young and old *Xenopus* tadpoles may originate, at least in part, from differences in the inflammatory reaction. This work should help to envision how to unlock the latent ability of human Müller glia to regenerate the retina and thereby fuel the area of regenerative medicine.

Keywords: retina, Müller cell, regeneration, inflammation

*Speaker

†Corresponding author: muriel.perron@universite-paris-saclay.fr

Hmmr modulates Wnt signaling to drive mesenchymal-to-epithelial transition in the developing forebrain

Fee Wielath * [1,2], Benjamin Wiest [2], Angela Nickel [2], Kerstin Feistel† [2]

[1] Universität Hohenheim Zoologie – Schloß Hohenheim 1, 70599 Stuttgart, Germany
[2] University of Hohenheim, Institute of Biology, Dept. Zoology – Germany

Mesenchymal-to-epithelial transition (MET) is a central morphogenetic process that is well orchestrated during development to form many of the epithelia in an embryo. However, MET also occurs in cancer metastasis, when secondary tumors are established from invading mesenchymal cancer cells that proliferate and epithelialize. We have previously shown that *hmmr*, a breast cancer susceptibility gene in humans, functions in several MET processes during *Xenopus* embryogenesis. *hmmr* is required for MET-driven epithelialization during epiboly, neurulation and in the forming pronephros, suggesting a general role in MET. In the developing nervous system, *hmmr* is essential to transform the multilayered neural plate into a single-layered epithelium. Hmmr is a microtubule (MT)-associated protein, and the intercalation of polarized neuroepithelial cells requires its MT-binding capacity. MET also depends on tight regulation of canonical Wnt signaling. Reporter assays were performed to assess the role of *hmmr* in both endogenous as well as in exogenously induced Wnt signaling. In either setting, *hmmr* was required for Wnt pathway activation, functioning at the level of β-catenin nuclear localization. Functional analysis of constructs in which specific domains were deleted showed that the MT-binding N-terminus of Hmmr negatively regulates Wnt signaling, while the C-terminus enhances nuclear localization of *ctnnb1.* Here we propose a model in which protein variants of Hmmr that lack MT-binding capacity preferentially localize to the nucleus and facilitate β-catenin-mediated Wnt signaling during the initiation of MET. As cells intercalate, Hmmr protein variants containing MT binding capacity are required to modulate the MT cytoskeleton, facilitating migratory movements. Our results suggest that Hmmr governs MET by controlling progression between canonical Wnt signaling and cell migration in a microtubule-dependent manner.

Keywords: Hmmr, MET, Wnt signaling, microtubule cytoskeleton, Xenopus laevis

*Speaker
†Corresponding author: k.feistel@uni-hohenheim.de

Xenopus tropicalis Immature Sertoli Cells show potential in promoting muscle regeneration in tadpoles

Qing Zhao * [1], Irem Mertova [1], Svetlana Zabkova¡ [1], Tereza Tlapakova [1], Vladimir Krylov† [1]

[1] Charles University, Faculty of Science, Vinicna 7, 128 44, Prague 2, Czech Republic – Czech Republic

Inherited muscle wasting diseases, accident muscle injuries in daily life or muscle loss with age, all can restrict human activity in varying degrees and affect life quality. Studying the mechanism behind muscle regeneration as well as how to promote muscle regeneration provide basis for clinical treatment of such illness. Sertoli cells, the essential somatic cells in testis, can not only "nourish" the developing germ cells, but also protect them against the host immune system. Its positive role in promoting spermatogonial proliferation, sperm motility, and in cell therapy transplantation for example diabetes, ischemic damage and Duchenne muscular dystrophy,etc. has been revealed in many studies. The fact that Xenopus tadpoles and mammals are similar in the cellular processes of muscle regeneration through PAX7+ satellite cell activation makes Xenopus as a favorable model in this field.

Our laboratory has successfully established a somatic cell line from testes of juvenile X. tropicalis frogs exhibiting morphological and gene expression characteristics of Sertoli cells (referred as XtiSCs).The microinjection to upper tail region of 2 weeks old X. tropicalis tadpoles showed that XtiSCs could survive in the injection site at least for 7 days and had the ability to migrate in tail region. After tail amputation, XtiSCs showed tendency to migrate and stay at injury site. PAX7+ satellite cells also increased significantly at 3 days post amputation (dpa) which hinted increased muscle repair ability.

The muscle regeneration is tightly coupled with the presence and activity of macrophages. Clodronate liposome was used for macrophage depletion and the efficiency was validated by isolectin B4 staining and by largely reduced tail regeneration outcomes. We found that XtiSCs injection increased macrophage cell signals at 7dpa, in both control liposomes and clodronate liposomes groups. Moreover, macrophage depletion made it difficult for XtiSCs to migrate or stay at injury site after tail amputation, as observed at 3dpa. Macrophage depletion reduced PAX7+ satellite cells at 1dpa. XtiSCs injection into depleted tadpoles showed significant increase of PAX7+ satellite cells at 7dpa compared with non-injected control groups (time points:1dpa, 3dpa, 7dpa).

In summary, XtiSCs can interact and stimulate the macrophages proliferation after tail amputation and thus benefits the subsequent muscle regeneration process through the Pax7+ satellite cells activation.

*Speaker

†Corresponding author: vladimir.krylov@natur.cuni.cz

Keywords: Xenopus tropicalis Immature Sertoli Cells (XtiSCs), Muscle Regeneration, Pax7+ Satellite Cells, Macrophage

TBC1D32/Bromi mutations are associated with retinitis pigmentosa: a study combining clinical ophthalmology, iPS-based disease modeling and Xenopus functional approaches

Caroline Borday * [1], Béatrice Bocquet [2,3], Daria Mamaeva [2], Nejla Erkilic [2], Alicia Donval [1], Christel Masson-Garcia [1], Karine Parain [1], Isabelle Meunier [2,3], Vasiliki Kalatzis† [2], Muriel Perron‡ [1]

[1] Paris-Saclay Institute of Neuroscience, CERTO-Retina France – CNRS, Université Paris-Saclay – France

[2] Institute for Neurosciences of Montpellier – Inserm, Montpellier University – France

[3] National Reference Centre for Inherited Sensory Diseases – Montpellier University, CHU – France

Inherited retinal diseases (IRDs) are a group of clinically and genetically heterogeneous disorders characterized by a dysfunction or degeneration of photoreceptors, and/or their underlying support tissue, the retinal pigment epithelium (RPE). The most common IRD is retinitis pigmentosa (RP). RP is initially characterized by night blindness appearing in childhood and followed by a progressive loss of peripheral vision. A teenager was diagnosed with RP, in absence of previous family history. It was determined that this patient does not present any mutation in the 80 causative RP genes, but has compound heterozygous mutations in *TBC1*D32, never associated with RP so far. Loss of function mutations in *TBC1D32* are lethal in human and lead to severe ciliopathy phenotypes and microphthalmia. In order to know whether an hypomorphic mutation in *TBC1*D32 could cause RP, we used Xenopus as a model, well suited to study gene knockdowns. We found that *Bromi*, the *Xenopus* homolog of *TBC1D32,* is expressed in developing photoreceptors and RPE cells, and that its reduced expression leads to impaired RPE cell differentiation during development and to defects in RPE ciliogenesis. These results were supported in RPE cells derived from the patient induced pluripotent stem (iPS) cells. Altogether, our results not only highlight a critical role for TBC1D32/Bromi in RPE ontology, they also suggest that *TBC1D32* hypomorphic mutations should be considered as a novel cause of retinal dystrophies.

Keywords: retinitis pigmentosa, retinal pigmented epithelium development, ciliogenesis, Induced pluripotent stem cells

*Speaker

†Corresponding author: vasiliki.kalatzis@inserm.fr

‡Corresponding author: muriel.perron@universite-paris-saclay.fr

Using CRISPR/Cas9 in Xenopus tropicalis to investigate cis-regulatory element variants in developmental eye anomalies : identification of an Otx2-binding site in a novel putative enhancer of the mab21l2 gene

Munevver Burcu Cicekdal * [1,2], Fabiola Ceroni [3,4], Richard Holt [3], Elfride De Baere [2], Nicola K Ragge [3,5], Kris Vleminckx† [1]

[1] Department of Biomedical Molecular Biology, Ghent University – Belgium
[2] Center for Medical Genetics and Department of Biomolecular Medicine, Ghent University and, Ghent University Hospital – Belgium
[3] Faculty of Health and Life Science, Oxford Brookes University – United Kingdom
[4] Department of Pharmacy and Biotechnology, UniversitÃ di Bologna – Italy
[5] West Midlands Regional Clinical Genetics Service and Birmingham Health Partners, Birmingham Women's and Children's Foundation Trust – United Kingdom

Genetic variants that disrupts the function of cis-regulatory elements (CREs), including enhancers and promoters, have been shown to conduce to a variety of human diseases. Here, we aimed to characterize a putative enhancer of *MAB21L2* in a 113.5 kb non-coding homozygous deletion identified in a proband with the phenotype of anophthalmia, micrognathia and microcephaly. The region was evaluated for the presence of putative enhancers using a genome-wide multi-omics database comprising ChIP-seq datasets of histone modification and transcription factors (TF) generated in human eye samples. Using TRANSFAC software, TF binding site (TFBS) prediction analysis for four conserved putative enhancers (M1-M4) was performed. These regions were further assessed in a Xenbase merged dataset, using ChromHMM to predict the state of CREs in Nieuwkoop and Faber (NF) stages (st) 10.5, 12.5 and 16 of *X.tropicalis*. One putative enhancer (M1) within the 39 kb region in *X. tropicalis* that corresponds to the 113.5 kb proband deletion, contained a conserved binding site for OTX2 , a TF that is critically involved in eye development. Furthermore, binding of Otx2 to the M1 enhancer was confirmed by ChIP-seq in mouse embryonic stem cells. Our further analysis in Xenbase indicated that M1 shows the epigenetic marks of a poised enhancer at NF st10.5, and an active enhancer at st12.5 and 16, in line with the initiation of *mab21l2* expression in *Xenopus*. CRISPR mediated interruption of the Otx2 TFBS (*CRE crispants*), as well as reproduction of the proband deletion (*del crispants*) resulted in eye defects, including misshapen eyes, lens defects and ocular coloboma, affecting approximately 50% of the injected embryos, and a significantly decreased eye size at st41. Lastly, at st20, which corresponds to an essential peak moment of *mab21l2* expression when the optic vesicles begin to evaginate, diminished levels of *mab21l2* mRNA expression in *CRE crispants* and *del crispants* were observed. In conclusion, our results showed for

*Speaker
†Corresponding author: Kris.vleminckx@irc.ugent.be

the first time a *X. tropicalis* disease model targeting a TFBS (for Otx2) in a putative enhancer of *mab21l2* using CRISPR/Cas9, promoting the use of *Xenopus tropicalis* as a promising animal model for in-depth investigation of non-coding gene regulatory networks and identification of CREs associated with both developmental and disease related phenotypes.

Keywords: cis regulatory elements, enhancers, noncoding, xenopus, CRISPR/Cas9, eye anomalies, xenopus

Xenopus: An in vivo model for studying skin response to UVB irradiation

Joudi El Mir * [1], Nadine Thézé [1], Sandrine Fedou [1], Hussein Fayyad-Kazan [2], Hamid-Reza Rezvani† [1], Pierre Thiébaud‡ [1]

[1] University of Bordeaux – Inserm U1312, Bordeaux, France – France
[2] University of Lebanon – Lebanon

Being the biggest and uppermost part of the body, skin acts as a protective shield against environmental factors such as light, heat, infection and chemicals. Among these factors, ultraviolet B rays (UVB) in sunlight cause skin damage ranging from wrinkles and irregular pigmentation to photoaging and skin cancer. Indeed, once in contact with skin cells, UVB can affect genomic DNA by creating cyclobutane pyrimidine dimers (CPDs) and pyrimidine-pyrimidine (6-4) photoproducts (6-4PPs) that, when accumulated, lead to serious physiopathological modifications and tumorigenesis. Nucleotide excision repair (NER), which is one of the powerful repair pathways and involves a large number of proteins such as XPC, XPA, XPB, XPD, XPE, XPF, XPG, is responsible of removing these lesions from genome. Besides, CPD and 6-4PP photolyases, which are expressed in a wide range of vertebrates and activated by blue light, provide another repair system of UV-induced damage. The main goal of this project is to validate the use of "*Xenopus laevis*" as an *in vivo* model system for investigating the impact of UVB on skin, owing to its unique advantage related to pigmentary analysis. To this end, the expression of *XPC* and CPD/6-4PP photolyases genes was examined using RT-PCR analysis. Results showed that the three genes are expressed at all stages of embryonic development and in all adult tissues tested. *Xenopus* embryos were then exposed to UVB irradiation. The removal kinetics of UVB-induced CPDs and the effects of UVB irradiation on cell proliferation, apoptosis, epidermal thickness and melanocytes behavior were studied at different time points after irradiation. We observed a gradual decrease in CPD levels, an increased number of apoptotic cells together with an epidermal thickening and an increased dendriticity of melanocytes. To confirm whether these modifications were dependent on UVB-induced DNA damage, embryos were exposed to blue light immediately after UVB irradiation. Quick removal of CPDs in blue-light exposed embryos confirmed the efficient activation of photolyases. A decrease in the number of apoptotic cells and an accelerated return to normal proliferation rate was noted in blue-light exposed embryos compared to their control counterparts. Overall, gradual decrease in CPD levels, detection of apoptotic cells, thickening of epidermis and increased dendriticity of melanocytes, which recapitulate the classical human skin responses to UVB, support *Xenopus* as an appropriate model for such studies.

*Speaker
†Corresponding author: hamid-reza.rezvani@u-bordeaux.fr
‡Corresponding author: pierre.thiebaud@u-bordeaux.fr

Keywords: Xenopus, Ultraviolet B rays (UVB), skin, DNA damage, XPC, Photolyases

A Progeroid Syndrome Caused by RAF1 deficiency Underscores the importance of RTK signaling for Human Development

Nathalie Escande-Beillard *† [1,2], Samantha Wong [2], Yu Xuan Tan [2], Kiat Yi Tan [2], Abigail Loh [2], Zainab Aziz [3], Engin Ã–zkan [3], Hülya Kayserili [1], Bruno Reversade [1,2]

[1] Medical Genetics Department, Koç University School of Medecine, Istanbul – Turkey
[2] Institute of Molecular and Cellular Biology, A*STAR, Singapore – Singapore
[3] Department of Biochemistry and Molecular Biology, University of Chicago – United States

Somatic and germline gain-of-function point mutations in RAF, the first oncogene to be discovered in humans, delineate a group of tumor-prone syndromes known as RASopathies. In this study, we document the first human phenotype resulting from the germline loss of function of the proto-oncogene RAF1 (a.k.a. CRAF). In a consanguineous family, we uncovered a homozygous p.Thr543Met mutation segregating with a neonatal lethal progeroid syndrome with cutaneous, craniofacial, cardiac and limb anomalies. Structure-based prediction and functional tests using human knock-in cells showed that threonine 543 is essential to: 1) ensure RAF1's stability and phosphorylation, 2) maintain its kinase activity towards substrates of the MAPK pathway and 3) protect from stress-induced apoptosis. When injected in *Xenopus* embryos, unlike RAF1 WT, mutant RAF1 T543M failed to phenocopy the effects of overactive FGF/MAPK signaling confirming its hypomorphic activity. Collectively, our data disclose the genetic and molecular etiology of a novel segmental progeroid syndrome which highlights the importance of RTK signaling for human development and homeostasis.

Keywords: RAF1, progeroid syndrome, MAPK pathway, RASopathy, birth defect, Mendelian genetics, Xenopus, proto oncogene, AcroCardioFacial Syndrome, RTK signaling

*Speaker
†Corresponding author: nbeillard@ku.edu.tr

Ttc30a affects tubulin modifications in a Xenopus model for ciliary chondrodysplasia with polycystic kidney disease

Maike Getwan * [1,2,3], Anselm Hoppmann [4], Pascal Schlosser [4], Kelli Grand [2,3,1], Weiting Song [3], Rebecca Diehl [3], Sophie Schroda [3], Florian Heeg [3], Konstantin Deutsch [5], Friedhelm Hildebrandt [5], Ekkehart Lausch [6], Anna Köttgen [4], Soeren Lienkamp† [1,2,3]

[1] Institute of Anatomy, University of Zurich, 8057 Zurich – Switzerland
[2] Swiss National Centre of Competence in Research Kidney Control of Homeostasis, University of Zurich, 8057 Zurich – Switzerland
[3] Renal Division, Department of Medicine, Faculty of Medicine, Medical Center - University of Freiburg, 79110 Freiburg – Germany
[4] Institute of Genetic Epidemiology, Faculty of Medicine, Medical Center - University of Freiburg, 79110 Freiburg – Germany
[5] Department of Pediatrics, Boston Children's Hospital, Harvard Medical School, Boston, MA 02115 – United States
[6] Department of Pediatrics, Faculty of Medicine, Medical Center - University of Freiburg, 79110 Freiburg – Germany

Skeletal ciliopathies (e.g. Jeune syndrome, short rib polydactyly, Sensenbrenner syndrome) frequently co-occur with nephronophthisis-like cystic kidney disease and other organ manifestations. Despite progress in genetic mapping of causative loci, no common molecular mechanism of cartilage defects and cystic kidneys was described so far.
Using CRISPR/Cas9 targeting of two ciliary chondrodysplasia genes (*ift80* and *ift172*), we established novel models for skeletal ciliopathies in *Xenopus tropicalis*. Metamorphic froglets had severely shortened limbs because of suppressed cartilage development, polydactyly, and cystic kidneys, closely matching the phenotype of skeletal ciliopathy patients. Due to a ciliogenesis defect in mulitciliated cells the fluid excretion in tadpoles was prevented and led to large edema. A data-mining based *in silico* screen uncovered another component of the IFT complex, *ttc30a,* to be related to known skeletal ciliopathy genes. Additionally *Ttc30a/b* transcripts are enriched in chondrocytes and osteocytes of single cell RNA-sequenced embryonic mouse limbs. CRISPR/Cas9 mutagenesis of *ttc30a* in *X. tropicalis* replicated the phenotype of *ift80* and *ift172* mutated froglets perfectly – limbs were shortened, toes multiplied, kidneys cystic and pronephric excretion inhibited. Further we detected altered posttranslational tubulin acetylation, glycylation and defective axoneme compartmentalization in *ttc30a* and *ift80* targeted multiciliated cells.
In conclusion, we identify TTC30A/B as an essential node in the network of ciliary chondrodysplasia and nephronophthisis-like disease proteins. We suggest that tubulin modifications and cilia segmentation contribute to skeletal and renal ciliopathy manifestations of ciliopathies in a

*Speaker
†Corresponding author: soeren.lienkamp@uzh.ch

cell type specific manner.

Keywords: chondrodysplasia, cilia, cystic kidney disease, tubulin modifications

The ribosomal proteins Bop1 and Rpl5 affect Xenopus laevis development

Corinna Gärtner * [1], Bianka Kernl [1], Annika Messmer [1], Petra Dietmann [1], Michael Kühl [1], Susanne J. Kühl† [1]

[1] Institute of Biochemistry and Molecular Biology, Ulm University – Germany

Ribosomes perform protein synthesis and hence are crucial for every cell in every organism. Ribosome biogenesis is a complex process involving hundreds of different factors. The two proteins block of proliferation 1 (Bop1) and ribosomal protein L5 (Rpl5) are involved in this process. Bop1 ensures the maturation of 5.8S rRNA and 28S rRNA. Rpl5 together with ribosomal protein L11 and 5S rRNA forms a ribonucleoprotein complex which is incorporated into the large ribosomal subunit.

Defects in ribosomal biogenesis lead to diseases called ribosomopathies. Patients show symptoms such as malformed cranial cartilage, bone marrow failure, and an increased incidence of tumor development. Mutations in *RPL5* are associated with the ribosomopathy Diamond Blackfan anemia. Bop1 is not associated with ribosomopathies yet.

Here, we show that the knockdown of Rpl5 and Bop1 via morpholino oligonucleotides resulted in severe phenotypes of anterior tissue in *Xenopus laevis* embryos including smaller eyes and brains and reduced cranial cartilages. Furthermore, expression of specific eye-, brain-, and neural crest cell marker genes were affected in Bop1 and Rpl5 morphants. A synergistic effect between Bop1 and the master regulator Pax6 indicates a common pathway between the two proteins.

In contrast to Bop1, Rpl5 depletion affected proliferative pathways. Furthermore, Rpl5 MO injection led to an increase in apoptotic cells. *Tp53* expression was increased and a more than additive effect was found between *tp53* and Rpl5 MO injection. Co-injection of the apoptosis blocker *bcl2* partially rescued the Rpl5 MO-induced phenotype – indicating that increased apoptosis contributes to this phenotype (Schreiner et al. 2022).

Keywords: Rpl5, Bop1, Ribosomal Biogenesis, embryonic development

*Speaker

†Corresponding author: susanne.kuehl@uni-ulm.de

Xenbase: latest support for genomics and disease models.

Christina James-Zorn * [1], Virgilio Ponferrada , Malcolm Fisher , Nivitha Sundararaj , Praneet Chaturvedi , Andrew Bell , Nadia Bayyari , Stan Chu , Kamran Karimi , Troy Pells , Vaneet Lotay , Peter Vize , Aaron Zorn†

[1] Xenbase, Dept. Developmental Biology, CCHMC – United States

Xenbase (www.xenbase.org) supports biomedical, developmental and cell biology using *Xenopus*, the African and Western clawed frogs. Xenbase is the central repository for *Xenopus* genetics and genomics data and provides researchers with bioinformatic resources and tools for complex analysis. Our mission is to 1) provide the latest genomes linked to genes and orthologs; 2) curate published research/literature for disease models, experimental phenotypes, and gene expression; 3) annotate *Xenopus* genes with GO terms (molecular functions, biological processes and cellular components); and 4) collate diverse genomics data from high throughput sequencing in a central, searchable, database. *Xenopus* genomes and *Xenopus* genes-to-human genes ortholog mappings (used in GO enrichment analysis) are available for download from out FTP site. The backbone of *Xenopus* gene expression curation is the Xenopus Anatomy Ontology (XAO) and phenotype curation uses the Xenopus Phenotype Ontology (XPO), linking Xenopus disease models to the Disease Ontology (DO) when appropriate. In addition, Xenbase has recently expanded our education resources including an anatomy atlas, normal tables of development, staging landmarks, marker genes, and a set of open access illustrations of embryonic development. Aggregating all of this information in an easy to use and free to access web portal, Xenbase effectively connects *Xenopus* genes and phenotypes to human genes and diseases via multiple data resources including Monarch and the Alliance of Genome Resources (AGR). Here we provide an overview of Xenbase resources, tools and curated *Xenopus* data, and data interconnectivity. Xenbase is funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD/NIH).

Keywords: database bioinformactics genome gene expression phenotype disease models

*Speaker

†Corresponding author: aaron.zorn@cchmc.org

Novel penetrant and short latency models for liposarcoma and wilms tumor using CRISPR multiplexing in Xenopus tropicalis

Dieter Tulkens *† [1,2], Marthe Boelens [1,2], Suzan Demuynck [1], Kelly Lemeire [1,3], David Creytens [2,4], Pieter Van Vlierberghe [2,5,6], Kris Vleminckx [1,2]

[1] Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium – Belgium
[2] Cancer Research Institute Ghent (CRIG), Ghent, Belgium – Belgium
[3] Inflammation Research Center, VIB, Technologiepark 71, Ghent, Belgium – Belgium
[4] Department of Pathology, Ghent University, Ghent, Belgium – Belgium
[5] Department of Biomolecular Medicine, Ghent University, Ghent, Belgium – Belgium
[6] Center for Medical Genetics, Ghent University, Ghent, Belgium – Belgium

Liposarcoma is one of major types of soft tissue sarcomas in adults. Of these, well-differentiated liposarcomas (WDLPS) represent more than 40%. Dedifferentiated liposarcomas (DDLPS) typically contain areas characteristic of WDLPS combined with areas of cellular non-lipomatous spindle-cell sarcoma. In contrast to WDLPS, DDLPS are very aggressive and often metastatic. Especially in the latter case patients have a poor clinical outcome. Both WDLPS and DDLPS are characterized by the presence of giant marker chromosomes originating from the q13-15 region of chromosome 12. Among the _˜150 genes located and amplified in this region are the *MDM2* and the *CDK4* genes, which negatively regulate the p53 and the RB proteins, respectively. Molecular targeted therapies have so far focused on compounds interfering with the MDM2/p53 interaction and/or CDK4 inhibitors. However, none of these have resulted in successful clinical therapies, urging the need for additional molecular targets. Interestingly, we found that by molecularly mimicking the clinically observed amplification of *MDM2* and *CDK4* in *Xenopus tropicalis* by CRISPR-based multiplexed inactivation of multiple tumor suppressor genes (*TP53*, *RB1*, *RBL1* and *RBL2*), together with the disruption of the *EP300* histone acetyltransferase, intra-abdominal liposarcomas were induced with high penetrance ($>$ 40%) and short latency ($<$ 50 days). In addition, these mosaic mutant animals also developed Wilms tumors. Furthermore, both the liposarcoma and Wilms tumors successfully engrafted upon intraperitoneal injection in immunodeficient *rag2* mutants. We will use these novel penetrant tumor models for identification of novel co-driver genes and for dependency mapping in order to find novel targets for molecular therapy.

Keywords: Cancer modeling, Driver screen, CRISPR/Cas9 multiplexing, Xenopus tropicalis

*Speaker
†Corresponding author: dieter.tulkens@irc.ugent.be

Phenotyping embryonic development and disease using deep learning and mesoSPIM light-sheet microscopy

Thomas Naert * [1], Ozgun Ciçek [2], Paulina Ogar [1], Max Bürgi [1], Nikko-Ideen Shaidani [3], Michael Kaminski [4,5], Yuxiao Xu [6], Kelli Grand [1], Marko Vujanovic [1], Daniel Prata [1], Friedhelm Hildebrandt [7], Thomas Brox [2], Olaf Ronneberger [2,8,9], Fabian Voigt [10], Fritjof Helmchen [10], Johannes Loffing [1], Marko Horb [3], Helen Rankin Willsey [6], Soeren Lienkamp† [1]

[1] Institute of Anatomy, University of Zurich , Zurich 8057, Switzerland ; Swiss National Centre of Competence in Research (NCCR) Kidney Control of Homeostasis (Kidney.CH) , Zurich 8057 – Switzerland

[2] Department of Computer Science , Albert-Ludwigs-University , Freiburg 79100 – Germany

[3] National Xenopus Resource and Eugene Bell Center for Regenerative Biology and Tissue Engineering , Marine Biological Laboratory, Woods Hole, MA 02543 – United States

[4] Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine in the Helmholtz Association , Berlin 10115 – Germany

[5] Department of Nephrology and Medical Intensive Care , Charité Universitätsmedizin Berlin , Berlin 10117 – Germany

[6] Department of Psychiatry and Behavioral Sciences , UCSF Weill Institute for Neurosciences, University of California , San Francisco, CA 94158 – United States

[7] Department of Pediatrics, Boston Children's Hospital, Harvard Medical School, Boston, MA 02115 – United States

[8] BIOSS Centre for Biological Signalling Studies, Albert-Ludwigs-University , Freiburg – Germany

[9] DeepMind , London WC2H 8AG – United Kingdom

[10] Laboratory of Neural Circuit Dynamics , Brain Research Institute, University of Zurich , Zurich 8057, Switzerland ; Neuroscience Center Zurich , Zurich 8057 – Switzerland

Autosomal dominant polycystic kidney disease (ADPKD) is caused by mutations in *PKD1* or *PKD2* and has an unmet need for new drugs and therapeutic targets. Ideally, these are identified in clinically relevant vertebrate disease models amenable to screening efforts.
We performed CRISPR/Cas9 genome engineering in *pkd1* or *pkd2*, which elicited cystic malformations in developing *Xenopus* renal tubules two-days post-fertilization ($p< 0.001$). We observed cystogenesis across different developmental stages and performed in-depth three-dimensional phenotypic analysis by employing tissue clearing and mesoSPIM light-sheet microscopy.
Notwithstanding the power of such novel disease models, the higher-throughput detailed and unbiased phenotypic assessment of altered embryonic development remains a challenge. This is especially relevant for analysis of light-sheet microscopy datasets, which easily encompass several gigabytes per embryo.
We explored how deep learning (U-Net) could automate segmentation tasks in various imaging

*Speaker

†Corresponding author: soeren.lienkamp@uzh.ch

modalities, and we automatically quantified phenotypes of altered renal, neural and craniofacial development in *Xenopus* embryos.
First, we demonstrated the utility of deep learning for automated phenotyping of embryos with polycystic kidneys (*pkd1* and *pkd2*). Second, we extended our approaches to craniofacial dysmorphia (*six1*) and neurodevelopmental disorders (*dyrk1a*). We highlighted how *in toto* light-sheet microscopy facilitates accurate reconstruction of kidney, brain or craniofacial structures within *Xenopus* embryos upon chemical or genetic disruption. Finally, we demonstrated compatibility of HCR whole-mount RNA-FISH protocols with our approaches, allowing to pin-point RNA expression domains in three-dimensions within a whole-mount embryo.
Taken together, deep learning increases the sensitivity and throughput of evaluating developmental malformations. We demonstrated the versatility, precision and scalability of deep neural network phenotyping on embryonic disease models and provide a resource of pre-trained deep learning models and instructional "how-to" videos (https://lienkamplab.org/deep-learning-models/). It is worth noting that training and deployment of U-Net deep learning models was done using graphical user-interfaces within the Fiji ecosystem, and does not require the user to be proficient at command-line coding.
In conclusion, by combining (light-sheet) microscopy and deep learning, we provide a framework for higher-throughput characterization of *Xenopus* embryonic development and disease.

Keywords: deep learning, light, sheet microscopy, disease models, kidney, CRISPR/Cas9

Genome-wide transcriptomics analysis of genes regulated by GATA4, 5 and 6 during cardiomyogenesis

Boni Afouda *† [1,2,3,4]

[1] Boni A. Afouda – United Kingdom
[2] Adam T Lynch – United Kingdom
[3] Eduardo de Paiva Alves – United Kingdom
[4] Stefan Hoppler – United Kingdom

The transcription factors GATA4, GATA5 and GATA6 are key regulators of vertebrate heart muscle differentiation (cardiomyogenesis), but specific target genes regulated by these individual cardiogenic GATA factors remain unknown. We have identified genes that are specifically regulated by each of them, as well as those regulated by either of them using genome-wide transcriptomics analysis in *Xenopus laevis*. The genes regulated by *gata4* are particularly interesting because GATA4 is able to induce differentiation of beating cardiomyocytes in Xenopus and in mammalian systems. Among the specifically *gata4*-regulated transcripts we identified two SoxF family members, *sox7* and *sox18*. Experimental reinstatement of gata4 restores *sox7* and *sox18* expression, and loss of cardiomyocyte differentiation due to gata4 knockdown is partially restored by reinstating *sox7* or *sox18* expression, while (as previously reported) knockdown of *sox7* or *sox18* interferers with heart muscle formation. In order to test for conservation in mammalian cardiomyogenesis, we confirmed in mouse embryonic stem cells (ESCs) undergoing cardiomyogenesis that knockdown of *Gata4* leads to reduced *Sox7* (and *Sox 18*) expression and that Gata4 is also uniquely capable of promptly inducing *Sox7* expression. Our genome-wide transcriptomics analysis therefore identifies an important and conserved gene regulatory axis from *gata4* to the SoxF paralogs *sox7* and *sox18* and further to heart muscle cell differentiation. Our identification of genes that are differentially regulated by each of cardiogenic gata factors also provides a platform for future investigations on the gene regulatory network underpinning embryonic cardiomyogenesis.

Keywords: GATA factors, SoxF family members, sox, cardiomyogenesis, heart

*Speaker
†Corresponding author: boni.afouda@abdn.ac.uk

Otic Neurogenesis in Xenopus: Proliferation, Differentiation, and the Role of Eya1

Suad Almasoudi [1], Gerhard Schlosser [*†] [1]

[1] National University of Ireland Galway – Ireland

Using immunostaining and confocal microscopy, we provide the first detailed description of otic neurogenesis in *Xenopus laevis*. We show that the otic vesicle comprises a pseudostratified epithelium with apicobasal polarity (apical enrichment of Par3, aPKC, Myosin light chain, N-cadherin) and interkinetic nuclear migration (apical localization of mitotic, pH3-positive cells). A Sox3-immunopositive neurosensory area in the ventromedial otic vesicle gives rise to neuroblasts, which delaminate through breaches in the basal lamina between stages 27 and 39. Delaminated cells congregate to form the vestibulocochlear ganglion, whose peripheral cells continue to proliferate (incorporate EdU), while central cells differentiate into Islet1/2-immunopositive neurons (stage 29) and send out neurites (stage 31). The central part of the neurosensory area retains Sox3 but stops proliferating from stage 33, forming the first sensory areas (utricular/saccular maculae). Eya1 protein localizes to both nuclei and cytoplasm in the otic epithelium, with levels of nuclear Eya1 declining in differentiating (Islet1/2+) ganglion neurons and in the developing sensory areas. Morpholino-based knockdown of Eya1 leads to reduction of proliferating, Sox3- and Islet1/2-immunopositive cells, redistribution of cell polarity proteins and loss of N-cadherin suggesting that Eya1 is required for maintenance of epithelial cells with apicobasal polarity, progenitor proliferation and neuronal differentiation during otic neurogenesis.

Keywords: ear, placodes, neurogenesis, sensory organs, development

[*]Speaker

[†]Corresponding author: gerhard.schlosser@nuigalway.ie

A testis ex vivo model to study and interfere with Xenopus laevis sperm epigenetic programming

Florian Berger * [1], Gaël Le Troadec [1], Cynthia Fourgeux [1], Jeremie Poschmann [1], Jérome Jullien† [1]

[1] CR2TI – Nantes Université, INSERM, Center for Research in Transplantation and Translational Immunology, UMR 1064, F-44000, Nantes, France – France

Sperm deliver to the embryos epigenetic information contributing to embryonic development (Teperek et al. 2016; Siklenka et al. 2015). However, the sperm epigenetic cues involved in this process are poorly defined. To better understand how sperm is epigenetically programmed for development we are combining scRNA-seq and RNA FISH approach to: (i) accurately characterize spermatogenesis intermediates and accessory cell types in *Xenopus laevis* testis and (ii) Identify chromatin pathways associated with male germ cells transition towards mature sperm. We use this *in vivo* spermatogenesis cell atlas as a benchmark to evaluate spermatogenesis in ex-vivo cellular explant (Risley et al. 1987). We aim to evaluate how spermatogenesis progress in such explant by tracking appearance of spermatogenesis marker from labelled progenitors. Once validated we plan to use this ex-vivo assay to interfere with sperm epigenetic programming and evaluate consequences on embryos development.

Keywords: spermatogenesis scRNA, seq FISH explant epigenetic

*Speaker

†Corresponding author: jerome.jullien@inserm.fr

Epigenetic contributions to Xenopus mucociliary development

Sarah Bowden * [1,2], Magdalena Engelhardt [1,3,4], Maximilian Haas [1,3], Peter Walentek† [4,1]

[1] Renal Division, Department of Medicine, University Freiburg Medical Centre, Freiburg – Germany
[2] International Max Planck Research School for Immunobiology, Epigenetics, and Metabolism, Max Planck Institute of Immunobiology and Epigenetics, Freiburg – Germany
[3] Spemann Graduate School of Biology and Medicine, Albert Ludwigs University Freiburg, Freiburg – Germany
[4] CIBSS - Centre for Integrative Biological Signalling Studies, Albert Ludwigs University Freiburg, Freiburg – Germany

It is a well-established fact that the transcriptional needs and activity of a cell change during development, in order to facilitate cell-type specification and differentiation. These changes are facilitated by regulatory elements such as promoters, enhancers, silencers, and insulators, which facilitate combinatorial transcription factor binding. Therefore, these regulatory elements are associated with open - or accessible - regions of chromatin.
It is also well established that the mucociliary epithelium of *Xenopus laevis* responds to Wnt and Notch signaling during development, and key transcription factors operating in these signaling pathways are being identified in specific cell types. However, there remains a large gap in understanding how these signaling pathways translate into cell specific specification or differentiation decisions during development.
To elucidate this relationship, we performed ATAC-seq on a stage series of animal cap organoids, which we are using to reveal which loci undergo accessibility changes, the dynamics of these changes over time, and subsequently, how these changes regulate mucociliary development.
A prototypic example of bridging the signaling-chromatin gap is demonstrated by the transcriptional co-repressor Groucho. Groucho has the capacity to silence gene expression through direct repression, or via long term chromatin silencing. Groucho is known to mediate repression in both Wnt and Notch signaling target genes, and inhibition of Groucho has negative influences on ciliation, mucus secretion and the mucociliary patterning program.
Combined, these data will expand our understanding of the regulatory mechanisms of tissue development and morphogenesis.

Keywords: Chromatin, Groucho, signaling, development

*Speaker
†Corresponding author: peter.walentek@medizin.uni-freiburg.de

A non-transcriptional function of Yap regulates the DNA replication program

Odile Bronchain *† [1], Rodrigo Meléndez GarcÃa [1], Olivier Haccard [2], Albert Chesneau [1], Hemalatha Narassimprakash [2], Jérome E Roger [1], Muriel Perron‡ [1], Kathrin Marheineke§ [2]

[1] Institut des Neurosciences Paris-Saclay – Centre National de la Recherche Scientifique : UMR9197, Université Paris-Saclay – France

[2] Institute for Integrative Biology of the Cell – Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Sud, Université Paris-Saclay, Gif-sur-Yvette, France. – France

In multicellular eukaryotic organisms, the initiation of DNA replication occurs asynchronously throughout S-phase according to a regulated replication timing program. Here, using *Xenopus* egg extracts, we showed that Yap (Yes-associated protein 1), a downstream effector of the Hippo signaling pathway, is required for the control of DNA replication dynamics. We found that Yap is recruited to chromatin at the start of DNA replication and that Yap depletion accelerates DNA replication dynamics by increasing the number of activated replication origins. Furthermore, we identified Rif1, a major regulator of the DNA replication timing program, as a novel Yap binding protein. In *Xenopus* embryos, using a Trim-Away approach during cleavage stages devoid of transcription, we found that both Yap and Rif1 depletion trigger an acceleration of cell divisions, suggesting a shorter S-phase by alterations of the replication program. Finally, our data show that Rif1 knockdown leads to defects in the partitioning of early versus late replication foci in retinal stem cells, as we previously showed for Yap. Altogether, our findings unveil a non-transcriptional role for Yap in regulating replication dynamics. We propose that Yap and Rif1 function as breaks to control the DNA replication program in early embryos and post-embryonic stem cells.

Keywords: Hippo/Yap pathway, Rif1, DNA replication, retinal stem cells

*Speaker

†Corresponding author: odile.bronchain@u-psud.fr

‡Corresponding author: muriel.perron@universite-paris-saclay.fr

§Corresponding author: kathrin.marheineke@i2bc.paris-saclay.fr

Role of foxn1 in innate T cells driven immune tolerance of X. laevis tadpoles.

Dionysia Dimitrakopoulou * [1], Francisco De Jesus [1], Eleanor Wise Bellin [1], Mark Horb [2], Jacques Robert† [1]

[1] University of Rochester Medical Center – United States
[2] National Xenopus Resource and Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, University of Chicago – United States

Xenopus laevis (*X. laevis*) is extensively used for comparative immunology studies. Our lab has established a research platform that, to date, is the only one outside mammals where an immune surveillance system based on non-polymorphic MHC class Ib directing the development and function of innate-like (i)T cells has been characterized. Notably, tadpoles rely on an adaptive immune system dominated by iT cells and elicit a delayed and less inflammatory anti-microbial immune response than adult frogs. We postulate that this larval tolerigenic capacity is related to the prominence of iT cells. Although all T cells differentiate in the thymus, only conventional T cells are selected by interacting with polymorphic MHC molecules expressed by thymic epithelial cells (TEC), whereas iT cells interact with nonpolymorphic class Ib molecules expressed by other thymocytes and then follow a distinct differentiation program. Since TEC cell development/function is controlled by the master regulator gene *foxn1*, we targeted this gene with the CRISPR/Cas9 system. While waiting for F1 and F2 lines, we used F0 mosaic progenies to assess the effect of *foxn1* deficiency on iT cell function. Our foxn1 F0 lines demonstrated high knock-out score (37%) with similar phenotypic characteristics as murine models (e.g., thymic aplasia, T-cell deficiency). Interestingly, besides a severe T cell deficiency, *foxn1* crispants exhibited a decline/reduction in invariant T cell receptor alpha (TCRA) rearrangement iVa6 and iVa45 T cells indicating the defective development of the corresponding iT subsets. To further investigate the consequence *foxn1* knockdown and iT cell deficiency, we assessed tadpole immune responses against the ranavirus *Frog Virus 3* (*FV3*) and *Mycobacterium marinum* (*Mm*), two major pathogens of aquatic vertebrates. *Mm*-infected *foxn1* crispant tadpoles showed a reduced gene expression encoding cytokines (*il18, il12, inos*) and myeloid markers (csf1r, csf3r). Similarly, FV3 infection revealed some impairment in the myeloid lineage (*csf1r, csf3r*). This transgenic model will contribute to gathering insight into the regulatory role of iT cells during early development against microbial infections.

Keywords: foxn1, thymic epithelial cells, innate like T, cells, Mycobacterium marinum, inflammation

---

*Speaker

†Corresponding author: Jacques_Robert@URMC.Rochester.edu

European Xenopus Resource Centre, modelling rare monogenic human disease in Xenopus: recent progress in the behavioural analysis of tadpole models of Neurodevelopmental disorders.

Annie Godwin *† [1], Matt Parker , Colin Sharpe , Sarah Ennis , Diana Baralle , Matt Guille

[1] European Xenopus Resource Centre, Portsmouth – United Kingdom

Global developmental delay is a term frequently used to describe a Neurodevelopmental Disorder that arises early in childhood development and is characterised by impaired intellectual function, often assessed as an inability to achieve set developmental milestones such as age-appropriate mobilisation, communication, and social interaction. The early emergence of severe cognitive impairment in these patients carries a significant socio-economic burden with well-recognised long-term impacts that extend well into adulthood. A delay in the accurate diagnosis of those patients suffering from genetic conditions can limit their ability to access key resources, including specialist education programs and novel or repurposed therapeutics. Behavioural analysis in animal models is becoming an integral part of diagnostic translational medicine. *Xenopus* have an extensive track record for cost-effective, high-throughput gene function analysis, allowing us to determine the gene-disease link in $> 80\%$ of variants of unknown significance with distinct anatomical phenotypes (passed to us by clinical geneticists). Nonetheless, *Xenopus* broadly lack the robust assays needed to measure higher executive neural functions. Here we describe a pipeline to screen variants of unknown significance collated in a UK Neurodevelopmental Disorder Cohort, including the generation and characterisation of behavioural phenotypes such as altered locomotion and anxiety in knock-out *Xenopus tropicalis* lines. This work also includes measuring a recently described vertebrate search behaviour strategy reliant on working memory in *Xenopus tropicalis* tadpoles, using the free movement pattern Y-maze (FMP Y-maze). Our work in press shows significant working memory deficits in two glutamate subunit, *gria1* knock-out lines screened using the FMP Y-maze, supplementing findings previously published in the mouse and providing evidence for the description of a novel neurodevelopmental disorder.

Keywords: Xenopus tropicalis, CRISPR/ Cas9, Gene editing, Neurodevelopmental disorder, Free movement pattern Y, maze

*Speaker

†Corresponding author: Annie.godwin@port.ac.uk

Hnf1b renal expression directed by a distal enhancer responsive to Pax8

Laura Goea [1], Isabelle Buisson [1], Valérie Bello [2], Alexis Eschstruth [1], Mélanie Paces-Fessy [1], Ronan Le Bouffant [1], Albert Chesneau [3], Silvia Cereghini [1], Jean-FranÃ§ois Riou [1], Muriel Umbhauer [*†] [1]

[1] IBPS - UMR7622 – Sorbonne Université - CNRS, Sorbonne Université - CNRS – France
[2] IBPS - UMR7622 – Sorbonne Université - CNRS, Sorbonne Université - CNRS – France
[3] Institute of Neuroscience, CERTO Retina – Sorbonne Université - CNRS – France

Xenopus provides a simple and efficient model system to study nephrogenesis and explore the mechanisms causing renal developmental defects in human. *Hnf1b* (hepatocyte nuclear factor 1 homeobox b), a gene whose mutations are the most commonly identified genetic cause of developmental kidney disease, is required for the acquisition of a proximo-intermediate nephron segment in *Xenopus* as well as in mouse. Genetic networks involved in *Hnf1b* expression during kidney development remain poorly understood. We decided to explore the transcriptional regulation of *Hnf1b* in the developing *Xenopus* pronephros and mammalian renal cells. Using phylogenetic footprinting, we identified an evolutionary conserved sequence (CNS1) harboring epigenomic marks characteristics of a distal enhancer in embryonic and adult renal cells in mammals. By means of functional expression assays in *Xenopus* and mammalian renal cell lines we showed that CNS1 displays enhancer activity in renal tissue. Using CRISPR/cas9 editing in *Xenopus tropicalis*, we demonstrated the *in vivo* functional relevance of CNS1 in driving *hnf1b* expression in the pronephros. We further showed the importance of Pax8-CNS1 interaction for CNS1 enhancer activity allowing us to conclude that *Hnf1b* is a direct target of Pax8. Our work identified for the first time a *Hnf1b* renal specific enhancer and may open important perspectives into the diagnosis for congenital kidney anomalies in human, as well as modeling *HNF1B*-related diseases.

Keywords: kidney, patterning, transcriptionla regulation

[*]Speaker
[†]Corresponding author: muriel.umbhauer@sorbonne-universite.fr

The Rho GEF Trio is a major regulator of neural crest cell migration and dynamically localized at microtubules in cranial NC cells

Stefanie Gossen * [1,2], Marie-Claire Kratzer [2,1], Sarah F.s. Becker [3], Jubin Kashef [4], Annette Borchers† [1,2]

[1] Department of Biology, Molecular Embryology, Philipps University Marburg – Germany
[2] DFG Research Training Group, GRK 2213, Phillipps University Marburg – Germany
[3] Department of Development and Stem Cells, Institut de Génétique et de Biologie Moléculaire et Cellulaire, – université de Strasbourg – France
[4] Karlsruhe Institute of Technology, Institute for Photon Science and Synchrotron Radiation, Eggenstain-Leopoldshafen – Germany

Directional cell migration is controlled by various external signals leading to a precisely orchestrated intracellular signaling response that converges at the level of small Rho GTPases. The Rho guanine nucleotide exchange factor (GEF) Trio features distinct catalytical domains to control the activity of Rac1 and RhoA and therefore is a suitable candidate to unite signaling pathways leading to directional cell movement. Recently, we showed that in *Xenopus* Trio is required for coordinated cranial neural crest (NC) cell migration and formation of the craniofacial cartilage. Furthermore, Trio is important for protrusion formation and Trio morphant NC cells show a blebbing phenotype. As expected, the Trio morphant phenotype can be rescued by low concentrations of constitutively active Rac1 and RhoA but not Cdc42. Interestingly, while the Trio GEF1 domain, activating Rac1, has no effect, the Trio GEF2 domain, activating exclusively RhoA, is sufficient to rescue the Trio morphant phenotype. In addition, the GEF2 but not the GEF1 domain of Trio colocalizes with EB3 at microtubule plus-ends in *Xenopus* NC cells. Low concentrations of a Trio GEF2 mutant, lacking the SxIP-motif responsible for microtubule plus-end localization, is no longer able to rescue the Trio morphant phenotype, leading to the assumption that the colocalization of Trio to microtubule plus-ends is required for its function. Furthermore, Trio loss of function seems to disrupt the microtubular network as well as focal adhesion assembly in NC cells. Currently, we are investigating the dynamic intracellular transport of Trio and its consequences for microtubule network and focal adhesion formation leading to coordinated NC cell migration.

Keywords: Rho GEF Trio, neural crest cell migration, microtubular dynamics, focal adhesions

*Speaker
†Corresponding author: borchers@staff.uni-marburg.de

TOMO-Seq technique for subcellular analysis of multiple types of cells (oocytes, tumor).

Viktoriia Iegorova *† [1], Lisa Andersson [2], Emma Jonasson [2], Ravindra Naraine [1], Anders Stahlberg [2], Radek Sindelka *

1

[1] Institute of Biotechnology of the Czech Academy of Sciences - BIOCEV, Prumyslova 595, Vestec, 252 50 – Czech Republic
[2] Sahlgrenska Center for Cancer Research, Medicinaregatan 1F, Gothenburg, 413 90 – Sweden

TOMO-seq is a relatively new method that has been used to assess the spatial transcriptome within tissues and even within the single cell. This approach is a combination of cryosectioning and RNA-seq on individual sections, which allows for the reconstruction of the spatial localization of the transcripts.
TOMO-seq was first applied to the oocytes and embryos of the models *Danio rerio* and *Xenopus laevis*. The oocyte is usually easier to handle and manipulate relative to a typical somatic cell given the size difference. Additionally, oocytes like those from the *X. laevis*, have specific coloring differences that differentiate the animal and vegetal hemispheres, which facilitates the correct orientation of the sample. As of recent, we have already analyzed various samples (oocytes, embryos) from multiple species (*X. laevis*, *D. rerio*, *Ambystoma mexicanum*, *Acipenser ruthenus*, *Oncorhynchus mykiss*, etc.).
In this research, we present our successful application of RT-qPCR and TOMO-seq analysis to determine the RNA expression within multiple types of cells with varying sizes (from bigger to smaller): *X. laevis* oocytes (diameter 1.5mm), *Homo sapiens* oocytes (diameter 110μm), and small tumor cells MLS1765 (diameter up to 17μm).

Keywords: RNA localization, TOMO, seq, oocyte, tumor, cell, Xenopus, human

*Speaker
†Corresponding author: viktoriia.iegorova@ibt.cas.cz

Exonization by the emergence of a cleavage-polyadenylation site

Agnès Mereau* [1], Hubert Lerivray [1], Justine Viet [1], Serge Hardy [1], Luc Paillard† [1], Yann Audic ‡§ [1]

[1] Institut de Génétique et Développement de Rennes – Universite de Rennes 1, Centre National de la Recherche Scientifique : UMR6290, Structure Fédérative de Recherche en Biologie et Santé de Rennes – France

Exonization is the evolutionary process of recruitment of new exonic regions from previously intronic regions. It is a major contributor to the increased complexity of alternative splicing. Here, we explore exonization mediated by the emergence of a novel cleavage and polyadenylation site in a previously intronic region. In *Xenopus laevis*, the *tpm1* gene, which encodes muscular tropomyosin, contains alternative terminal exons. In adult muscles and embryonic hearts, exon 9A is joined to the terminal exon 9B. In embryonic somites, it is joined to the exonic region 9', which is transcribed from the intron immediately downstream of exon 9A. Consequently, exon 9A is either an internal exon when ligated to exon 9B, or a part of a terminal exon along with region 9'. We show here that region 9' is present only in amphibians and coelacanths. This suggests that it emerged in sarcopterygians and was lost in amniotes. We used antisense morpholino oligonucleotides to mask the regulatory sites in living *Xenopus* embryos. This revealed that the definition of exon 9A9' relies on a weak cleavage-polyadenylation site and an intronic enhancer, but is independent of the 3' splice site. We demonstrate that exon 9B is toxic when accumulated in somites. We propose that this toxicity contributed to the evolutionary pressure that led to the exonization of region 9' in sarcopterygians. These findings provide new insights into the mechanisms and driving forces of exonization-mediated diversification of terminal exons.

Keywords: exonization, 3' terminal exons, alternative splicing, amphibians, Xenopus.

*Corresponding author: agnes.mereau@univ-rennes1.fr
†Corresponding author: luc.paillard@univ-rennes1.fr
‡Speaker
§Corresponding author: yann.audic@univ-rennes1.fr

Let's make a quantum leap: *Xenopus laevis* advantages to shed light on axonal circRNAs

Linda Masante *† [1], Eloina Corradi [1], Ilaria Sanvido [2], Archana Iyer [3], Jelena Pistolic [4], Paul Collier [5], Thomas Hansen [6], Vladimir Benes [5], Marie-Laure Baudet‡ [1]

[1] Centre for Integrative Biology (CIBIO), University of Trento – Italy
[2] Institut für Physiologie Medizinische Universität – Austria
[3] Tata Institute of Fundamental Research – India
[4] TRON Mainz – Germany
[5] Genomics Core Facility, EMBL Heidelberg – Germany
[6] MBG, Aarhus University – Denmark

Xenopus laevis and circRNAs have something in common: they are ignored by most people. *X. laevis* community is widely underrepresented compared to the most famous *Mus musculus, Homo sapiens* and even *Danio rerio* ones. However, *X. laevis* offers several technical advantages compared to other animal models. During development, embryos, which can be obtained in very large numbers in a very short time, can be easily manipulated. Neurons are maintained in culture without the need of any growth factors. Several small molecules can be *in vivo* electroporated and detected both *ex vivo* and *in vivo*. Moreover, single axons can be easily visualized, *ex vivo* and *in vivo*, allowing to perform complex experiments such as uncover RNA subcellular localization.

CircRNAs are covalently closed molecules produced by the canonical spliceosome in a non-canonical splicing process, known as back-splicing. First believed to merely be splicing errors, after fifty years, a clear definition - and function - is yet to be found. Thousands of circRNA molecules have been detected in a plethora of different eukaryotes, showing an enrichment in brain synapses, where their levels peak specifically during synaptogenesis. Synapses are composed of a pre- and post- synaptic compartment – the axon and the dendrites - widely different in terms of morphology, physiological roles and mechanisms of compartmentalization. It is important – since still unknown - to elucidate where, within synapses, circRNAs are preferentially localized. The proper development of the axon is crucial to guarantee the correct synapse formation. Impairments in this process can lead to severe neurodevelopment diseases. We have thus focused on the developing axon to shed light on circRNA preferential localization.

Exploiting *in vivo* laser capture microdissection and *ex vivo* isolation of *X.laevis* retinal ganglion cells (RGCs) axons, we have uncovered an abundant population of circRNAs in the axonal compartment, where they are enriched compared to the somatic one. The enrichment in the axonal compartment led us to explore deeper their local distribution with the final aim to uncover the molecular mechanism driving their preferential subcellular translocation. To this end, we developed a novel, modified nucleic-acid based tool - circTracker - which enables to specifically target and study circRNA transport. Through live imaging of single axons, we described an

*Speaker
†Corresponding author: linda.masante@unitn.it
‡Corresponding author: marielaure.baudet@unitn.it

uncommon distribution of an axonal circle, circDDX17, along the axon.
Thanks to the advantages *X.laevis* provides, our results propose circRNAs as a novel class of regulatory RNAs acting locally in the axonal compartment during the most critical phases of its development.

Keywords: Xenopus laevis, circRNAs, axon, retinal ganglion cells, RNA transport, RNA localization

CRISPR-CAS13 knockdown in Xenopus laevis

Sofia Moreira * [1], Elias H. Barriga† [2]

[1] Mechanisms of morphogenesis lab, Gulbenkian Institute of Science (IGC), Oeiras,Portugal – Portugal
[2] Mechanisms of morphogenesis lab, Gulbenkian Institute of Science (IGC), Oeiras,Portugal – Portugal

RNA "knockdown" strategies have been widely used to explore biological processes. These transient downregulation strategies are especially important in cases where lethality and infertility are associated with gene disruption. Recent advances reveal that CRISPR-CAS13 defense mechanism of prokaryotes can be effectively used as a knockdown tool in fission yeast, plants, mammalian cell lines and zebrafish as well as mouse embryos. While several knockdown tools have been successfully applied in *Xenopu*s, whether CRISPR-CAS13 works in *Xenopus leavis* remains to be determined. In our lab, we were able to implement this cutting-edge strategy to downregulate both exogenous and endogenous transcripts in *Xenopus laevis*. Remarkably, our results recapitulate described loss-of-function phenotypes of known developmental markers. Here, we aim to provide a workflow on how to use CRISPR-CAS13 for downregulation of target mRNA in a comparatively efficient, specific, cost-effective and fast manner. We hope this tool will be used within our *Xenopus* community to generate well controlled and in turn reproducible data.

Keywords: CRISPR, CAS13, RNA knockdown, Xenopus laevis

*Speaker
†Corresponding author: ebarriga@igc.gulbenkian.pt

Temporal integration of Notch signaling in mucociliary epithelia cell fate specification

Magdalena Brislinger Engelhardt * [1,2,3], Fabian Lorenz [3,4], Sarah Bowden [1,5], José Luis Gomez VÃ¡squez [6], Maximilian Haas [1,2], Alexia Tasca [1], Charlotte Softley [1], Clemens Kreutz [3,4], Peter Walentek† [1,3,7]

[1] Renal Division, Department of Medicine, University Freiburg Medical Center, Freiburg – Germany
[2] Spemann Graduate School of Biology and Medicine, Albert Ludwigs University Freiburg, Freiburg – Germany
[3] CIBSS - Center for Integrative Biological Signalling Studies, Albert-Ludwigs-University Freiburg – Germany
[4] Institute of Medical Biometry and Statistics, Albert Ludwigs University Freiburg – Germany
[5] International Max Planck Research School for Immunobiology, Epigenetics, and Metabolism, Max Planck Institute of Immunobiology and Epigenetics, Freiburg – Germany
[6] Renal Division, Department of Medicine, University Freiburg Medical Center, Freiburg – Germany
[7] Molecular and Cell Biology Department, University of California at Berkeley, Berkeley, CA – United States

In mucociliary epithelia, Notch signaling regulates identities and numbers of epithelial cell types. A correct composition of ciliated, secretory and stem cells is required for clearing pathogens from the respiratory tract, and defects can lead to severe diseases such as chronic obstructive pulmonary diseases. Notch regulation of mucociliary epithelia was discovered over 20 years ago, but it remains unresolved how exactly cell fates, numbers and pattern formation are regulated due to its reiterative use in different tissues during establishment, homeostasis, and regeneration. It is our objective to uncover which general mechanisms are used and how they can be modified in a context-dependent manner. To uncover these objectives, we use the embryonic epidermis of *Xenopus* as a powerful model for the airway mucociliary tissue.
We found that different cell types require differential Notch levels and that they arise sequentially, starting with low Notch-requiring cell types and later switching to high Notch-cell types. This mode of specification is induced by an increasing signaling strength over time, generated by increasing numbers of Notch ligand presenting cells. With increasing cell numbers, Notch signaling is elevated to suppress earlier and to promote later cell fates. Our most recent data suggests that these Notch levels are translated by a set of direct target Hes genes into their respective cell fates.
We conclude that the cell-cell contact-dependent Notch signaling pathway acts like a developmental morphogen in the temporal dimension. Such a mechanism can generate repetitive local patterns, adaptable and scalable to organ size and physiological needs.

Keywords: Notch pathway, signaling, cell type specification, mucociliary epithelia, development

*Speaker
†Corresponding author: peter.walentek@medizin.uni-freiburg.de

Novel insights into Myosin1d function during left-right axis determination in Xenopus laevis

Szilvia Csaba * [1], Melanie Tingler† [1], Axel Schweickert‡ [1], Martin Blum [1]

[1] University of Hohenheim – Germany

The left-right body axis is defined by the asymmetric arrangement of the inner organs. Establishment of the left-right axis therefor requires the asymmetric induction of the highly conserved Nodal-Cascade in left lateral plate mesoderm. During *Xenopus* development, a cilia-driven leftward-fluid flow during neurulation is the initial step that leads to post-transcriptional repression of the Tgf-β/Wnt/BMP antagonist *dand5* on the left side and thus to the release of Nodal1. The activation of the highly conserved Nodal-cascade then regulates asymmetric development of inner organs.
We recently showed that the unconventional myosin Myo1d is required for the symmetry breakage process by directing cilia polarity, which is important for directional leftward-flow. New functional data, generated by side-directed *myo1d* knockdown, revealed that only right-sided loss of function of myo1d leads to ectopic induction of the Nodal-Cascade. Our findings argue for a second important function of Myo1d during laterality determination, independent of cilia polarization.

Keywords: Myosin1d, Dand5, laterality

*Speaker
†Corresponding author: melanie.tingler@uni-hohenheim.de
‡Corresponding author: axel.schweickert@uni-hohenheim.de

ENTPD5: a new player during kidney formation

Fanny Hardet , Eric Boué-Grabot , Karine Masse *† [1]

[1] Institut des Maladies Neurodégénératives – Université de Bordeaux (Bordeaux, France) – France

ENPTDase are ectonucleotidases which regulate purinergic signalling pathways by controlling the extracellular purine concentrations. The implications of this signalling pathway have been shown during the physiology of several organs, such as the kidney and deregulation of this pathway, especially ectonucleotidases expression is correlated with renal pathologies. ENTPDase5 is an intracellular transmembrane enzyme with several enzymatic activities. It has been shown that it can also be located at the plasma membrane et secreted, suggesting it can regulate the activation or inhibition of several purinergic receptors. Furthermore, ENTPDase5 is identical to the proto-oncogene PCPH, whose truncated oncogenic form, cph, has been involved in several cancer. This enzyme is expressed in the adult human kidney but its renal functions remain unknown.

We previously showed that ENPTDase 5 is highly and specifically expressed in the *Xenopus* pronephros. Our functional data demonstrate that this enzyme is required for nephrogenesis and is sufficient to generate ectopic kidneys. Over-expression of the cph protein induces similar but less severe renal phenotypes than ENTPDase5 overexpression induced ones. We show that these roles are dependant of its enzymatic activities and the pronephric alterations are linked to its subcellular localization. Finally, our preliminary data suggest that ENPTDase5 overexpression induces an increased apoptosis at the early steps of kidney formation which could be the cause of the reduced pronephric phenotype.

Our findings are the first evidence of entpd5 roles during embryogenesis and bring novel elements to decipher its mechanism by which ENTPDase5 regulates kidney formation and renal carcinogenesis.

Keywords: ectonucleotidase, purinergic signalling pathway, PCPH proto, oncogene, ectopic renal structures

*Speaker

†Corresponding author: karine.masse@u-bordeaux.fr

The endocytic receptor Lrp2 orchestrates apical constriction and cell polarity to drive cranial neural tube closure

Josefine Hoeren * [1], Kerstin Feistel† [1]

[1] University of Hohenheim, Institute of Biology, Dept. Zoology – Germany

Neural tube closure defects (NTDs) are common malformations affecting embryogenesis. Impaired morphogenetic movements such as cell intercalation or apical constriction can cause NTDs. Convergent extension (CE) is the dominant morphogenetic movement of caudal neurulation. It requires cell polarity mediated by the planar cell polarity (PCP) pathway and is brought about by mediolateral intercalation of neural plate cells. Apical constriction (AC), on the other hand, creates hinge points that facilitate neural fold elevation and is essential for neural tube closure in the cranial region.

Cranial NTDs occur in human patients with pathogenic variants of the endocytic receptor Lrp2. Using *Xenopus laevis* and mice, we have shown that Lrp2 is essential for neural fold elevation and neural tube closure in the forebrain area. Lrp2-mediated endocytosis drives AC by interacting with scaffold proteins and removing excess apical membrane in constricting cells. At the same time, Lrp2 is required for cell polarity. Despite the lack of PCP-mediated CE in the cranial region, cells exhibit planar polarity involving the core PCP protein Vangl2. During cranial neurulation, the subcellular localization of the Vangl2 changes dynamically. Lrp2 regulates Vangl2 localization in a temporospatial manner, suggesting a direct interaction between the two proteins. Lrp2 and Vangl2 both contain a C-terminal PDZ-binding domain (PBD). Since PBD-containing proteins often form multiprotein complexes coupled by PDZ domain-containing scaffold proteins, the functional interaction between Lrp2 and Vangl2 may be mediated by such a complex.

To test this hypothesis, CRISPR/Cas9 was used to either induce loss of the entire protein or to eliminate the C-terminal PBD of *lrp2*. Loss of the entire protein, either using a translation blocking morpholino oligomer or N-terminal CRISPR/Cas9, impaired AC and disturbed dynamic cell polarization. CRISPR/Cas9-mediated deletion of the C-terminal PBD of Lrp2, however, increased AC while cell polarization was unaffected. Taken together, these results suggest that protein interactions via the C-terminal PBD of Lrp2 drive scaffold accumulation to regulate AC. The C-terminal PBD is dispensable for cell polarization, suggesting that the influence of Lrp2 on PCP-dependent cell polarity must be mediated by additional motifs in the intracellular domain of Lrp2. Our preliminary functional approaches suggest that an additional PBD acts as a regulator of cell polarity.

*Speaker

†Corresponding author: k.feistel@uni-hohenheim.de

Keywords: Lrp2, neural tube closure, endocytosis, Xenopus laevis, planar polarity, PDZ, binding

R-spondin 2 as a BMP receptor 1A antagonist in the Spemann Organizer function to regulate Xenopus axial patterning

Hyeyoon Lee * [1], Carina Seidl [1], Christof Niehrs [1]

[1] DKFZ – Germany

How vertebrate embryo tell apart head from tail, is a fundamental question of developmental biology. In *Xenopus* embryo, the Spemann Organizer is a small signaling center which plays an eminent role in regulating embryonic axis formation and neural induction. It is known that the molecular mechanism underlying the Spemann Organizer resides in inhibition of BMP signaling. However, how BMP signaling receptors are extracellularly modulated in this context, is still poorly understood. R-spondins (RSPO1-4) are long-held secreted WNT signaling agonists implicated in development and cancer. Unexpectedly, here we show by gain and loss of function experiments that in *Xenopus* embryos R-spondin 2 (RSPO2) functions as a BMP receptor antagonist and a negative feedback inhibitor of BMP4 to regulate the Spemann Organizer signaling. Notably, genome editing mutagenesis of *rspo2* and either *chordin* or *noggin* hyperactivates BMP signaling thus yields strongly ventralized embryos. We provide the underlying molecular mechanism and demonstrate that unlike RSPO2 and -3, RSPO1 and -4 do not inhibit BMP signaling, the key difference residing in the TSP1 domain, which mediates the type I BMP receptor (BMPR1A) binding. Building on the specific interaction of the TSP1 domain of RSPO2 with BMPR1A, we further identify a 10-mer peptide which prevents binding between RSPO2 and BMPR1A. Administration of the peptide in *Xenopus* early embryos induces ventralization by upregulating BMP signaling without affecting WNT signaling. Our study invites RSPO2 as a new regulator of the Spemann organizer signaling by antagonizing BMPR1A, and further provides a proof-of-concept that BMP antagonism and WNT agonism can be uncoupled by specific pharmacological intervention with RSPO2-BMPR1A binding.

Keywords: RSPO, Spemann Organizer, BMP signaling, WNT signaling

*Speaker

Exploration of pronephric precursor specification using single-cell transcriptomics

Jean-Francois Riou *† [1], Isabelle Buisson [1], Laura Goea [1], Ronan Le Bouffant [1], Mélanie Paces-Fessy [1], Valérie Bello [1], Muriel Umbhauer [1]

[1] Institut de Biologie Paris-Seine, Laboratoire de Biologie du Développement, UMR7622 – Sorbonne Université, CNRS – France

Renal function at larval stages is accomplished by a pair of pronephroi located on each side of the Xenopus tadpole. Development of the pronephros starts after completion of gastrulation with the upregulation of a set of transcription factors (TF)- encoding genes in a group of dorso-lateral mesoderm cells called the kidney field. This process is dependent on retinoic acid (RA). Although every renal precursor (RP) of the kidney field expresses *pax8* and *lhx1*, regionalized expressions of other genes such as *hnf1b*, *mnx1* or *evi1/mecom* suggest that RPs are not a homogeneous population of cells. What RPs subpopulations form during neurulation and how these different cells will acquire the capability to initiate pronephros morphogenesis that is taking place at tailbud stages remains poorly understood.
We have explored RP diversity using subsets of whole *X. tropicalis* embryo single-cell RNA seq data (ventral/intermediate mesoderm, pronephric mesenchyme) produced in A. Klein and M Kirschner laboratories (Department of Systems Biology, Harvard Medical School, tinyurl.com/scXen2018). Cell clustering performed with Seurat tools clearly identifies RP populations from early neurula (st13-14) to early tailbud stages (st22). Together with pseudotime analysis with Monocle3, we have been able to identify early RP transcribing master renal regulators (*osr2*, *pax8*, *lhx1*) while down-regulating genes expressed in other mesodermal lineages (*actc1*, *hand1*...). These early RP are most abundant during early-mid neurula stages and appear to produce strong autocrine RA signaling (high *aldh1a2* expression). Later RP rather appear to switch off *aldh1a2*. They segregate into several subclusters reflecting RP subpopulations of the kidney field. Differential expression analysis between early and late RP, as well as among late RP clusters allows identification of candidate actors of early pronephros morphogenesis.
This switch from early to late RP state is likely to reflect the onset of transcriptional regulation dependent on master renal regulators. To further characterize this process, we have performed transcriptional profiling of genes dependent on pax8 depletion in kidney field explants isolated at late neurula stage (st18-19), when pax8 protein is detected in nuclei. Besides TFs acting downstream of pax8, identified targets suggest a role of pax8 in the early control of RP proliferation, growth and epithelialization.

*Speaker
†Corresponding author: jean-francois.riou@sorbonne-universite.fr

Keywords: renal precursors, kidney field, pronephros, single cell analysis, pax8

R-spondins are BMP receptor antagonists in Xenopus early embryonic development

Carina Seidl * [1], Hyeyoon Lee [1], Christof Niehrs [1,2]

[1] DKFZ – Germany
[2] IMB – Germany

Bone Morphogenetic Proteins (BMPs) are a subfamily of TGFβ growth factors with crucial functions in development, stem cells, adult tissue homeostasis, and cancer. The regulation and extracellular modulation is therefore of high importance and of particular interest as therapeutic target. BMPs signal through a tetrameric receptor kinase complex composed of type I and type II receptors. R-spondins (RSPOs) are a family of four secreted proteins that function as potent stem cell effectors and oncogenes. They are known as agonists of WNT-signaling by preventing Frizzled/LRP5/6 receptor ubiquitination and degradation via transmembrane E3 ubiquitin ligases ring finger 43 (RNF43) and zinc and ring finger 3 (ZNRF3). In this study we investigate WNT-independent functions of RSPOs and show the physiological relevance in development and disease. We found that RSPO2 and RSPO3, not RSPO1 and RSPO4, act as BMP antagonists. RSPO2 and RSPO3 are high affinity ligands for the type I BMP receptor BMPR1A/ALK3. RSPO2 forms a ternary complex between BMPR1A and the E3 ligase ZNRF3, which triggers internalization and degradation of the BMP receptor. We further found that, in *Xenopus* early embryonic development, Rspo2 is a negative feedback inhibitor in the BMP4 synexpression group and a regulator of dorsoventral axis formation. Our study reveals that R-spondins are bifunctional ligands, which activate WNT- and inhibit BMP signaling via ZNRF3, inviting re-interpretation of the mode of action of R-spondins in stem cell and cancer biology.

Keywords: RSPO, BMP, embryonic development, axis formation

*Speaker

The endocytic receptor Lrp2 modulates canonical Wnt signaling during neural crest specification

Valentina Trivigno * [1], Kerstin Feistel† [1]

[1] University of Hohenheim, Institute of Biology, Dept. Zoology – Germany

During embryogenesis, the neural crest gives rise to a set of different cell types which are involved in the formation of structures as diverse as the facial skeleton or the peripheral nervous system. Development of the neural crest requires a complex gene regulatory network. The understanding of this network is of major importance for medicine. Developmental malformations caused by defective neural crest development are referred to as neurocristopathies, often characterized by craniofacial defects. Craniofacial malformations (CFM) are observed in patients with Donnai-Barrow syndrome (DBS), a genetic disorder caused by pathogenic variations in *lrp2*, encoding a multifunctional endocytic receptor. DBS-related CFM show the requirement for Lrp2 in craniofacial development and strongly suggest a role for Lrp2 in neural crest formation. During neural crest specification in *Xenopus laevis*, Lrp2 was expressed in cells at the neural plate border, the precursors of the neural crest, and subsequently in cranial neural crest cells proper. Loss of Lrp2 in the neural crest severely affected cranial cartilage formation and induced eye defects, a phenotype that resembled the malformations of DBS patients. The neural crest-related phenotype established early during neurulation, when Lrp2 was required for neural crest specification. The Wnt signaling pathway, an important player throughout neural crest development, relies on endocytosis, suggesting that the endocytic receptor Lrp2 could modulate Wnt signaling. Luciferase reporter assays were performed to assess the role of Lrp2 in both endogenous as well as in exogenously induced Wnt signaling. In either setting, loss of Lrp2 reduced Wnt signaling activity, which was partially rescued by co-injection of ß-catenin, confirming the role of Lrp2 during ß-catenin-mediated canonical Wnt signaling. Intracellular Lrp2 sequences were detected in the nucleus, indicating that the intracellular domain, which can be released by proteolytic cleavage, may play a role in the intracellular transduction of Wnt signals. In addition, the influence of Lrp2 on Wnt signaling depended on its extracellular ligand-binding domain, suggesting the involvement of Lrp2 ligands in the process. Together, these data strongly suggest that Lrp2 is an essential protein for neural crest development, regulating neural crest specification via an influence on canonical Wnt signaling.

Keywords: Lrp2, neural crest, endocytosis, Wnt signaling, Xenopus laevis

*Speaker

†Corresponding author: k.feistel@uni-hohenheim.de

European Xenopus Resource Centre (EXRC): Our current research directions

Melek Ersin * [1], Anita Abu-Daya , Anna Noble , Matthew Guille , Kayleigh Adamson , Viki Allan , Kelvin Bateman , Annie Godwin , Sian Martin , Magda Mozsynska, , Gretel Nicholson , Colin Sharpe , Greg Widzik

[1] European Xenopus Resource Centre (EXRC), University of Portsmouth – United Kingdom

The EXRC is one of the main providers of molecular resources, animals, embryos, testes, sperm and oocytes to support researchers using the *Xenopus* model for developmental and cell biology, supplying thousands of resources annually. With ever changing conditions such as Brexit, Covid-19 and the outbreak of *M. liflandii,* the centre has adapted to be able to offer resources to the community in forms that are easier to transport and more biosecure than animals – for example embryos and frozen sperm. The EXRC has taken quick action to combat the *M. liflandii* outbreak. As a result, the centre has expanded, and with the installation of new MBK systems, the holding capacity of the EXRC has doubled allowing the centre to produce and house more lines, more wild-type animals and to provide larger quantities of the animal derived resources. Nanopore sequencing of EXRC *Xenopus* and animals from external labs is contributing to understanding whether *Xenopus* populations are surviving with endemic *M. liflandii.*
To further expand the EXRC, new transgenic lines have been generated and the centre is focused on producing lines in response to suggestions from the *Xenopus* community. Transgenic lines remain a powerful tool for screening changes after CRISPR/Cas9 gene-editing allowing researchers to study *in situ* the development of knock-out phenotypes at varying time points. Alongside, the EXRC is currently testing commercially available monoclonal antibodies raised against immunogens very similar to the frog to detect *Xenopus* proteins. Results have indicated a lack of consistency between commercially available antibodies, and as a result, testing has shifted to an older collection of antibodies from the Maller lab. Western blotting has demonstrated the antibodies are still functional in detecting *Xenopus* proteins. Further testing will allow the centre to determine which bleeds work, enabling researchers to order specific antibodies from the EXRC that have been tested on *Xenopus* extracts.
Come and visit the poster to talk over what you want from your resource centre and how we can develop and improve what we provide for the community.

Keywords: EXRC, Xenopus

*Speaker

Role of the transmembrane protein TUSC5 in neural development

Santosh Kumar * [1], Pauline Cabochette [1], Alba Sabaté San José [1], Aurore Latragna [2], Eric Bellefroid† [1]

[1] Génétique du développement, Université Libre de Bruxelles – Belgium
[2] Laboratory of Neuroscience, UMons – Belgium

Tusc5 (Tumor suppressor candidate 5) is a transmembrane protein expressed in adipose tissue where it was found to control insulin-induced glucose uptake through physical interaction with the glucose transporter Glut4. Tusc5 knockout mice were shown to have impaired insulin sensitivity leading to reduced glucose clearance and increased body weight, suggesting its important role in maintenance of healthy metabolism in mouse and human. In our lab, transcriptomic analysis of murine developing dorsal root ganglia in which the transcriptional regulator Prdm12 has been knocked-out in post-mitotic neuron revealed a strong downregulation of Tusc5 following Prdm12 depletion. These observations suggest that Tusc5 is expressed in developing sensory neurons and, maybe more specifically, in the lineage of pain sensing neurons which selectively require Prdm12 for their proper development. Moreover, *In situ* hybridization of developing and adult murine dorsal root ganglia have been performed to further validate Tusc5 expression in the mouse peripheral nervous system. This unanticipated expression of TUSC5 in peripheral sensory neurons and given literatures suggesting that the peripheral nervous system responds to adipose derived signaling factors and vice-versa provides largely unexplored links between environmental factors, inputs from CNS and adipose tissue physiology. We thus decided to extend our study to explore the function of Tusc5 in the neural specification of mouse and *Xenopus* to further explore its expression and potential connections in regulation of neural patterning genes. Characterization of such link between adipocytes physiology and nervous system function could have significant implication towards understanding the biology of metabolic diseases related neuropathies.

Keywords: Tusc5, Prdm12, Xenopus, Murine

*Speaker
†Corresponding author: Eric.Bellefroid@ulb.be

CRISPR/Cas Based Methods For Precise, Non-Mosaic DNA Integration in Xenopus

Sian Martin * [1,2], Annie Godwin† [3,4], Melek Ersin‡ [3,4], Matt Guille§ [1,2]

[1] University of Portsmouth – United Kingdom
[2] European Xenopus Resource Centre – United Kingdom
[3] University of Portsmouth – United Kingdom
[4] European Xenopus Resource Centre – United Kingdom

Xenopus have long had a reputation for being a powerful model organism for use in developmental, cell and biochemistry research. With the advent of gene editing technologies, and full genome sequencing of *Xenopus* genomes revealing the full extent of the genetic conservation between *Xenopus* and humans, it was expected that *Xenopus* would soon become a popular model for human disease. However, the inability to produce non-mosaic, precise DNA insertions through homology directed repair has limited the strength of *Xenopus* in this field. Furthermore, it has prevented researchers from taking full advantage of fusion tagging, a method for directly tagging genes with either epitope or fluorescent tags, allowing the visualisation, quantification and tracking of proteins without the use of protein-specific antibodies, which are often unreliable or not reactive in *Xenopus*. Here we demonstrate a method for precise DNA insertion into *Xenopus* oocytes using CRISPR/Cas9, followed by *in vitro* maturation and fertilisation by intracytoplasmic sperm injection, culminating in the production of embryos carrying a non-mosaic, heterozygous HA tag fused endogenous RUNX1 protein. We also demonstrate an improvement to HDR insertion using long homology compared to short homology in embryos and make a move toward trialling other emerging HDR insertion methods, prime editing and base editing in *Xenopus*. The comparison of these methods will help carve a clear path to further optimising HDR insertion in both oocytes and fertilised eggs, which will strengthen the frog as a genetic model organism.

Keywords: Disease modelling, Homology directed repair, precise knockin, Oocytes

*Speaker
†Corresponding author: annie.godwin@port.ac.uk
‡Corresponding author: melek.ersin@port.ac.uk
§Corresponding author: matthew.guille@port.ac.uk

Functional E3 ubiquitin ligase Trim29 is required for the differentiation of the mucociliary epidermis in Xenopus

Herbert Neuhaus [*†] , Astrid Vess , Marie Schomburg , Stefanie FÃ¶rster , Thomas Hollemann [1]

[1] Martin-Luther University Halle-Wittenberg, Institute of Physiological Chemistry, HollystraÃŸe 1, 06114 Halle – Germany

During frog embryogenesis the E3 ubiquitin ligase *trim29* was exclusively expressed in the basal layer of the entire developing epidermis. Only in the developing neural plate *trim29* was not detectable. Down regulation of Trim29 expression blocked the differentiation of the typical cell types, that compose the mucociliary epidermis. In epithelial structures lacking Trim29 expression, epidermal differentiation markers like *a-tubulin*, *E-cadherin* and *E-keratin* were not detectable, indicating that differentiation was blocked. Instead, a massive accumulation of undifferentiated cells underneath the ectoderm could be observed. Since reduced expression of the transcription factor tp63 causes a similar phenotype, we asked whether *trim29* expression is regulated by tp63. Injection of tp63 morpholinos resulted in down regulation of *trim29* expression, indicating that tp63 induces the transcription of *trim29*.
To better understand the functional role of Trim29 during epidermis formation we wanted to identify proteins that interact with Trim29. Therefore, we immuno precipitated Trim29 overexpressed in different cell lines and identified several potential interaction partners by mass spectometry Interestingly during further interaction studies we could demonstrate, that Trim29 binds to tp63. This indicates that tp63 not only regulates *trim29* expression, but also the direct interaction of these two proteins is required for the formation of a functional mucociliary epidermis.

Keywords: mucociliary epidermis, E3 ubiquitin ligase, tp 63, differentiation

[*]Speaker
[†]Corresponding author: herbert.neuhaus@medizin.uni-halle.de

Transcriptional control of early nephrogenesis in Xenopus tropicalis

Paulina Ogar * [1], Thomas Naert [1], Soeren Lienkamp† [1]

[1] Institute of Anatomy, University of Zurich, Zurich – Switzerland

In mammalian kidney specification, signalling from the metanephric mesenchyme (MM) drives the growth of the ureteric bud (UB) out from the nephric duct, which in turn branches into the MM eventually forming the final kidney, the metanephros. The interaction between the epithelium (e.g., the UB) and the mesenchyme (e.g., the MM) is an essential driver of nephron formation. In *Xenopus* pronephros development, the tubule anlage condenses, mesenchymal to epithelial transition occurs and functional tubules subsequently form, but the available analysis has yet to uncover distinct tissue domains. Moreover, transcription factors such as *pax8*, *hnf1b*, and *sall1* that underpin early kidney development, are localised in distinct regions within the UB and MM in mammals. Curiously, however, these factors are roughly co-expressed within the same pronephric region in *Xenopus*. Here, we outline an imaging strategy combining fluorescence *in situ* hybridisation (FISH), immunostaining and light-sheet microscopy to examine the epithelial and mesenchymal domains in 3D within the developing *Xenopus* kidney, and to correlate these morphogenetic events to the expression of *pax8*, *hnf1b*, *sall1*. Lastly, to further investigate transcriptional circuitry, we used CRISPR/Cas9 to knock out *pax8*, *hnf1b* and *sall1* in *Xenopus tropicalis* and aim to conduct RNA-seq on individual embryos (single embryo transcriptomics). Through RNA-seq analysis, we will examine key interacting genes, roles and hierarchies of *pax8*, *hnf1b*, *sall1*, and relevant transcription factors underlying early kidney development.

Keywords: kidney, FISH, microscopy, CRISPR/Cas9

*Speaker

†Corresponding author: soeren.lienkamp@anatomy.uzh.ch

Skin depigmentation in Xenopus tropicalis: heavy metal toxicity and/or hypervitaminosis A+D?

Julia Traversari *† [1], Udo Hetzel [2], Soeren Lienkamp‡ [1]

[1] Institute of anatomy, University of Zurich – Switzerland
[2] Institute of Veterinary Pathology, University of Zuric – Switzerland

One month after arrival in a new temporary facility with recirculating tap water system, a group of twenty post-metamorphic *X. tropicalis* froglets displayed skin depigmentation involving head and dorsum, which progressed to erosions and joint swelling. Similar symptoms were observed in home-bred animals, which additionally showed oedema, growth retardation and increased mortality before reaching metamorphosis. At necropsy, the kidneys were enlarged and presented mild radial whitish striation. The histological examination led to following diagnosis: erosive-ulcerative dermatitis with skin calcification, dermal vasculitis, renal tubular atrophy with calcifications and periarticular fibrosis and calcification (pseudogout). Rhodanine stain of copper deposits was positive in the gut of a tadpole, water and organ analysis revealed increased copper and zinc concentration. Other management-related issues were excluded after colony management and technical verification, infectious diseases were ruled out by PCR, transmission electron microscopy and histology. The findings indicated heavy metal toxicity-induced renal insufficiency, possibly leading to uraemia and consequent dermatitis, as well as to pseudogout due to renal hyperparathyroidism.
The colony was transferred to the new permanent facility and young animals raised in reconstituted deionized water. After approximately 9 months, recently bred froglets manifested symptoms similar as previously observed. The calcifications, however, were more extensive and sometimes affected the corneas, skin ulcerations were absent. Moreover, metamorphic animals presented with poly- and/or amelia. Adult frogs showed round enlargements – histologically identified as nodular fibrosis with mineralization – arising from the distal phalanges of the clawed digits of the hind feet, as well as digital joint swelling. The abovementioned inquiries were conducted and consistently with the clinical findings, excessive vitamin A and D content was revealed by the feed analysis, the composition of which changed dramatically compared to the previous commercial declarations.
The present case highlights the complexity of multifactorial diseases, which albeit presenting with overlapping symptoms, were probably caused by different underlying pathophysiological processes, and stresses the vital role played by management conditions and the regular monitoring thereof.

*Speaker
†Corresponding author: julia.traversari@anatomy.uzh.ch
‡Corresponding author: soeren.lienkamp@anatomy.uzh.ch

Keywords: toxicity

Cyst initiation and development in autosomal dominant polycystic kidney disease in the Xenopus model

Taiyo Yamamoto * [1], Thomas Naert [1], Soeren Lienkamp [1]

[1] University of Zurich – Switzerland

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common monoallelic disorders, affecting between 1:400 to 1:1'000 of the population worldwide. It is typically caused by mutations in the *PKD1* or *PKD2* genes. The symptoms encompass progressively growing renal cysts leading to kidney enlargement, fibrosis, and eventually end-stage renal disease (ESRD), requiring dialysis or kidney transplants in patients.
PKD1 and *PKD2* encode for polycystin-1 and polycystin-2 respectively, which associate and form a transmembrane complex. Homozygous loss of one of the PKD genes leads to rapid renal cyst development and embryonic death, while monoallelic mutations lead to lowered but sufficient polycystin protein levels. The onset of the disease is generally explained through a 2-hit hypothesis, where somatic mutations further reduce polycystin expression or availability in individual cells and induce cyst nucleation sites. However, the molecular mechanisms involved in cyst nucleation and development are still largely unknown.
A better understanding of the disease progression and the process of cyst formation is necessary to develop novel therapeutic avenues. Current animal models of ADPKD rely on inducible full tissue knockouts of *PKD1* or *PKD2*. This leads to rapid renal deterioration upon knockout and does not replicate the single-cell origin of cysts in the human disease. Therefore, the process of cyst nucleation and the interactions of diseased cells with a healthy environment cannot be assessed in these models.
The *Xenopus* model is well suited to address these issues. Established protocols for targeted mosaic gene editing and in vivo imaging will allow us to observe the cystic development of individual cells. We will use in vivo spinning-disk and light-sheet microscopy to observe changes in cell morphology, motility, or proliferation during initial cyst development in a mosaic *PKD1* knockout model, in which gene edited cells are trackable via fluorescence expression. By observing the transition of cells from healthy to cystic, hypotheses of the involved molecular pathways can then be established. We will investigate and develop the hypotheses through KO/rescue experiments and compound screenings in wild type and transgenic animals and aim to elucidate the mechanisms of cyst initiation.

Keywords: Autosomal Dominant Polycystic Kidney Disease, Disease modeling, Live imaging

*Speaker

The European Xenopus Resource Centre V2.0?

Matt Guille * [1], Anita Abu-Daya , Kayleigh Adamson , Viki Allan , Kelvin Bateman , Mel Ersin , Annie Godwin , Sian Martin , Magda Moszynska , Gretel Nicholson , Ania Noble , Colin Sharpe , Greg Widzik

[1] European Xenopus Resource Centre (EXRC) – University of Portsmouth, School of Biological Sciences King Henry Building, Portsmouth PO1 2DY UK, United Kingdom

The EXRC is designed as a "one stop shop" where animal and molecular resources for working with *Xenopus* have been collected, quality assured, stored and distributed since 2006. A combination of Brexit, the sars-cov-2 pandemic and particularly an outbreak of *M. ulcerans* ecovar *liflandii* have made running the centre very challenging over the last 2 years. The way the centre works has adapted, and it has been significantly rebuilt to meet these challenges.
To partially address the obstacles that have arisen to moving any perishable material due to both Brexit and the post-covid travel decrease we have sent many more *Xenopus* "products" rather than live animals. These include embryos, oocytes, sperm and testes. Recovery from the *M. liflandii* outbreak has been a major piece of work, although our biosecurity measures meant that no frog lines were lost and limited the damage done. The tanks and systems in our two largest frog rooms have subsequently been replaced with new state of the art kit. This has greatly enlarged the centre and we have also taken the opportunity to improve Biosecurity.
Recently we have been testing commercially available, monoclonal antibodies predicted to recognise *Xenopus* proteins. The results have been disappointing, however the results with what are now a very old collection of antibodies from the Maller lab are much more promising. Alongside this, new transgenic lines have been created and many visitors to the centre have undertaken a variety of experiments, particularly CRISPR knockouts, and training. We have also developed an assay that detects asymptomatic *M. liflandii* and initial results suggest it is present across a number of labs in both the UK and Europe; we should be able to confirm whether these results are robust at the meeting.
Looking to the future, the new tanks are filling with tadpoles generated from cleaned embryos and some of these are now large froglets. In terms of supplying large numbers of *X. tropicalis*, these should be available early next year but female *X. laevis* will not be adults until Easter 2023.
We need you to prioritise your needs to enable us to plan the next 5 years of development of the EXRC and will ask you to fill in a survey to help us.

Keywords: resources, trangenics, animals, antibodies, oocytes, training

*Speaker

Investigating EZH2 as a druggable mediator of immune cell exclusion in desmoid tumors

Marthe Boelens [*†] [1,2], Dieter Tulkens [3,4], Thomas Naert [5], Suzan Demuynck [6], David Creytens [4,7], Kris Vleminckx [6]

[1] Cancer Research Institute Ghent (GRIG), Ghent – Belgium
[2] Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium – Belgium
[3] Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium – Belgium
[4] Cancer Research Institute Ghent (CRIG), Ghent, Belgium – Belgium
[5] Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium – Belgium
[6] Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium – Belgium
[7] Department of Pathology, Ghent University, Ghent, Belgium – Belgium

CRISPR/Cas9 based dependency mapping in *Xenopus tropicalis* identified EZH2, a member of the polycomb repressive complex 2, as a druggable dependency factor for desmoid tumors, a rare tumor specifically and exclusively driven by Wnt pathway activation. Furthermore, the EZH2 inhibitor Tazemetostat led to rapid desmoid tumor shrinkage in the *in vivo* model. In contrast, the compound did not have an overt effect on cell proliferation or cell death in cultured human desmoid tumor cells. However, Tazemetostat treatment significantly decreased transcriptional responses to the Wnt pathway. Therefore, given the well-established fact that solid tumors in which the Wnt/β-catenin pathway is activated are immunologically cold and thereby insensitive to immune checkpoint inhibition, we postulate that desmoid tumor regression by Tazemetostat in the *Xenopus* model may be due to counteracting this immune suppressive environment and allowing the engagement of a natural anti-tumor immune response. This hypothesis is on the one hand tested using a novel *Xenopus rag2* knockout line, which besides has been validated in tumor cell transplantation studies. Desmoid tumor appearance (*i.e.* size and number) following Tazemetostat treatment in immunocompromised animals will be compared to the situation in wild type animals and heterozygous siblings. According to our hypothesis, no tumor-specific effect of Tazemetostat treatment is expected in animals that lack an adaptive immune system since natural immunity cannot be induced in this context. In parallel with the above, we will exploit a *rag2*::GFP *Xenopus* reporter line to detect increased T-cell infiltration in desmoid tumors upon Tazemetostat treatment relative to a non-treated control arm. Moreover, *pdcd1* and *ctla4* mosaic mutants are generated in this genetic background, which will be screened for desmoid tumor incidence. If immune checkpoints are engaged in desmoid tumors it should be reflected by a reduced number or decreased size of desmoid tumors in the crispant animals. In conclusion, we aim at exposing the interaction of desmoid tumors with the immune environment and thereby providing insight into the mechanism of action of Tazemetostat. Overall, the knowledge obtained in our desmoid tumor model, may provide critical insight into more complex cancers associated with Wnt pathway activation.

[*]Speaker
[†]Corresponding author: marthe.boelens@irc.vib-ugent.be

Keywords: Desmoid tumor, cancer modeling, Tazemetostat, EZH2, immunological cold, Wnt pathway activation

Modelling USH2A-associated retinal disease in Xenopus tropicalis to investigate the pathogenicity of human missense variants implicated in inherited blindness

Marjolein Carron * [1,2], Suzan Demuynck [2], Silke Maes [2], Kristof Van Schil [1], Elfride De Baere [1], Kris Vleminckx [2]

[1] Centre for Medical Genetics, Ghent University and Ghent University Hospital, Ghent – Belgium
[2] Developmental Biology Unit, Department of Biomedical Molecular Biology, Ghent University – Belgium

Introduction: The *USH2A* gene, coding for the usherin protein, is one of the most frequently mutated genes involved in inherited retinal diseases (IRDs). It is implicated in 7Â-14% of autosomal recessive retinitis pigmentosa (RP) and in up to 80% of USH2, a syndromic autosomal recessive IRD characterized by moderate to severe hearing loss together with progressive RP. *USH2A* is known for its tremendous amount of missense variation. Most of this variation is classified as variants of uncertain significance (VUS), creating an unmet need for functional assessment of the numerous detected variants. We created a *Xenopus tropicalis ush2a-/-* model and aimed to introduce selected VUS in the *Xenopus genome* by use of base editing techniques whereby we can assess the causality of the variant *in vivo*.
Methods: An *ush2a-/- Xenopus tropicalis* model was generated via CRISPR/Cas9 genome editing. At regular time points we performed a histological examination of the *Xenopus* retina. As a proof-of-concept for the introduction of VUS, embryos were injected with the base editor (BE) BE4max and a sgRNA in order to introduce a pathogenic missense variant. Genotyping of the KO model and VUS model is performed via targeted MiSeq sequencing. Furthermore we have constructed an electroretinography (ERG) setup, which we are currently calibrating. Via electroretinogram recordings we are able to measure the functioning of the different cell types in the retina *in vivo*, in order to pick up larger but also subtle differences in functionality caused by missense mutations and follow the progression of the disease.
Results: Histological examination at the age of 9 months showed aberrations at the height of the photoreceptor cell layer. All rods were degenerated, leaving only cones, which corresponds with the early phase of RP in human patients. After a titration experiment we confirmed the optimal dose for the bilateral injection of BE and sgRNA. We were able to introduce the pathogenic variant via base editing in the *Xenopus* genome. However, editing efficiencies were quite low and further optimization is needed.
Conclusion: We were able to create an *ush2a-/- Xenopus tropicalis* model that mimics the patient phenotype, which can serve as a baseline for the disease phenotype. Furthermore, we successfully inserted a pathogenic missense variant in the *Xenopus* genome via base editing, but additional optimization is necessary. This proof-of-concept can be used to study VUS of interest

*Speaker

in more detail in order to reclassify the VUS and provide a clear molecular diagnosis to the patient.

Keywords: Xenopus tropicalis, CRISPR/Cas9, retinal diseases, USH2A, VUS

Impact of glyphosate-based herbicide on early embryonic development of the amphibian Xenopus laevis

Hannah Flach * [1], Alexander Lenz [1], Sarah Pfeffer [2], Michael Kühl [1], Susanne J. Kühl† [1]

[1] Institute of Biochemistry and Molecular Biology, Ulm University – Germany
[2] Institute of Neurobiology, Ulm University – Germany

Background: Amphibian populations are declining drastically worldwide. One reason for this might be the use of pesticides. A large group of pesticides used are the herbicides. The worldwide used herbicide glyphosate is an inhibitor of the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase of plant shikimate pathway, which prevents the formation of aromatic amino acids, inducing plant death. Due to this specific action, glyphosate-based herbicides (GBH), which contain other ingredients in addition to the active ingredient glyphosate, are considered non-toxic to non-target organisms. However, GBH affect embryonic development of chickens, amphibians and fishes.
Results: We demonstrated that GBH RoundupÂ® LB plus has a negative effect on embryonic development of the South African clawed frog *Xenopus laevis*. Treatment with GBH at sublethal concentrations resulted in a decreased body length and mobility of embryos. In addition, incubation with GBH resulted in smaller eyes, brains, and cranial cartilages compared to untreated embryos. GBH incubation also resulted in shorter cranial nerves and affected heart development including reduced heart rate and atrium size. On a molecular basis, GBH treatment led to reduced expression of marker genes in the different tissues and developmental stages.
Conclusion: GBH leads to impaired embryonic development of *Xenopus laevis*.

Keywords: pesticide, glyphosate, based herbicide, embryonic development

*Speaker
†Corresponding author: susanne.kuehl@uni-ulm.de

Ptk7 is dynamically localized at NC cell-cell contact sites and interacts with the RhoGEF Trio

Katharina Till * [1], Anita Grund [1], Laurence Riesselmann [1], Annette Borchers† [1]

[1] Philipps Universität Marburg – Germany

Neural crest (NC) cells are highly migratory cells contributing to a broad range of vertebrate tissues and their migration behavior resembles cancer cell invasion. The directional migration of NC cells is controlled by various mechanisms including information exchange via dynamic NC cell-cell contacts. A transmembrane protein that is likely involved in this process is PTK7 (protein tyrosine kinase 7), an evolutionary conserved Wnt/PCP co-receptor, which is required for *Xenopus* NC migration. Our data demonstrate that Ptk7 is dynamically localized at NC cell-cell contacts and plays a role in contact inhibition of locomotion (CIL), a phenomenon whereby NC cells change their polarity and directionality upon cell-cell contact. We found that loss of Ptk7 results in disturbed CIL behavior. Conversely, non-NC cells were protected from NC invasion by ectopic expression of Ptk7, but not by a Ptk7 deletion construct lacking the extracellular domain. These data suggest that Ptk7 is not only necessary but also sufficient for CIL. Recently, we identified the Rho guanine exchange factor (GEF) Trio as an interaction partner and possible downstream effector of Ptk7 during NC cell migration. Trio is especially well suited to relay signals, as it features two GEF domains, which specifically activate Rac1 and RhoA. Like Ptk7, Trio is also required for NC migration and ectopic expression of Trio rescues the Ptk7 morphant phenotype. Currently, we investigate the dynamic subcellular interaction of Ptk7 and Trio and the molecular mechanisms by which they control NC migration.

Keywords: neural crest, contact inhibition of locomotion, Ptk7, RhoGEF Trio

*Speaker

†Corresponding author: borchers@staff.uni-marburg.de

Using Xenopus laevis tadpoles to study basic principles underlying vertebrate motor control

Wen-Chang Li *† [1]

[1] School of Psychology and Neuroscience, the University of st Andrews – United Kingdom

How different groups of brainstem neurons in the reticular formation control movements in vertebrates has generally been difficult to study. Pre-feeding *Xenopus laevis* tadpoles have been used as a model animal to study the spinal and hindbrain circuits controlling movement for four decades. Much of the work has been focusing on delineating classes of neurons in the spinal cord and how they are synaptically connected to mediate simple flexion responses and generate rhythmic motor outputs like swimming and struggling. Research methods include conventional electrophysiology recordings, anatomical tracing of neurons, pharmacology and computer modelling. Current projects have moved to the hindbrain and midbrain circuits that are potentially involved in the turning behaviour, the initiation, cessation and modulation of tadpole swimming and struggling behaviour New molecular biological tools enabling GCamP-based calcium imaging and optogenetic manipulation of the activity in neuronal groups with specific markers will greatly facilitate the precise interrogation of neuronal roles in motor control. These markers may lie in the transcription factors identified in the early differentiation of neurons or proteins specific to neurons of certain neurotransmitter phenotypes like cholinergic or GABAergic neurons.

Keywords: brainstem, motor control, transcription factor, neurotransmitter

*Speaker

†Corresponding author: wl21@st-andrews.ac.uk

Table of contents

List of sponsors **1**

Mechanical and Chemical signals interplay during neural crest migration, Roberto Mayor 4

The role of memories in axolotl regeneration, Elly Tanaka 5

The drivers and blockers of retinal regeneration, Muriel Perron 6

Mechanics and development of the mucociliary epithelium, Jakub Sedzinski . . . 7

Oxygen-producing microalgae in the brain of Xenopus tadpoles rescue neuronal activity, Hans Straka 8

In vivo time-lapse imaging of olfactory sensory neuron birth, differentiation, and axogenesis, Ivan Manzini 9

Re-generating the principles of appendage regeneration in the single-cell era, Can Aztekin 10

Uncovering the cells and genes responsible for limb regeneration, Nicolas Leigh . 11

Modeling spinal cord regeneration in the axolotl, Osvaldo Chara 12

The Axolotl limb skeleton through growth and regeneration, Tatiana Sandoval-Guzman 13

A functional Operon imparts laterality at the ciliated LR organizer, Bruno Reversade 14

SOX transcription factors direct tissue-specific WNT responsive transcription independent of TCFs, Aaron Zorn [et al.] 15

Cross-talk between thyroid hormone and glucocorticoid signalling, Laurent Sachs 16

CHARGEd with neural crest defects - using Xenopus to characterize neurocristopathies, Janina Schwenty-Lara [et al.] 17

The PKA-PP2A dynamics keep oocytes arrested in meiosis I, Aude Dupré 18

Subcellular translation in neurons: when mRNAs meet endosomes, Jean-Michel Cioni 19

Trim29: Ubiquitin signaling in ectodermal development, Thomas Hollemann . . . 20

Development and Evolution of Tetrapod Motor Circuits, Lora Sweeney 21

Receptor-mediated endocytosis orchestrates anterior neural tube closure, Kerstin Feistel 22

microRNAs: novel transport route and action in axons development, Marie-Laure Baudet 23

Dr. Strangefoot (aka, Xenopus) or: How did I Learn to Stop Worrying and Love Metabolism, Enrique Amaya 24

A new regulator of the cerebellar granular neuron stem/progenitors niche size and behavior: A study in amphibian, Béatrice Durand 25

Mucus, ciliation, stemness - Spdef does it all, Maximilian Haas [et al.] 26

The tadpole foregut as new model to study mucociliary epithelia in Xenopus, Mona Hansen [et al.] 27

Tra2b – a new paradigm in coordinated post-transcriptional control of ciliogenesis, Charlotte Softley [et al.] 28

dmrt2 and myf5 link early somitogenesis to left-right axis determination in Xenopus laevis, Melanie Tingler [et al.] 29

CP110, a surprising new regulator of cilium disassembly, Alexia Tasca [et al.] . . 30

Timing of swim and limb motor neuron generation in the Xenopus spinal cord, Clara Becker [et al.] 31

Redox control of stemness: insights from neural stem cells of the Xenopus retina, Alicia Donval [et al.] 32

Link between Müller cell proliferation and inflammation in Xenopus laevis upon retinal injury., Karine Parain [et al.] 33

Hmmr modulates Wnt signaling to drive mesenchymal-to-epithelial transition in the developing forebrain, Fee Wielath [et al.] 34

Xenopus tropicalis Immature Sertoli Cells show potential in promoting muscle regeneration in tadpoles, Qing Zhao [et al.] 35

TBC1D32/Bromi mutations are associated with retinitis pigmentosa: a study combining clinical ophthalmology, iPS-based disease modeling and Xenopus functional approaches, Caroline Borday [et al.] 37

Using CRISPR/Cas9 in Xenopus tropicalis to investigate cis-regulatory element variants in developmental eye anomalies : identification of an Otx2-binding site in a novel putative enhancer of the mab21l2 gene, Munevver Burcu Cicekdal [et al.] 38

Xenopus: An in vivo model for studying skin response to UVB irradiation, Joudi El Mir [et al.] 40

A Progeroid Syndrome Caused by RAF1 deficiency Underscores the importance of RTK signaling for Human Development, Nathalie Escande-Beillard [et al.] . . 42

Ttc30a affects tubulin modifications in a Xenopus model for ciliary chondrodysplasia with polycystic kidney disease, Maike Getwan [et al.] 43

The ribosomal proteins Bop1 and Rpl5 affect Xenopus laevis development, Corinna Gärtner [et al.] 45

Xenbase: latest support for genomics and disease models., Christina James-Zorn [et al.] 46

Novel penetrant and short latency models for liposarcoma and wilms tumor using CRISPR multiplexing in Xenopus tropicalis, Dieter Tulkens [et al.] 47

Phenotyping embryonic development and disease using deep learning and mesoSPIM light-sheet microscopy, Thomas Naert [et al.] 48

Genome-wide transcriptomics analysis of genes regulated by GATA4, 5 and 6 during cardiomyogenesis, Boni Afouda 50

Otic Neurogenesis in Xenopus: Proliferation, Differentiation, and the Role of Eya1, Suad Almasoudi [et al.] 51

A testis ex vivo model to study and interfere with Xenopus laevis sperm epigenetic programming, Florian Berger [et al.] 52

Epigenetic contributions to Xenopus mucociliary development, Sarah Bowden [et al.] 53

A non-transcriptional function of Yap regulates the DNA replication program, Odile Bronchain [et al.] 54

Role of foxn1 in innate T cells driven immune tolerance of X. laevis tadpoles., Dionysia Dimitrakopoulou [et al.] 55

European Xenopus Resource Centre, modelling rare monogenic human disease in Xenopus: recent progress in the behavioural analysis of tadpole models of Neurodevelopmental disorders., Annie Godwin [et al.] 56

Hnf1b renal expression directed by a distal enhancer responsive to Pax8, Laura Goea [et al.] 57

The Rho GEF Trio is a major regulator of neural crest cell migration and dynamically localized at microtubules in cranial NC cells, Stefanie Gossen [et al.] . 58

TOMO-Seq technique for subcellular analysis of multiple types of cells (oocytes, tumor)., Viktoriia Iegorova [et al.] 59

Exonization by the emergence of a cleavage-polyadenylation site, Agnès Mereau [et al.] 60

Let's make a quantum leap: *Xenopus laevis* advantages to shed light on axonal circRNAs, Linda Masante [et al.] 61

CRISPR-CAS13 knockdown in Xenopus laevis, Sofia Moreira [et al.] 63

Temporal integration of Notch signaling in mucociliary epithelia cell fate specification, Magdalena Brislinger Engelhardt [et al.] 64

Novel insights into Myosin1d function during left-right axis determination in Xenopus laevis, Szilvia Csaba [et al.] 65

ENTPD5: a new player during kidney formation, Fanny Hardet [et al.] 66

The endocytic receptor Lrp2 orchestrates apical constriction and cell polarity to drive cranial neural tube closure, Josefine Hoeren [et al.] 67

R-spondin 2 as a BMP receptor 1A antagonist in the Spemann Organizer function to regulate Xenopus axial patterning, Hyeyoon Lee [et al.] 69

Exploration of pronephric precursor specification using single-cell transcriptomics, Jean-Francois Riou [et al.] 70

R-spondins are BMP receptor antagonists in Xenopus early embryonic development, Carina Seidl [et al.] 72

The endocytic receptor Lrp2 modulates canonical Wnt signaling during neural crest specification, Valentina Trivigno [et al.] 73

European Xenopus Resource Centre (EXRC): Our current research directions, Melek Ersin [et al.] 74

Role of the transmembrane protein TUSC5 in neural development, Santosh Kumar [et al.] 75

CRISPR/Cas Based Methods For Precise, Non-Mosaic DNA Integration in Xenopus, Sian Martin [et al.] 76

Functional E3 ubiquitin ligase Trim29 is required for the differentiation of the mucociliary epidermis in Xenopus, Herbert Neuhaus [et al.] 77

Transcriptional control of early nephrogenesis in Xenopus tropicalis, Paulina Ogar [et al.] 78

Skin depigmentation in Xenopus tropicalis: heavy metal toxicity and/or hypervitaminosis A+D?, Julia Traversari [et al.] 79

Cyst initiation and development in autosomal dominant polycystic kidney disease in the Xenopus model, Taiyo Yamamoto [et al.] 81

The European Xenopus Resource Centre V2.0?, Matt Guille [et al.] 82

Investigating EZH2 as a druggable mediator of immune cell exclusion in desmoid tumors, Marthe Boelens [et al.] 83

Modelling USH2A-associated retinal disease in Xenopus tropicalis to investigate the pathogenicity of human missense variants implicated in inherited blindness, Marjolein Carron [et al.] 85

Impact of glyphosate-based herbicide on early embryonic development of the amphibian Xenopus laevis, Hannah Flach [et al.] 87

Ptk7 is dynamically localized at NC cell-cell contact sites and interacts with the RhoGEF Trio, Katharina Till [et al.] 88

Using Xenopus laevis tadpoles to study basic principles underlying vertebrate motor control, Wen-Chang Li 89

List of participants

- Afouda Boni
- Amaya Enrique
- Audic Yann
- Aztekin Can
- Baudet Marie-Laure
- Becker Clara
- Beillard Nathalie
- Bellefroid Eric
- Bergen Max
- Berger Florian
- Boelens Marthe
- Borchers Annette
- Borday Caroline
- Bowden Sarah
- Brislinger Magdalena
- Bronchain Odile
- Chara Osvaldo
- Chesneau Albert
- Cicekdal Munevver Burcu
- Cioni Jean-Michel
- Csaba Szilvia
- Dimitrakopoulou Dionysia
- Dupré Aude
- Durand Beatrice
- El Mir Joudi

- Ersin Melek
- Feistel Kerstin
- Flach Hannah
- Gärtner Corinna
- Getwan Maike
- Godwin Annie
- Gossen Stefanie
- Guille Matt
- Haas Maximilian
- Hansen Mona
- Hernandez Puente Cinthia Violeta
- Hoeren Josefine
- Hollemann Thomas
- Hoppler Stefan
- Hormanseder Eva
- Iegorova Viktoriia
- James-Zorn Christina
- Jullien Jerome
- Kodjabachian Laurent
- Kühl Susanne
- Kühl Michael
- Kumar Santosh
- Kumar Vijay
- Lee Hyeyoon
- Leigh Nicholas
- Li Wenchang
- Lienkamp Soeren
- Locker Morgane
- Manzini Ivan
- Martin Sian
- Martin Sian
- Masante Linda

- Masse Karine
- Mayor Roberto
- Monsoro Burq Anne-Helene
- Moreira Sofia
- Naert Thomas
- Neuhaus Herbert
- Ogar Paulina
- Parain Karine
- Perron Muriel
- Qing Zhao
- Reversade Bruno
- Riou Jean-Francois
- Sachs Laurent
- Salonna Marika
- Sandoval-GuzmÃ¡n Tatiana
- Schlosser Gerhard
- Schweickert Axel
- Sedzinski Jakub
- Seidl Carina
- Softley Charlotte
- Straka Hans
- Sweeney Lora
- Tasca Alexia
- Till Katharina
- Tingler Melanie
- Traversari Julia
- Trivigno Valentina
- Tulkens Dieter
- Umbhauer Muriel
- Vleminckx Kris
- Walentek Peter
- Wheeler Grant

- Wielath Fee
- Wiest Benjamin
- Yamamoto Taiyo
- Zorn Aaron

Keynote lecture

Roberto Mayor, UK
Muriel Perron, FR
Elly Tanaka, AT

Organizers

Kris Vleminckx, BE
Eric Bellefroid, BE
Peter Walentek, DE
Muriel Perron, FR
Eva Hoermanseder,DE
Marie-Laure Baudet, IT
Yann Audic, FR
Soeren Lienkamp, CH

Confirmed Speakers

Can Aztekin, CH
Annette Borchers, DE
Osvaldo Chara, UK
Jean-Michel Cioni, IT
Aude Dupré, FR
Béatrice Durand, FR
Thomas Hollemenn, DE
Nicolas Leigh, SE
Ivan Manzini, DE
Anne-Hélène Monsoro-Burq, FR
Bruno Reversade,SG
Laurent Sachs, FR
Tatiana Sandoval Guzman, DE
Jakub Sedzinski, DK
Hans Straka, DE
Aaron Zorn, US

https://amphiclub2022.sciencesconf.org/

POSTER_NUMBER	TYPE	STATUT	TYPDOC	TOPIC	TITLE
1	ABSTRACT	Accepted	poster	POSTER_SESSION	A Progeroid Syndrome Caused by RAF1 deficiency Underscores the importance of RTK signaling for Human Development
2	ABSTRACT	Accepted	poster	POSTER_SESSION	A testis ex vivo model to study and interfere with Xenopus laevis sperm epigenetic programming
3	ABSTRACT	Accepted	poster	POSTER_SESSION	CP110, a surprising new regulator of cilium disassembly
4	ABSTRACT	Accepted	poster	POSTER_SESSION	CRISPR-CAS13 knockdown in Xenopus laevis
5	ABSTRACT	Accepted	poster	POSTER_SESSION	Cyst initiation and development in autosomal dominant polycystic kidney disease in the Xenopus model
6	ABSTRACT	Accepted	poster	POSTER_SESSION	CRISPR/Cas Based Methods For Precise, Non-Mosaic DNA Integration in Xenopus
7	ABSTRACT	Accepted	poster	POSTER_SESSION	Epigenetic contributions to Xenopus mucociliary development
8	ABSTRACT	Accepted	poster	POSTER_SESSION	European Xenopus Resource Centre (EXRC): Our current research directions
9	ABSTRACT	Accepted	poster	POSTER_SESSION	Exonization by the emergence of a cleavage-polyadenylation site
10	ABSTRACT	Accepted	poster	POSTER_SESSION	Exploration of pronephric precursor specification using single-cell transcriptomics
11	ABSTRACT	Accepted	poster	POSTER_SESSION	Functional E3 ubiquitin ligase Trim29 is required for the differentiation of the mucociliary epidermis in Xenopus
12	ABSTRACT	Accepted	poster	POSTER_SESSION	Genome-wide transcriptomics analysis of genes regulated by GATA4, 5 and 6 during cardiomyogenesis
13	ABSTRACT	Accepted	poster	POSTER_SESSION	Link between Müller cell proliferation and inflammation in Xenopus laevis upon retinal injury.
14	ABSTRACT	Accepted	poster	POSTER_SESSION	Novel insights into Myosin1d function during left-right axis determination in Xenopus laevis
15	ABSTRACT	Accepted	poster	POSTER_SESSION	R-spondins are BMP receptor antagonists in Xenopus early embryonic development
16	ABSTRACT	Accepted	poster	POSTER_SESSION	Role of the transmembrane protein TUSC5 in neural development
17	ABSTRACT	Accepted	poster	POSTER_SESSION	Skin depigmentation in Xenopus tropicalis: heavy metal toxicity and/or hypervitaminosis A+D?
18	ABSTRACT	Accepted	poster	POSTER_SESSION	The endocytic receptor Lrp2 modulates canonical Wnt signaling during neural crest specification
19	ABSTRACT	Accepted	poster	POSTER_SESSION	The endocytic receptor Lrp2 orchestrates apical constriction and cell polarity to drive cranial neural tube closure
20	ABSTRACT	Accepted	poster	POSTER_SESSION	The tadpole foregut as new model to study mucociliary epithelia in Xenopus
21	ABSTRACT	Accepted	poster	POSTER_SESSION	Timing of swim and limb motor neuron generation in the Xenopus spinal cord
22	ABSTRACT	Accepted	poster	POSTER_SESSION	TOMO-Seq technic for subcellular analysis of multiple types of cells (oocytes, tumor)
23	ABSTRACT	Accepted	poster	POSTER_SESSION	Tra2b – a new paradigm in coordinated post-transcriptional control of ciliogenesis
24	ABSTRACT	Accepted	poster	POSTER_SESSION	Transcriptional control of early nephrogenesis in Xenopus tropicalis
25	ABSTRACT	Accepted	poster	POSTER_SESSION	Ttc30a affects tubulin modifications in a Xenopus model for ciliary chondrodysplasia with polycystic kidney disease
26	ABSTRACT	Accepted	poster	POSTER_SESSION	Xenbase: latest support for genomics and disease models.
27	ABSTRACT	Accepted	poster	Fast Talks + poster	Impact of glyphosate-based herbicide on early embryonic development of the amphibian Xenopus laevis
28	ABSTRACT	Accepted	poster	Fast Talks + poster	Investigating EZH2 as a druggable mediator of immune cell exclusion in desmoid tumors
29	ABSTRACT	Accepted	poster	Fast Talks + poster	Modelling USH2A-associated retinal disease in Xenopus tropicalis to investigate the pathogenicity of human missense variants implicated in inherited blindness
30	ABSTRACT	Accepted	poster	Fast Talks + poster	Ptk7 is dynamically localized at NC cell-cell contact sites and interacts with the RhoGEF Trio
32	ABSTRACT	Accepted	poster	Fast Talks + poster	Using Xenopus laevis tadpoles to study basic principles underlying vertebrate motor control