1-3 JULY 2024 - BORDEAUX, France

Keynotes

Jean Secondi
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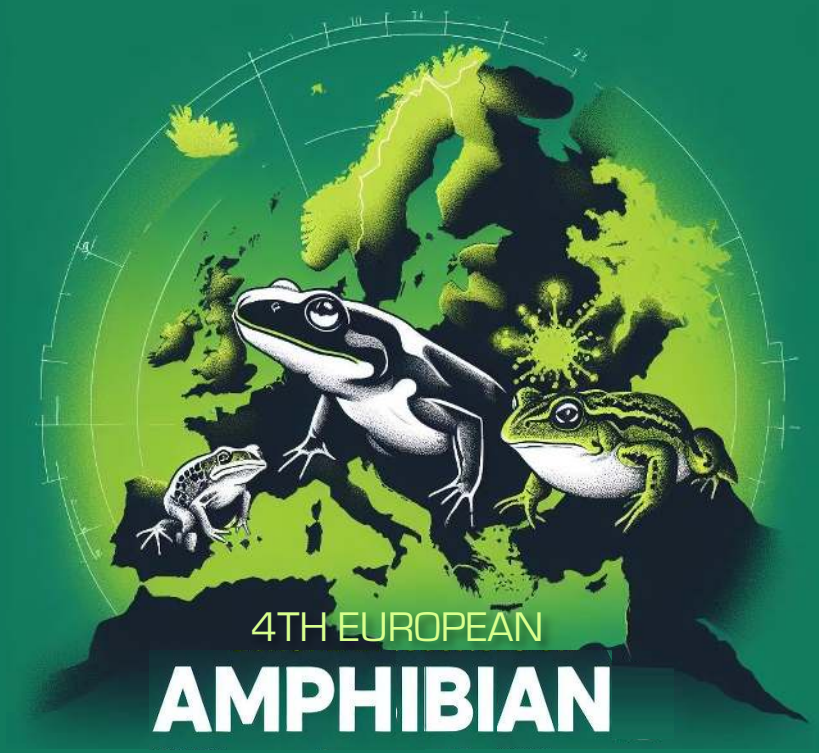

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Clotilde Cadart (France)
David Dupasquier (France)
Aude Dupré (France)
Karen Echeverri (USA)
Kristian Franze (UK)
Romain Gibeaux (France)
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Eva Hormanseder (Germany)
François Lambert (France)
Soeren Lienkamp (Switzerland)
Kathrin Marheineke (France)
Roberto Mayor (UK)
Anne-Hélène Monsoro-Burq (France)
Leon Peshkin (USA)
Jacques Robert (USA)
Aida Rodriguez (Spain)
Gerhard Schlosser (Ireland)
Grant Wheeler (UK)
Sarah Woolner (UK)
Aaron Zorn (USA)

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Introduction into mucociliary biology in Xenopus

Laurent Kodjabachian * [1], Peter Walentek *

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*Speaker

SUV4-20H1/KMT5b regulates Xenopus multiciliogenesis

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Histone post-translational modifications (PTM) greatly influence gene expression and are widely considered to regulate progression through development. However, the function of some PTMs remains elusive. H4K20 is sequentially methylated in concert with the cell cycle. In proliferating cells, SET8/KTM5A writes the monomethyl mark in G2/M phase, which then is converted to the di- and trimethylated states by SUV4-20H1/H2 (KMT5B/KMT5C) methyltransferases in the next G1 and S phase. In quiescent, differentiated cells, H4K20me2 represents the most abundant histone modification present in vertebrate chromatin. To address the function of H4K20 methyl states in development, we blocked the deposition of H4K20me2 and H4K20me3 by depleting the SUV4-20H1/2 enzymes in Xenopus embryos. This results in a severe ciliogenic defect in multiciliated cells (MCCs), as well as the repression of hundreds of cytoskeleton and cilium related genes. Further, we demonstrate that this defect can be rescued by wildtype, but not catalytically inactive SUV4-20H1, as well as by overexpressing PHF8, an H4K20me1-specific histone demethylase. Taken together, this indicates that SUV4-20H1 plays a critical role in multiciliogenesis.

*Speaker

A workflow to observe single-cell morphogenetic features of developing Xenopus mucociliary epidermis

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As the developing embryo forms, the simple mass of cells becomes more and more complex throughout the morphogenetic shaping of tissues. Morphogenetic processes display an astounding level of tissue self-organization, where an initially unorganized mass of cells rearranges to form a functional tissue. One example of such process is the formation of regularly patterned, multilayered tissue, such as mucociliary epithelia (MCE); however, the formation of this tissue from initially pluripotent cells remains uncharacterized.
The morphogenetic shaping and cell fate choices in the MCE takes place both collectively and individually. Cells exhibit collective movement, but single cells in in the deep cell layer can also migrate individually and remodel their environment. To resolve the morphogenetic behaviors across time and across the scale of individualism-collectivity, we quantify morphological and kinetic phenotypes of single cells in the embryonic frog (Xenopus laevis) MCE. Using explanted prospective MCE, we can image and quantify developmental dynamics in single-cell resolution.

To achieve this, we have developed a state of the art quantitative imaging pipeline to track cell dynamics in the bottom layer of developing Xenopus epidermal explants. The detailed backtracking of the cell's histories allows us to connect individual cell features to formation of collective behaviors that arise in the tissue over developmental time.
By assaying the embryonic epidermis, we aim to provide an unprecedented detailed view of the developmental dynamics of a mucociliary epithelium. Understanding these fundamentals of mucociliary differentiation could provide a better understanding of pathological conditions arising from defective development of airway epithelia.

Keywords: Xenopus, mucociliary epithelium, image analysis, quantitative biology

*Speaker

Xenopus as a model for the ultra-rare condition, Alström Syndrome

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Alström Syndrome (AS) is an ultra-rare caused by mutations in Alms1 that leads to systemic symptoms. It is caused by alteration to cilia development and function and leads to progressive sight and hearing loss, renal and cardiac conditions and metabolic and developmental differences, among others. This huge range of symptoms and patient phenotypes, along with its rarity, makes it challenging to study patterns in the progression of the condition.

I am developing a Xenopus laevis model for this ciliopathy to study both the intracellular mechanisms underpinning the condition and its progression across time and developmental stages.

Morpholino knockdown of Alms1 in Xenopus leads to reduced multiciliation and fluid flow in the epithelium. Alms1 knockdown embryos also showed morphological changes and changes to cilia-dependent signalling in the neural tube. This indicates that Alms1 in Xenopus is analogous to human Alms1 and could therefore be used as a model of AS.

I use a CRISPR/Cas9 approach to knockout Alms1 in Xenopus laevis embryos, targeting both alloalleles. Initial phenotypic studies in tadpoles have shown promise; I am now aiming to set up a genetic line for this condition in collaboration with the European Xenopus Resource Centre for further work and future screening.

The large clutch sizes of Xenopus and simple animal husbandry allows large sample sizes for studying phenotypes and their combinations in vivo. This will allow us to answer the question that is pressing for many people living with the condition – how does AS typically progress and what patterns might there be that the small patient sample size has so far kept hidden?

*Speaker

Deciphering Deuterosome composition to understand sizing of ciliary function in MCCs

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Cells bearing multiple motile cilia are found in a wide variety of metazoa, from marine invertebrates to humans. An early step of multiciliated cell (MCC) formation is the mass production of centrioles, which after conversion into basal bodies serve as anchoring units for cilia. The construction of a precise number of cilia on the surface of MCCs is essential for the emergence of physiological ciliary function. The production of multiple centrioles is a key step in MCC differentiation that is essential to understand the control of cilia number. Most centrioles in normal MCCs are produced from specialized organelles called deuterosomes. More than 40 years after its initial description, and despite recent advances, the deuterosome remains a relatively unknown organelle. In particular, little is known about its composition. Using a new model of multiciliated cells culture, we isolated deuterosomes and analyzed their protein composition. I will illustrate how these results allow us to progress in the understanding of the mechanisms controlling the number of centrioles in MCCs.

Keywords: Multiciliated cells, deuterosome, centrioles, proteomics, advanced microscopy

*Speaker

R-Spondin 2 governs Xenopus left-right body axis formation by establishing an FGF signaling gradient

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Establishment of the left-right (LR, sinistral, dextral) body axis in many vertebrate embryos relies on cilia-driven leftward fluid flow within an LR organizer (LRO). A cardinal question is how leftward flow triggers symmetry breakage. The chemosensation model posits that ciliary flow enriches a signaling molecule on the left side of the LRO that promotes sinistral cell fate. However, the nature of this sinistralizing signal has remained elusive. In the *Xenopus* LRO, we identified the stem cell growth factor R-Spondin 2 (Rspo2) as a symmetrically expressed, sinistralizing signal. As predicted for a flow-mediated signal, Rspo2 operates downstream of leftward flow but upstream of the asymmetrically expressed gene *dand5*. Unexpectedly, in LR patterning, Rspo2 acts as an FGF receptor antagonist: Rspo2 via its TSP1 domain binds Fgfr4 and promotes its membrane clearance by Znrf3-mediated endocytosis. Concordantly, we find that at flow-stage, FGF signaling is dextralizing and forms a gradient across the LRO, high on the dextral- and low on the sinistral side. Rspo2 gain- and loss-of function equalize this FGF signaling gradient and sinistralize and dextralize development, respectively. We propose that leftward flow of Rspo2 produces an FGF signaling gradient that governs LR-symmetry breakage.

*Speaker

The Xenopus tadpole as a model for mucin biology

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Mucus is vital for the protection of tissues in the human body. The principal structural components of mucus are the gel-forming mucins, which are large, polymeric glycoproteins that form a hydrated 'mesh' network with other proteins. Defects in mucin structure, secretion and hydration are central to diseases such as cystic fibrosis and ulcerative colitis, but since most mucosal surfaces in mammals are found in internal cavities, studying mucins live, in their native environment, is challenging and requires invasive techniques.
The *Xenopus tropicalis* tadpole skin is a mucociliary epithelium, composed of multiciliated cells, ionocytes and secretory cell types, and with broad similarities to the human airway. We have used the tadpole skin to study aspects of mucin biology: most recently, we have characterised a role for the chloride channel Tmem16a, a therapeutic target for cystic fibrosis, in the secretion and maturation of the MucXS mucin secreted from this cell layer. Our current work hints at a role for Tmem16a in the differentiation of cell types in the tadpole epidermis.

Mucus is also employed throughout the animal kingdom for functions like defence, locomotion and adhesion. The Xenopus cement gland, at the anterior of the tadpole, secretes a sticky substance that the tadpoles use to anchor to surfaces, including the oxygenated water surface. Our research shows that the gland comprises elongated cells packed full of electron-dense vesicles. It expresses canonical secretory cell markers, including those associated with differentiation (*spdef*), mucin production (*ire1-beta*), mucin processing (*agr1-3*) and mucin secretion (*vamp8*). By mass spectrometry, we have identified a cement gland mucin, Muc5j, that forms the large, heavily-glycosylated polymers typical of mucins. By morpholino knockdown, we have found that Muc5j accounts for most of the adhesiveness of material secreted from the cement gland; this is, to our knowledge, the first time a mucin has been identified as the "cement" in an aquatic cement gland.
Despite being secreted from the cement gland like a gel-forming mucin, Muc5j (unlike the epidermal gel-forming mucin) readily forms strands under tensile stress, and this atypical mucus conformation resembles strands secreted by human airway submucosal glands that can sweep large debris over the mucus gel surface. Unlike the epidermal gel mucin, cement gland strands stain prolifically with multiple lectins, indicating a rich and diverse sugar modification profile. Knockdown of a key O-glycosylation enzyme disrupts strand formation, prompting thicker, more elastic strands that appear to lack typical packing density, hinting at a role for O-glycosylation and these sugar side groups in the gross structural properties of mucins.

*Speaker

Calcium signals shape the apical actin network of multiciliated cells through myosin and RhoA

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Motile cilia perform crucial physiological functions in the airways, the central nervous system, reproduction and embryonic development. They are anchored by a specialized apical actin network, which is embedded into the plasma membrane and forms microridge-like structures on the surface of multiciliated cells. Here, we study the role of calcium signaling in the formation of these structures. We identify stochastic bursts in intracellular calcium concentration of developing multiciliated cells. Using Optogenetics to manipulate calcium, we find that calcium bursts promote coherence and growth of the developing actin network, dependent at least partially on non-muscle myosin. Inhibition of an endogenous calcium signaling pathway disrupts the formation of apical actin/microridge-like structures by reducing local RhoA signaling. Our findings shed light on the mechanisms driving the self-organization of a highly specialized cytoskeleton.

Keywords: Calcium, Cilia, Cytoskeleton, Actin

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A decade of CRISPR-mediated disease modeling in Xenopus tropicalis, a time to reflect and look forward

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*Speaker

Transcriptional control of early nephrogenesis

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The kidneys of all vertebrates originate from the intermediate mesoderm. In *Xenopus*, tissue condensation leads to mesenchymal to epithelial transformation and subsequent development of the pronephric tubule. However, nephrogenic tissue is already specified, and nephrogenic factors are detectable at neurula stages prior to tissue condensation. Mutations in early transcriptional regulators are frequently the cause of genetic kidney diseases. Here, we investigated the interplay and transcriptional regulatory network of three prominent factors with an early role in nephrogenesis: Pax8, Hnf1b and Sall1. We chose to employ a combination of CRISPR/Cas9 targeting and synchronized single-embryo transcriptomics to determine differential gene expression and infer a gene regulatory network of target genes. Intersection with transcriptomes of microdissected tubules at three stages identified kidney enriched genes not previously associated with renal development or congenital urogenital malformations. In situ hybridization confirmed expression in or in proximity to the nephrogenic zone of identified transcripts. Our findings demonstrate that a single-embryo sequencing approach can successfully detect tissue specific transcriptional targets and substantially expands the list of genes involved in early kidney formation. Novel nephrogenic genes may also be involved in congenital anomalies of the kidney and urinary tract (CAKUT).

*Speaker

Getting your eye in shape: A role for miR-204-1 in eye development

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MicroRNAs (miRNAs) are short RNA molecules involved in the regulation of gene expression. In our lab, we study miRNAs that are involved in neural crest development. To do so, we use Xenopus tropicalis. We have developed a novel method using CRISPR/Cas9 gene editing to knockdown miRNAs involved in development. In this talk I will present our work on miR-204-1 (homolog of human miR-211). We show, using knockdown and recue experiments that miR-204-1 is involved in the etiopathogenesis of coloboma and microphthalmia, which are developmental defects of the eye.

*Speaker

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

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[2] Max-Delbruück-Center for Molecular Medicine in the Helmholtz Association (MCD) – Germany

Neural tube closure defects (NTDs) are developmental malformations occurring upon impairment of morphogenetic movements during neurulation. While caudal neurulation, forming the spinal cord, is dominated by planar cell polarity (PCP)-mediated convergent extension (CE), apical constriction (AC) is essential for neural tube closure in the cranial region, which gives rise to the brain. Pathogenic variants of LRP2 have been identified in humans with NTDs. Patients carrying autosomal-recessive mutations in LRP2 develop Donnai-Barrow-Syndrome which includes severe forebrain defects. Modelling of lrp2-related NTDs in *Xenopus* and mouse showed that Lrp2 was essential for cranial neural tube closure. While loss of Lrp2 delayed or entirely inhibited neurulation, editing of Lrp2's C-terminal PDZ-binding domain (PBD) strikingly accelerated neural tube closure. We found that Lrp2 controlled AC - and that velocity and spatial distribution of AC determined the correct timing of cranial neural tube closure. In addition, Lrp2 connected AC to cell polarity. Despite the absence of PCP-driven CE in the cranial region, cells expressed the core PCP protein Vangl2 and acquired planar polarity during cranial neurulation. Lrp2 regulated the dynamic subcellular localization of Vangl2. Our data suggest that interaction of the Lrp2- / Vangl2-PBDs via PDZ-containing proteins is key to orchestrate the occurrence and mode of AC. Thus, Lrp2-mediated endocytosis emerges as a major regulator of morphogenetic movements essential for cranial neural tube closure, shedding new light on NTD etiology.

Keywords: neural tube closure, apical constriction, Xenopus, PCP

*Speaker

An Investigation into the Effect of Different Strain Rates on the Mechanical and Geometric Properties of Xenopus laevis Epithelia and the Role of Membrane Tension as a Mechanism in Mechnaotransduction

Iona Norwood * [1], Irwin Phanada† [1], Emma Johns [1], Joseph Hetmanski [1], Patrick Caswell [1], Oliver Jensen [2], Sarah Woolner‡ [1]

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Cells within our tissues are constantly subjected to internal and external mechanical forces and must detect and respond to these forces appropriately. Responses to mechanical force result in physical, geometric, and behavioural changes – such as changes in cell division rate and orientation. These mechano-responses play a key role in maintaining tissue homeostasis, morphogenesis, and the prognosis of many common diseases, such as cancer.
Most experiments looking into the effects of force on cell properties to date have only used instant applications of stretch in single cells or monolayers, whereas slower rates of stretch in 3D tissues may be more representative of the forces experienced during development or disease. Furthermore, the exact method by which cells can detect forces is not fully understood. One potential mechanism for this is through detecting changes in membrane tension which has been shown to be involved in processes such as cell fate determination and cell migration.

Therefore, we aimed to characterise the dynamic response of cells within *Xenopus laevis* epithelia to different rates of external stretch application and determine the role of membrane tension in the detection of different rates of stretch.

Using a vertex-based model, we can highlight how strain rate affects cell properties and behaviour in 3D tissues during development. Our preliminary results indicate that the rate at which a tissue experiences stretch can have an impact on the physical properties of cells within a tissue, such as compression and elongation. Furthermore, there was a significant increase in the number of cells undergoing mitosis in tissues 20 minutes after the application of fast stretch. However, this was not seen in the slow stretch conditions implying that the rate at which a stretch is applied is important in determining the downstream response of cells in tissues.
We also investigated the role that direct detection of changes in membrane tension may play in regulating the mechano-response. We developed protocols using the fluorescent membrane tension probe, FLIPPER-TR, and a probe for membrane proximal actin to measure membrane tension during cell division *in vivo*. We aim to use these techniques to measure changes in membrane tension in *Xenopus laevis* epithelia under stretch to investigate the molecular mechanisms underlying the response to varying stretch regimes.

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Keywords: developmental biology, mechanobiology, Xenopus laevis, Flipper, TR, membrane tension, membrane tension sensors, epithelea, early embryo, morphogenesis, cell division, mitosis, morphogenesis, strain rate

Drp1, a regulator of mitochondrial fission, affects Xenopus tail regeneration

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Mitochondria are highly plastic and dynamic organelles that serve as the "powerhouse" of the cell by producing energy in form of ATP. However, mitochondria also trigger cell growth, apoptosis, produce reactive oxygen species and regulate calcium homeostasis. In regenerative tissue such as skeletal muscles, mitochondria fuse to form a mitochondrial network. However, to maintain a healthy network, mitochondria not only undergo fusion, but also fission. An imbalance in fusion and fission of mitochondria results in a decrease of muscle mass and negatively affects muscle health. Dynamin-Related Protein 1 (Drp1) is a cytoplasmic GTPase that controls mitochondrial fission. Here, we have investigated the role of Drp1 during muscle and neuronal regeneration after tail amputation. We found that Drp1 is upregulated after tail amputation. Morpholino-mediated down-regulation of Drp1 slows down tail regeneration. In addition, the transcription of muscle and neuronal stem cell markers are reduced. Our findings suggest that mitochondrial fission is important for cell proliferation and stem cell formation.

Keywords: mitochondria, fission, Drp1, regeneration

*Speaker

Mechanics of gastrulation: The cellular and biophysical basis of mesoderm intercalation.

Francois Fagotto * [1], Artur Ruppel *

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Xenopus represents the basal archetype of vertebrate gastrulation. It involves a series of concurrent and coordinated collective tissue movements, which have been described in great detail. However, while we have good knowledge of the upstream gene regulatory network that determines the various regions of the gastrulating embryo, the cellular mechanisms responsible for their morphogenetic properties remain poorly understood. Our team focuses on what controls the active flow of the involuting mesoderm. This crucially relies on cells dynamically exchanging contacts with neighbours (intercalation). Importantly, rearrangement of this mesenchymal-like tissue does not rely on standard intercalations through "classical" T1 transition, but on a distinct type of intercalation based on differential migration. To get to the core of this process, we combine embryo/tissue analysis with minimalistic in vitro approaches. We find 1) that tissue dynamics are stimulated by controlled repression of actomyosin contractility; 2) that cell-cell contact remodelling involves fast reversible cadherin disengagement through a mechanism of peeling and lateral diffusion. 3) Furthermore, we have succeeded at reconstituting intercalation in a minimal system of cell quadruplets, allowing the first direct measurement of force dynamics through TFM. Altogether, our approach unveils unique characteristics of mesoderm dynamics, which are markedly different from the standard epithelial models.

Keywords: Morphogenesis, collective cell migration, cell biology, biophysics

*Speaker

(Phospho)-proteomics of oocyte meiotic division

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Oocyte meiotic divisions represent a critical process in sexual reproduction, as diploid non-dividing oocyte transforms into a haploid fertilizable egg, preparing embryonic divisions and differentiation. Although cell differentiation and proliferation are governed by transcription, oocyte maturation and early embryonic divisions depend entirely on changes in protein abundance and post-translational modifications. Here, we analyze the abundance and phosphorylation of proteins during *Xenopus* oocyte meiotic maturation. We reveal significant shifts in protein stability, related to spindle assembly, DNA replication and RNA-binding. Our analysis pinpoints broad changes in phosphorylation correlating with key cytological meiotic milestones, noteworthy changes in membrane trafficking, nuclear envelope disassembly and modifications in microtubule dynamics. Additionally, specific phosphorylation events target regulators of protein translation, Cdk1 and the Mos/MAPK pathway, providing insights into the dynamics of Cdk1 activity related to the meiotic cell cycle. This study sheds light on the orchestration of protein dynamics and phosphorylation events during oocyte meiotic divisions, providing a rich resource for understanding the molecular pathways orchestrating meiotic progression in the frog, and most likely applicable to other vertebrate species.

*Speaker

Wnt signalling-controlled Gene Regulatory Networks in early Xenopus embryonic development.

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The early embryo of Xenopus is an ideal experimental model system for studying how Wnt signalling regulates context-specific gene expression and subsequent cell differentiation. This is because uniquely there is a dramatic shift from maternal Wnt signal transduction regulating early expression of genes that promote dorsal development, to then zygotic Wnt8 signalling regulating genes that promote almost the opposite, ventral mesoderm development. Both are mediated by the so-called canonical Wnt pathway, involving translocation of β-catenin protein into the nucleus to regulate expression of direct target genes by binding to DNA-binding proteins, principally of the TCF/LEF family.
Combining RNA-seq and β-catenin ChIP-seq analysis, our investigation unearths not two but more like five different classes of direct Wnt target genes, which highlights important roles for co-regulation with other developmental signalling pathways (such as nodal, FGF, BMP) but also identifies feed-forward regulation, blurring any clear distinction between direct versus indirect target gene.

Mathematical modelling of these Gene Regulatory Networks controlled by canonical Wnt signalling during early Xenopus embryonic development helps test and largely validate the logic of our understanding. Our modelling additionally highlightes the importance of epigenetic accessibility to direct Wnt target gene loci, and it identifies remaining gaps in our understanding.
We are now re-examining any role for the TCF/LEF transcription factors and particularly for alternatively spliced isoforms of TCF7 in context-specific regulation of these direct Wnt target genes during early Xenopus embryonic development.

*Speaker

Prdm12 in nociceptor specification and nociception: what the mouse and frog have told us

Simon Desiderio [1], Simon Vermeiren [1], San Jose Sabaté [1], Aurore Latragna [1], Aurore Thélie [1], Claude Van Campenhout [1], Pauline Cabochette [1], Maya Dannawi [1], Eric Bellefroid * [1]

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Nociceptors, the pain sensing neuron, constitute an attractive therapeutic target as their sensitization makes an important contribution to many chronic pain states. Better understanding how nociceptors function is critical for the development of new pain therapies. Our work over the past few years has identified the epigenetic regulator PRDM12, mutated in humans affected by congenital insensitivity to pain, as essential in the mouse for the development and proper functioning of nociceptors.
I will review what we have learned about Prdm12 function in developing and mature nociceptive neurons. I will then describe our ongoing efforts to approach its mechanism of action with the hope to develop novel nociceptor selective strategies to treat pain.

*Speaker

A retinoic acid-RFX6-Wnt regulatory network patterns the developing digestive system: implications for understanding Mitchell-Riley syndrome.

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Many different signaling pathways and transcription factors (TFs) are implicated in digestive system development, but how these are integrated into networks coordinating organogenesis and how disruptions to those networks can cause congenital syndromes is poorly understood. Using human pluripotent stem cell cultures and Xenopus embryos, we uncovered a conserved transcriptional program downstream of retinoic acid (RA) crucial for embryonic gut tube patterning. We found that RA directly activated expression of the TF *RFX6* in the posterior foregut, where it accounts for much of RA-mediated patterning. Disruption of either RA signaling or Rfx6 function in Xenopus leads to reduced foregut-midgut identity and an expansion of intestinal and pharyngeal fates, similar to findings in mouse mutants and Mitchell-Riley Syndrome patients with *RFX6* mutations. We show that Rfx6 directly regulates the transcription of foregut TFs (Pdx1 and Onecut1) and secreted Wnt-antagonists (Sfrp2/5), crucial for anterior-posterior patterning, while suppressing hindgut (Cdx2) and pharyngeal (Nkx2-3/5/6) TFs as well as Bmp ligands that promote pharyngeal fate. These results integrate RA, Wnt, and Bmp pathways into a network with multiple lineage-specific TFs, providing a mechanistic understanding of Mitchell-Riley Syndrome.

*Speaker

CRISPR/Cas9-mediated disruption of an evolutionarily conserved enhancer in the mab21l2 locus induces disease related anomalies in eye morphogenesis in Xenopus tropicalis

Munevver Burcu Cicekdal * [1,2], Fabiola Ceroni [3,4], Richard Holt [5], Elena Sorokina [6], Thomas Naert [1,7], Soeren Lienkamp [7], Elena Semina [6], Nicola Ragge [3,8], Elfride De Baere [2], Kris Vleminckx† [1]

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Anophthalmia, microphthalmia, and coloboma **(AMC)** are developmental eye disorders that account for approximately 20% of childhood visual impairment. Despite growing recognition of the role of non-coding regulatory regions in disease, their impact on gene function and phenotype remains challenging to characterize. Specifically, the contribution of variants in these regions to AMC phenotypes is not well understood. In this study, we aimed to characterize a putative enhancer of the *MAB21L2* gene, located within a 113.5kb non-coding homozygous deletion identified in a proband presenting with anophthalmia, micrognathia, and microcephaly. A putative enhancer **(CRE14)** within the corresponding 39kb region in the *Xenopus tropicalis* genome, containing a conserved binding site for transcription factor OTX2 (involved in eye development), was identified using a genome-wide multi-omics dataset. The binding of OTX2 to the CRE14 enhancer was confirmed by ChIP-seq in mouse embryonic stem cells. During early Xenopus development, CRE14 exhibited epigenetic marks of a poised enhancer at the mid-gastrula stage (NF-St.10.5) and an active enhancer at the early-neurula stage (NF-St12.5), coinciding with the initiation of *mab21l2* expression and eye specification. CRISPR-mediated disruption of the OTX2 binding site (CRE14-crispants) and replication of the proband's deletion (del-crispants) resulted in reduced *mab21l2* transcript levels and structural eye defects, predominantly ocular coloboma. Furthermore, *in situ* hybridization revealed that disruption of the OTX2 binding site in the CRE14 enhancer led to the loss of *mab21l2* expression in the lens placode and midbrain. Comprehensive 2D and 3D phenotyping using *in toto* light-sheet microscopy and deep neural network-assisted image reconstruction revealed a significant decrease in both the volume and sphericity of the retina in CRE14-crispants compared to controls.

*Speaker
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This study presents the first CRISPR/Cas9-mediated *Xenopus tropicalis* eye disease model targeting a specific transcription factor binding site in a non-coding cis-regulatory element. Additionally, we applied advanced phenotyping techniques using light-sheet imaging and deep neural network-based anatomical reconstruction to sensitively and accurately characterize eye defects.

Mechanical regulation of cell division orientation: investigating the role of nuclear mitotic apparatus protein

Nawseen Tarannum * [1], Dionn Hargreaves [1], Oliver Jensen [1], Sarah Woolner [1]

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Cells within an organism constantly experience a variety of mechanical forces from their surrounding tissue environment. Reading and responding to these forces is crucial to shape and maintain tissues, with errors in this process contributing to failures in embryogenesis and diseases such as cancer. One of the cellular functions regulated by mechanical force is cell division orientation, which is determined by the position of the mitotic spindle. For example, stretching a tissue leads the majority of divisions to align along the axis of stretch. For external forces to regulate division orientation, they must be sensed and relayed to the mitotic spindle, and spindle-associated cortical proteins are likely key to this process. One such candidate, nuclear mitotic apparatus protein (NuMA) has been implicated in orienting the spindle according to force although the mechanistic details remain uncharacterised, especially in a tissue context. Therefore, we utilised the *Xenopus laevis* embryonic animal cap tissue to which reproducible tensile forces can be applied externally, to understand the role of NuMA in mechanosensitive spindle orientation. Using GFP-tagged NuMA, we show that cortical localisation of GFP-NuMA is dynamic and sensitive to mechanical stretch, with recruitment to the polar cortex earlier during mitosis in stretched tissues. Furthermore, we use mathematical modelling of spindle movements to show that amplifying microtubule-pulling at sites of experimentally observed cortical NuMA, as opposed to other pulling regimes, moves the spindle to an orientation that most closely matches experimental data. Using morpholino-targeted knockdown of endogenous *Xenopus* NuMA in early embryos, we also show that a reduction in NuMA levels disrupts the ability of cells to orient divisions along the axis of mechanical stretch and cell shape. Interestingly, our data suggest that mechanosensitive spindle orientation through NuMA is an effect of direct force sensing rather than sensing changes in cell shape. Furthermore, by comparing two different tissue stretch regimes, we demonstrate that NuMA responds specifically to anisotropic tension to orient cell divisions in stretched *Xenopus* tissues. Overall, with a combination of live tissue imaging and mathematical modelling, our results indicate that NuMA is vital to orient a mitotic spindle according to external force.

Keywords: cell division orientation, forces, NuMA, mitosis, spindle orientation

*Speaker

Polyploidy in Xenopus lowers metabolic rate by decreasing total cell surface area

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Although polyploidization is frequent in development, cancer, and evolution, impacts on animal metabolism are poorly understood.
We generated triploid *Xenopus laevis* embryos and showed that triploid tadpoles are made of fewer, larger cells than diploids and consume oxygen at a lower rate. To understand the underlying basis of such decrease, we developed a mathematical framework to quantify the energy budget of tadpoles and combined it with quantitative measurements of the energy allocated to proliferation, growth, and maintenance. We show that treatments altering biosynthesis pathways and plasma membrane ionic pumps such as the Na+/K+ ATPase abolish the metabolic difference across ploidies. We propose that the increase in cell size in triploids causes a decrease in total cell surface area and a reduction of costs associated with production and activity at the plasma membrane which explains the overall lower metabolic rate. Crucially, comparison of three *Xenopus* species that evolved through polyploidization reveals that metabolic differences emerge in development only once cell size scales with genome size. Thus, cell size increase, not ploidy or genome size, causes the reduction in embryo metabolic rate.
Future work will further investigate the connection between energy expenditure, ploidy, and organogenesis.

*Speaker

Using Xenopus to unpick how mechanical force regulates cell division in complex tissue environments

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Living tissues are constantly being pushed and pulled and must sense and respond to these mechanical forces appropriately, for example by modifying patterns of cell division in a co-ordinated fashion. Whilst we are beginning to understand the cellular mechanisms that link cell behaviour and force in single cells, a major gap in our knowledge is understanding how mechanical force is transmitted and sensed across complex tissues. Bridging this gap is particularly important considering that many common diseases, such as cancer, alter the mechanical properties of our tissues. Using Xenopus animal caps and embryos, alongside a combination of biological and mathematical approaches, we investigate how cell division is regulated by mechanical force in complex tissue environments. We have developed methods to stretch and image animal cap explants and combined these with new mathematical models for inferring mechanical stress across a tissue. In my talk, I will describe how we have used these approaches to uncouple the effects of mechanical stress and cell shape on cell division regulation and reveal how the speed of tissue-stretch impacts division responses. Moreover, I will describe work using Xenopus embryos as a model for early-stage cancer, where we find that oncogenic cells pull on their wild type neighbours and coerce them to divide aberrantly, contributing to tumour growth.

*Speaker

XPC-Deficient Xenopus Embryos: An In Vivo Model System for Studying the Mechanisms Underlying Pigmentary Abnormalities in XPC Patients

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Xeroderma pigmentosum (XP) is a rare autosomal recessive disorder characterized by defective DNA repair mechanisms. Patients with XP exhibit clinical features such as photosensitivity, actinic skin damage, and an increased risk of skin cancers. Early clinical signs of XP often include changes in skin pigmentation and xerosis, as reflected in the name of the disease. Despite the importance of these pigmentary abnormalities, research in this area has been limited due to the lack of appropriate experimental models. This project seeks to model pigmentary abnormalities in XP disorders, specifically focusing on XP-C patients.
We chose the amphibian *Xenopus laevis* as a potential *in vivo* model due to its evolutionary proximity to human and the ease of studying melanocyte development thanks to its transparency, making it a suitable alternative to the mouse model. Initially, we evaluated whether *Xenopus laevis* could effectively model the impact of UVB radiation on skin and melanocyte physiology. Subsequent experiments aimed to replicate the pigmentary abnormalities observed in XP-C patients using *Xenopus laevis*.

Our studies demonstrated that exposure of *Xenopus* embryos to UVB irradiation activated the DNA damage response (DDR) network, including induction of DNA repair systems, apoptosis, epidermal thickening, and increased melanocyte dendricity. These responses mirrored the skin reactions observed in human skin following UVB exposure, validating *Xenopus* as a suitable model for studying the effects of UVB on skin physiology.

By the use of antisense oligonucleotide morpholinos, we successfully downregulated *xpc* expression in *Xenopus* embryos. Knocking down *xpc* resulted in developmental abnormalities and pigmentary disturbances, closely resembling the clinical manifestations of XP-C patients. These findings underscored the model's potential to elucidate the cellular and molecular mechanisms contributing to pigmentary abnormalities in XP-C patients. Proteomic analysis of xpc morphant embryos provided further insights into the molecular mechanisms underlying these abnormalities.
Altogether, our findings highlight the sensitivity of *Xenopus* embryos to UVB irradiation and establish them as a novel model for investigating the DNA damage response and the interaction between melanocytes and keratinocytes. The ability to observe and experiment on large numbers of synchronously developing embryos further supports *Xenopus* as a valuable *in vivo* model for studying pigmentary abnormalities in XP-C patients.

*Speaker
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Keywords: Ultraviolet B, Cyclobutane pyrimidine dimers, Xenopus laevis, Xeroderma pigmentosum C, XPC, Pigmentation

Deciphering the Mechanisms Behind the Immunologically Cold Phenotype of Wnt-Positive Desmoid Tumors

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With the advent of CRISPR/Cas9 technology, *Xenopus tropicalis* is emerging as a powerful model for studying human cancers. Targeted injections of multiple sgRNAs and NGS mediated genotyping of the emerging tumors in the F0 mosaic mutants, allows mapping of (co-)driver genes and identification of dependency genes within specific cancer contexts.
CRISPR/Cas9-based dependency mapping has pinpointed Ezh2, part of the polycomb repressive complex 2, and Creb3l1, a central mediator of collagen gene expression, as essential genes for desmoid tumors, which are uniquely driven by Wnt pathway hyperactivation. Additionally, treatment with the EZH2 inhibitor Tazemetostat resulted in a significant reduction/stasis in desmoid tumor size in the *in vivo* model. Contrastingly, this compound did not significantly affect cell proliferation or death in cultured human desmoid tumor cells but markedly reduced the transcriptional activity of the Wnt pathway. The latter observation points to a possible involvement of the tumor microenvironment (TME) in the anti-tumor effect of Tazemetostat.

Given that tumors with activated Wnt/β-catenin pathways are known to be immunologically cold, we hypothesize that the regression of desmoid tumors observed upon Tazemetostat treatment in the *Xenopus* model may be due to its ability to mitigate this immune suppression, thereby enabling a natural anti-tumor immune response. To investigate this, we aim to determine the mechanism underlying immune cell exclusion in desmoid tumors. Specifically, we seek to uncover whether this exclusion results from reduced T cell infiltration due to the tumor cells hijacking immune checkpoints and diminished production of chemoattractants and interleukins. Alternatively, given the identification of *creb3l1* as an additional dependency gene, we want to investigate where the extensive collagen composition of desmoid tumors physically restricts T cell access.
These hypotheses will be addressed using novel *rag2* knockout and *rag2*::GFP reporter lines in *Xenopus*, as well as *pdcd1* and *ctla4* (central immune checkpoint mediators) mosaic mutants in the desmoid tumor model, and whole-mount 3D imaging. Insights gained from this desmoid tumor model could be pivotal for understanding more complex cancers associated with Wnt pathway activation.

Keywords: Xenopus tropicalis, Cancer Modeling, Desmoid Tumors, Immunologically Cold, Wnt

*Speaker

Pathway Hyperactivation

Establishment of a framework to study and interfere with Xenopus laevis spermatogenesis

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Sperm deliver to the embryo epigenetic information contributing to embryonic development (Siklenka et al. 2015; Teperek et al. 2016). However, 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 approaches to: (i) accurately characterize germ cells and accessory cell types in *Xenopus laevis* testis and (ii) identify chromatin pathways associated with male germ cells transition towards mature sperm. Integrating public and in-house scRNA-seq datasets, we developed a cell atlas to benchmark spermatogenesis evolution 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, RNA, FISH, explant, epigenetic

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European Xenopus Resource Centre Update

Matt Guille * [1], Anna Noble , Anita Abu-Daya , Annie Godwin [2,3], Gemma Bavister , Colin Sharpe , Sarah Woolner [4]

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Sitting in the EXRC allows a unique overiew of research activity using Xenopus, there has been a significant increase in commercial use fo the model for both environmental and biomedical resarch since the pandemic and it is great to see new academic labs being established that use the model (including one a Portsmouth!). The commercial use of the centre is important since it now makes much of the academic research at cost sustainable in a time when the Wellcome Trust has moved away from Resource funding. We have to balance work carefully since, for example, one company is requesting tissue from 80% of the X. laevis we will have available in 2025 so the earlier academic users (who have precedence) can let us know their needs the sooner we can manage commercial expectations. In terms of developing resources, an inducible cas9 line in X. laevis is growing up and in build in X. tropicalis, a line that expresses in the antimicrobial peptide secreting cells has been made for conservation research, a range of signaling reporter lines are now well in build. As part of the last, the most efficient way to combine G-blocks and the pTransgensis system has been ectablished, particularly for difficult to clone sequences. As lines from the rare disease diagnostics programme (XenMD) become avaialble they will be added to the website and advertised in the newsletter. The Amphiclub is a great opportunity to talk to us about what you need as we start to develop the plans for the next funding period.

*Speaker

Role of nonclassical MHC-Ib and innate-like T cells in Xenopus tadpole immunity

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Expanded nonclassical MHC-Ib gene lineages distinct from classical polymorphic MHC-Ia genes are found across jawed vertebrates' genomes, although their significance in immunity is unclear. To date *Xenopus laevis* is the only species outside of mice and humans where the requirement of nonpolymorphic MHC-Ib for the development and function of antimicrobial innate (i)T cells has been shown. While mammalian-like conventional T cells are dominant in adult frogs, tadpoles rely on a few distinct prominent iT cell subsets interacting with cognate nonpolymorphic MHC-Ib (XNC) molecules. Thus, *Xenopus* provide an ideal comparative model for investigating the roles of MHC-Ib and iT cells in immune surveillance during evolution.

To examine iT cell responses *in vivo*, we determined their proliferation during microbial infection, which was more limited in tadpoles than in adult frogs. However, tadpole T cells proliferated at a high rate upon *in vitro* stimulation with PHA that directly cross-linked the T cell Receptor (TCR). This suggests that T cell hypo-responsiveness in tadpoles is in part due to MHC-Ib expression on antigen presenting cells.

To investigate the cellular requirements of MHC-Ib cell surface expression, we adapted a Lentiviral-VSV hybrid system to transduce and express tagged recombinant MHC-Ib molecules more efficiently in *X. laevis* cell lines. In contrast to classical MHC-Ia, our preliminary results suggest that b2-microbglobulin is not strictly required for MHC-Ib translocation at the cell surface, as for some mammalian MHC-Ib such as CD1 and HLA-F.
Overall, our studies provide new functional insight into an immune surveillance system more widely represented in jawed vertebrates than previously thought.

Keywords: MHC, T cell development, immune response

*Speaker

The Xenopus Eleuthero-embryonic Thyroid Assay (XETA): Applications in Ecotoxicology for Identifying Thyroid-Active Chemicals

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Amphibians are primary targets for environmental pollutants in ecosystems. Numerous chemicals and environmental samples have been identified as having thyroid activity. Amphibians are particularly sensitive to thyroid disruption because metamorphosis, a critical process for their development, is thyroid-dependent. Therefore, identifying thyroid disruptors in amphibians is of great concern, especially in the context of declining amphibian populations.
The Organization for Economic Cooperation and Development (OECD) has set up a conceptual framework for the testing and assessment of endocrine disrupters. Different *in vitro* and *in vivo* standardized methods (test guidelines, TG) are categorized into five levels of increasing biological complexity. Detection of thyroid active molecules is addressed by mammalian and amphibian testing. Amphibian testing takes advantage of the THs' exclusive control of anuran metamorphosis. The Amphibian Metamorphosis Assay (AMA) detects the actions of thyroid active molecules via physiological endpoints relevant for metamorphosis. In addition, in June 2019, the OECD validated the *Xenopus* Eleutheroembryonic Thyroid Assay (XETA) in the test guideline program.
We developed the Xenopus Embryonic Thyroid Assay to offer an amphibian screen for thyroid active molecules and environmental samples. The XETA utilizes eleutheroembryos to detect modulation of thyroid signalling by thyroid active chemicals. The assay is transcription-based and uses a transgenic line expressing the Green Fluorescent Protein (GFP) under the control of a promoter directly regulated by TH. The response measured is fluorescence of embryos. When transcription of the genomic construct is activated or inhibited following chemical exposure, the embryos express more or less GFP and therefore emit more or less fluorescence compared to unexposed embryos. The XETA TG has been validated through an international effort via the OECD. The test by seven laboratories of a set of 14 reference thyroid active chemicals with various known MoA (Mode of Action) demonstrated the transferability, reliability and reproducibility of the XETA. This presentation will illustrate various applications of the XETA in ecotoxicology, ranging from its regulatory use in pesticide assessment to its role in collaborative research projects.

*Speaker

Histone Acetylation: A Barrier to Cell Fate Reprogramming

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As the zygote undergoes cell divisions and differentiation to form all cell types of an organism, cell fates are established and faithfully transmitted to the progeny. This is tightly regulated by epigenetic mechanisms that safeguard cell identities. Introducing a differentiated somatic nucleus to a vertebrate egg induces cell fate reprogramming and produces cloned animals in a process called nuclear transfer (NT). This process is inefficient, partly due to epigenetic memory of the transcriptional profile of the starting cell type (donor), leading to aberrant gene expression in the wrong cell type of the NT embryo. Previous work by our group has identified high H3K4me3 levels on the promotors of such reprogramming-resistant genes, termed ON-memory genes (Hörmanseder et al 2017). Overexpression of a histone demethylase corrected the wrongful gene expression of ON-memory genes in NT embryos and improved their development. However, not all reprogramming-resistant genes were rescued with this approach, hinting that other epigenetic features could act as reprogramming barriers. Thus, we performed a multi-omics screen combining transcriptome and epigenome data from donor tissue, as well as transcriptome data from NT and IVF embryos, developed a machine learning analysis approached termed "digital reprogramming" aiming to predict reprogramming resistance from epigenetic barcodes in the donor cell. We detected strong H3K27ac enrichment levels on promotors and putative enhancers of ON-memory genes. We hypothesized that H3K27ac could stabilize active transcription in the donor nucleus, which persists in the resulting NT embryo and poses a barrier for cell fate reprogramming. Thus, we perturbed H3K27ac levels in the donor nucleus using p300 inhibitors and detected a significant reduction of strongly expressed ON-memory genes in NT embryos. This points towards a new role for histone acetylation as a roadblock to reprogramming and a potential mechanism for stabilizing cell fates by maintaining active chromatin states.

Keywords: Epigenetics, Reprogramming, Histone Acetylation, Machine Learning, Genomics

*Speaker
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Investigating mechanisms of cyst formation in a Xenopus model for autosomal dominant polycystic kidney disease (ADPKD)

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Background

ADPKD is a genetic kidney disease typically caused by mutations in *PKD1* or *PKD2*, and has a prevalence exceeding 1:1000. It is characterized by progressively growing renal cysts, leading to kidney enlargement, fibrosis, and eventually end-stage renal disease. Despite the high prevalence, no satisfactory treatment options are currently available. This is mainly due to the incomplete understanding of the *PKD1/2* gene products and the molecular events leading up to cyst development.

In this project, we aim to further the understanding of the molecular mechanisms that define the cyst initiation event. Leveraging the unique advantages of the *Xenopus* model allows us to intravitally observe and manipulate renal tubules as they transform from healthy to cystic.

Methods

In the context of this project, we have developed a high-throughput intravital live imaging setup, where we can follow pronephric tubule and cyst development of up to 72 tadpoles in parallel. Using mutant lines, CRISPR-Cas9, morpholino, and drug-based interventions, we are investigating the contributions of cytoskeletal components, primary cilia, and mechanosensors to the cyst initiation event.

Results & conclusions

Before the onset of flow, *pkd1* knockout tubules are indistinguishable from wild type controls. It is only as luminal flow begins that tubules rapidly start dilating. Interestingly, these dilations are also observable in wild type pronephros, where they are closely followed by reconstriction back to the original tubular diameter. This constrictional response is missing in the *pkd1* knockout animals, leading to continued tubule dilation and cyst formation. This finding raises the possibility that cyst initiation in ADPKD is due to an insufficient tubular constrictional response, a conceptually new hypothesis.
We have successfully phenocopied these tubule dilations through flow disruption, myosin II inhibition, and cAMP activation, indicating the involvement of flow-sensing and the actomyosin network in dilation-driven cyst formation.

*Speaker

Keywords: Disease modeling, live imaging, kidney, pronephros, ADPKD, CRISPR

Maintenance of H3K4me3 in early Xenopus laevis embryos is indicative of the developmental gene expression program

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Transmission of active chromatin states from mother to daughter cells is typically intertwined with active transcription. H3K4 trimethylation, a chromatin mark closely linked to active states, typically depends on active transcription for its propagation but was recently found on chromatin in embryos after several transcriptionally silent cell divisions. This raises pertinent questions about if and how H3K4me3 domains persist throughout early embryonic cell divisions in the absence of transcription, and their significance for embryonic development. Here, we show that H3K4me3 is maintained during early embryonic cell divisions, identify underlying mechanisms and address the role of this maintenance in embryonic transcription and development. We reveal the presence of H3K4me3 and other active histone marks on embryonic chromatin from an early cleavage stage until the initiation of zygotic genome activation (ZGA). The duration of maintenance in early embryos closely correlates with higher H3K4me3 peak intensities and breadths at gene promoters. Interestingly, zygotically expressed genes important for gastrulation and embryonic development correlate with broad H3K4me3 domains, are enriched for unmethylated CpGs and are more accessible at their promoters prior to ZGA. Using knockdowns of specific H3K4 methyltransferases that function independently of transcription, we demonstrate their role in embryonic development and instructing expression patterns at ZGA. In summary, this project uncovers transcription-independent maintenance of H3K4me3 during cell divisions in pre-ZGA embryos, and highlights its significance for accurate gene expression and embryonic development. Importantly, we provide evidence for the existence of epigenetic memory of active states by demonstrating the transmission of active chromatin states independently of transcription, and providing valuable insight into the signal that induced the state.

Keywords: Epigenetics, ZGA, chromatin, H3K4me3, development

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Predictable and precise CRISPR/Cas9-mediated integration

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The precise and targeted integration of transgenes via CRISPR/Cas9 technology holds significant promise for applications in biotechnology and gene therapy. However, it is paramount that such integration maintains genomic integrity to avoid unintended side-effects and is suitable for the intended target cell types. Typically, CRISPR/Cas9-mediated integration relies on homology-directed repair (HDR), which necessitates large homology arms on either side of the cargo and is only active in proliferating cells, or on non-homologous end joining (NHEJ) and microhomology-mediated end joining (MMEJ). However, NHEJ and MMEJ may result in unintended genomic alterations at transgene-genome borders, including genetic deletions within the surrounding genome or transgene, and the potential disruption of neighboring genes. Enhancing our ability to predict the repair process at the interface between the genome and transgene MMEJ repair arms has the potential for rational repair arm design towards gene editing outcomes of interest.
Here, we explored the potential for harnessing deep learning models, trained on DNA repair outcomes, to develop the optimal rules for designing DNA repair arms for the integration of large genetic cargo. This will allows for predictable editing outcomes driving intended integrations, but not bystanders mutations.

For this, we used tandem repeats of small homologies (trimology) to facilitate on-target integration via MMEJ using CRISPR/Cas9. We show that CRISPR/Cas9 repair is non-random on the interface between the genome and such repair arms of large transgenic cassettes ($>$ 2.5kb), *in vitro* and *in vivo*, unveiling an unanticipated predictability. Moreover, such repair arms safeguard the boundaries during integration. We further deducted the design rules for optimal integration and show trimology integration to be effective in cell contexts where HDR is largely ineffective, such as rapidly cycling vertebrate embryos (*Xenopus*) and adult post-mitotic mouse neuronal cells.
We have demonstrated that trimology integration enables efficient and predictable cargo insertion in *Xenopus tropicalis* at a newly identified stable landing site. A key advantage of trimology repair arms are their exceptionally short lengths, which simplifies the generation of repair templates. Together, we show that integration with tandem repeat repair arms is sufficient for predictable in-frame repair and offers higher predictability then NHEJ-based methods.

*Speaker

Le monde mal connu et menaçé des amphibiens

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*Speaker

The salamander thymus, a new paradigm of regeneration and anti-ageing

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Extensive regeneration of the body plan is found in a few exceptional vertebrates, including salamanders such as the axolotl (*Ambystoma mexicanum*) and the Spanish ribbed newt (*Pleurodeles waltl*). In these organisms, regeneration of complex structures relies on the modulation of cellular plasticity for the generation of regenerative progenitors, which often arise from dedifferentiation or transdifferentiation of mature adult cells instead of stem cells. Further to this, salamanders display additional noteworthy traits, namely extraordinary longevity and lack of traditional signs of age-related decay or 'negligible senescence'. As such, they constitute valuable models for addressing the nature of organismal senescence and the interplay between regeneration and ageing. Here, I will present a new paradigm of regeneration, namely the *de novo* regeneration of the axolotl thymus. Further, I will discuss distinct aspects of its involution, and how these features can inform new strategies towards the promotion of healthspan.

*Speaker

Deciphering Early Neural Crest Patterning at a single cell resolution

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Neural crest cells exemplify cellular diversification from a multipotent progenitor population. However, the full sequence of early molecular choices orchestrating the emergence of neural crest heterogeneity from the embryonic ectoderm remains elusive. Gene-regulatory-networks (GRN) govern early development and cell specification towards definitive neural crest. We have combined ultra-dense single cell transcriptomes with machine-learning and large-scale transcriptomic and epigenomic experimental validation of selected trajectories, to provide the general principles and highlight specific features of the GRN underlying neural crest fate diversification from induction to early migration stages using Xenopus frog embryos as a model. Our main results include that during gastrulation, a transient neural border zone state precedes the choice between neural crest and placodes which includes multiple converging gene programs; and that during neurulation, transcription factor connectome and bifurcation analyses demonstrate the early emergence of neural crest fates at the neural plate stage, alongside an unbiased multipotent-like lineage persisting until epithelial-mesenchymal transition stage. We also decipher circuits driving cranial and vagal neural crest formation and provide a broadly applicable, high-throughput, in vivo, experimental validation strategy for investigating single cell transcriptomes in vertebrate GRNs in development, evolution, and disease.
Reference: Kotov, Seal et al., PNAS 2024, PMID: 38683994

*Speaker

The chemo-mechanical regulation of neuronal growth in the developing Xenopus laevis brain

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During brain morphogenesis, neurons extend axons over large distances along well-defined pathways. Axon pathfinding is regulated by both chemical and mechanical signals. However, we currently know very little about how these signals interact. Using the African clawed frog, *Xenopus laevis,* as a model system, we here show how local mechanical brain tissue properties contribute to guiding neuronal axons. *In vivo* time-lapse atomic force microscopy revealed stiffness gradients in developing brain tissue, which axons followed towards soft. Interfering with brain stiffness and mechanosensitive ion channels *in vivo* both led to aberrant neuronal growth patterns with reduced fasciculation and pathfinding errors. Tissue stiffness not only directly impacted neuronal growth but also indirectly by regulating neuronal responses to and the availability of chemical guidance cues in the surrounding tissue, strongly suggesting that chemical and mechanical signaling pathways are intimately linked, and that their interaction is crucial for morphogenetic events.

*Speaker

Inhibition acts as a conductor of vestibulospinal reflex in postural control

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Postural control is an adapted feature allowing animals to behave in their environment. Vestibulospinal (VS) nuclei in the brainstem participate in the regulation of this postural control, by integrating multisensory information and sending adapted commands to spinal motor networks. However, it remains unclear how such an excitatory system, very well conserved through Evolution, can allow postural control accuracy in so different animal species, with so distinct biomechanics. We started to investigate the putative implication of inhibitory networks in regulating VS sensory-motor integration and adapting VS operation in two phylogenetically distant species, the *Xenopus laevis* and the adult mouse. Here, we present evidences in *Xenopus* tadpoles that both GABA and glycine neurotransmissions are involved in local and commissural interactions in the VS system, and that blocking either or both inhibitions dramatically impacts the expression and the accuracy of VS reflexes.

Keywords: Inhibition, Posture, Vestibulospinal

*Speaker

Animal caps: the Xenopus gastruloids perfect for mechanobiology of induction

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Embryonic induction is the process by which signals produced by one embryonic tissue (the inducer) transform the fate of another tissue (the responder). Since its discovery more than a hundred years ago, embryonic induction has been the focus of intense research, leading to the identification of numerous inductive molecules and signalling pathways. Xenopus animal caps, a section of animal hemisphere ectoderm, have been instrumental in identifying many mesodermal and neural inductive signals. In recent years, it has become evident that cells can respond not only to molecular signals but also to mechanical cues. However, the role of mechanics in embryonic induction has been largely unexplored. Here, we utilize the power of Xenopus and its animal caps to study the role of mechanics in mesodermal and neural crest induction.

*Speaker

Maturation in central vestibular neurons during frog metamorphosis: one rule to rule them all... or not

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*Speaker

Optimizing two photon scanning microscopy for in-vivo brain-wide functional imaging in Xenopus Laevis tadpoles

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In neuroscience, two photon scanning microscopy is commonly used to record brain activity in species that differ greatly in brain size and their properties and distributions of neurons. Accordingly, tailoring the properties of the imaging system to the experimental model in question is critical. These include adjustments in the size of the field-of-view, the shape and curvature of the scan-plane, or adjustment in excitation PSF sizes. Here, we report our progress to optimising these and other imaging parameters for high-signal-to-noise imaging of whole-brain neuronal activity in the large nervous system of *Xenopus laevis* tadpoles.
Building on nTC (1), our recently published approach for flexible selection of key optical parameters, we test excitation efficiency with different sizes of PSFs for a field of view in a 3D range of 1.2x1.2x0.6 mm up to 3.5x3.5x0.6 mm. We also optimized collection efficiency using a custom design substage optical system. Combining those two factors allows imaging from Xenopus tadpole brains with comparable quality compared to what is currently possible in juvenile zebrafish. We also built a light stimulator for a full flash stimulus in a range of 296-680 nm.
(1) Janiak, F.K., Bartel, P., Bale, M.R. *et al.* Non-telecentric two-photon microscopy for 3D random access mesoscale imaging. *Nat Commun* **13**, 544 (2022). https://doi.org/10.1038/s41467-022-28192-0

Keywords: two photon, neuroscience, functional imaging, brain

*Speaker

Memory of somatic cell identity affects specific cell differentiation programs in cloned organisms

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Nuclear reprogramming can change cellular fates. Yet, reprogramming efficiency is low and the resulting cell types are often not functional. Our work will be presented where we used nuclear transfer to eggs to follow single cells during reprogramming *in vivo*. We show that the differentiation success of reprogrammed cells varies across cell types and depends on the expression of genes specific to the previous cellular identity. We find subsets of reprogramming resistant cells that fail to form functional cell types, undergo cell death, or disrupt normal body patterning. Reducing expression levels of genes specific to the cell-type of origin leads to better reprogramming and improved differentiation trajectories. Thus, our work demonstrates that failing to reprogram *in vivo* is cell-type specific and emphasises the necessity of minimising aberrant transcripts of the previous somatic identity for improving reprogramming.

*Speaker

Marcks and Marcks-like 1 proteins promote spinal cord development and regeneration in Xenopus

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Marcks and Marcksl1 are abundant proteins that shuttle between the cytoplasm and membrane to modulate multiple cellular processes, including cytoskeletal dynamics, proliferation, and secretion. Here, we performed loss- and gain-of-function experiments in *Xenopus laevis* to reveal the novel roles of these proteins in spinal cord development and regeneration. We show that Marcks and Marcksl1 have partly redundant functions and are required for normal neurite outgrowth and proliferation of neuro-glial progenitors during embryonic spinal cord development and for its regeneration during tadpole stages. Rescue experiments in Marcks and Marcksl1 knockout animals further suggested that some of the functions of Marcks and Marcksl1 in the spinal cord are mediated by phospholipase D (PLD) signaling. Taken together, these findings identify Marcks and Marcksl1 as critical new players in spinal cord development and regeneration and suggest new pathways to be targeted for therapeutic stimulation of spinal cord regeneration in human patients.

Keywords: spinal cord regeneration, axon outgrowth

*Speaker

Investigating the molecular network underlying Wilms' tumor formation using CRISPR multiplexing in Xenopus tropicalis

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Wilms' tumor (WT), also known as nephroblastoma, is the most common kidney cancer in young children. WTs have a triphasic histological phenotype, containing three cell types that are present in the fetal kidney: blastemal, stromal, and epithelial. While a multitude of driver mutations have been documented in the formation of these tumors, including genes involved in kidney development, genome maintenance, and microRNA processing, a unifying molecular model for WT formation is still lacking.

We have recently established a short-latency, highly penetrant model for WT in *Xenopus tropicalis*, using multiplexed CRISPR/Cas9-mediated targeting of the p53 and RB pathways. Interestingly, triphasic histology of the experimental *Xenopus* WTs was maintained upon allografting in *rag2* KO animals.

Our WT model is currently being expanded through targeting of genes involved in microRNA processing and the let-7 miRNA signaling axis. Via transcriptomic and epigenomic profiling of these *Xenopus* tumors, combined with an interspecies comparison using data from experimental mouse and clinical human samples, we aim to better understand the molecular signatures that underly WT formation. We foresee to identify potential WT vulnerabilities, which we will further explore and validate in vivo via CRISPR/Cas9-mediated dependency mapping in the *Xenopus* WT models, with the ultimate aim of finding novel targets for therapy.

Keywords: Tumor modeling, cancer, kidney, CRISPR, genome editing

*Speaker
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Sox9 and Sox10 regulate different target genes during neural crest and placode development

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Neural crest (NC) cells and cranial placodes share evolutionary and embryological origins and are of fundamental importance to vertebrate development, evolution, and disease. NC cells are multipotent and form much of the cranial skeleton and peripheral nervous system. Placodes form the paired sense organs including the eye lenses, olfactory, otic, and lateral line organs, and some sensory neurons. The SOX E proteins SOX9 and SOX10 are transcription factors acting as neural crest specifiers in vertebrates. They are also expressed in the otic placode, which gives rise to the inner ear. However, little is known about their different roles during NC and placode development. To analyse the specific roles of Sox9 and Sox10 for NC and cranial placode development, we identified their candidate target genes during two developmental stages in *Xenopus laevis*: neurulation and organogenesis. We first FACS-sorted cells from NC and otic explants after overexpression of SOX9 and SOX10 followed by bulk RNA sequencing allowing us to identify candidate target genes in these two tissues. To confirm the binding sites of these candidate genes, we then performed CUT&RUN sequencing on DNA extracted from NC and otic explants overexpressing SOX9 and SOX10 mRNA. The data obtained from differential gene expression studies, gene set enrichment analysis and peak calling analysis revealed these transcription factors' individual and shared roles between the NC and placodes in *Xenopus laevis.* We observed that SOX10 repressed placodal and non-neural ectodermal genes in the NC whilst activating them in the placode during neurulation. SOX9 on the other hand, activated NC genes in the NC whilst suppressing them in the placodes. The present study provides us with a broader understanding of the Sox9 and Sox10 gene regulatory networks involved in NC and placodal development.

Keywords: Neural crest, Placode, Development, SOX transcription factors, Neurocristopathies

*Speaker

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Regulation of the spatiotemporal DNA replication program in early Xenopus laevis embryos

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Vertebrate genomes are duplicated by the coordinated activation of tens of thousands of replication origins at different times during the S phase. Deregulation of this spatio-temporal replication program provokes genomic instability and cancer, but the underlying mechanisms remain unclear, especially during early development. We have investigated the regulation of origin activation during the rapid S phase in early embryos using sperm nuclei replicating in *Xenopus* egg extracts, single DNA molecule analysis, numerical simulations, and an autocorrelation analysis approach. We found that the depletion of the replication timing factor Rif1 accelerates cell cycles in pre-MBT *Xenopus laevis* embryos and DNA replication in egg extracts due to a global increase in origin activation at the level of replication clusters and chromatin domains. This clearly demonstrated that an embryonic replication timing program exists in early embryos. Furthermore, we showed that the depletion of the checkpoint recovery kinase Polo-like kinase (Plk1) inhibited DNA synthesis by decreasing the number of active replication clusters and by locally increasing origin distances. We used quantitative chromatin proteomics and co-immunoprecipitations to demonstrate that Plk1 interacts with Rif1. Phosphoproteomic analysis showed that Plk1 phosphorylates and inhibits Rif1, thereby linking Plk1 to the temporal program. In addition, we showed that Plk1 also interacts with several rate-limiting initiation factors such as MTBP/Treslin and TopBP1. Plk1 depletion inhibited the chromatin binding dynamics of these factors, probably explaining its local effect on origin distances. Altogether, our results show that while Rif1 slows down the temporal replication program and can be counteracted by Plk1, Plk1 is also necessary to positively regulate the spatial program in early *Xenopus laevis* embryos.

*Speaker

Counting Oocyte Divisions: The cyclin B symphony

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Meiotic cells undergo two consecutive divisions, meiosis I and meiosis II, without an intermediate S-phase, to become haploid. To ensure correct ploidy of the progeny, female meiosis further comes to a halt at metaphase of meiosis II (metaphase II) for fertilization in vertebrates. Failure to stop at the correct cell-cycle stage, or not to stop at all, results in non-viable embryos, often leading to infertility, particularly in women. Importantly, meiotic progression and arrest depend on three temporally regulated B-type cyclins: cyclin B1, cyclin B2 and the more-divergent cyclin B3. Cyclin B1 and B2 have similar turnover and exhibit redundant functions in controlling meiotic progression. Together with Cdk1, they phosphorylate common substrates and must be degraded for both meiosis I and meiosis II exit. In contrast, when associated with Cdk1, cyclin B3 positively regulates exit from meiosis I and is degraded with a delay, in anaphase I. In meiosis II, cyclin B3 must be absent for the oocyte to arrest in metaphase II for fertilization. How B-type cyclins work together to bring about meiotic progression in oocytes remains poorly understood. In addition, it is not known whether cyclin B3 has additional functions during the meiosis I-meiosis II transition in vertebrates. Using either cyclin B1 or cyclin B3 in *Xenopus* oocytes, we discovered that, when incompletely degraded after meiosis I, cyclin B3 behaves as a super-oscillatory cyclin by indirectly targeting Cdk1-cyclin B1/B2 through specific cell-cycle regulators of meiosis II. As a result, oocytes undergo multiple cell divisions after meiosis I. Thus, our results highlight that the tight coordination of cyclin B1/B2 and cyclin B3 turnover is crucial to limit the number of cell divisions to only two during the meiosis I-meiosis II transition in vertebrates.

*Speaker

Expansion microscopy of Xenopus egg extract spindles

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The spindle is a bipolar structure, composed of hundreds of thousands of microtubules, which can adapt its size and architecture among cell types and organisms to ensure the faithful segregation of chromosomes. However, how specific spindle morphologies are established is poorly understood. It also remains unclear how different microtubule subpopulations organize into complex tri-dimensional spindles. To address this question, we use *Xenopus* egg extracts. Yet, the high density of microtubules within *Xenopus* spindles makes it impossible to resolve individual bundle or microtubule level using classical light microscopy, preventing a complete understanding of architectural details. To solve this limitation, we set out to adapt expansion microscopy to the study of *Xenopus* egg extract spindles. We developed an optimized expansion protocol by comparing various conditions and measuring their expansion factor, the conservation of the spindle architecture, as well as possible deformations due to expansion. Still, thanks to increased resolution provided by expansion microscopy, we could clearly observe artefacts resulting from the fixation procedure classically used in the field. We thus compared various fixation conditions to reach an improved fixation protocol compatible with subsequent expansion microscopy. Having optimized both fixation and expansion, we are now able to analyze microtubule organization within egg extract spindles and compare them between *Xenopus* species with unprecedented details.

*Speaker

Oocytes maintain ROS-free mitochondrial metabolism by suppressing complex I

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Oocytes form before birth and remain viable for several decades before fertilization. Although poor oocyte quality accounts for most female fertility problems, little is known about how oocytes maintain cellular fitness, or why their quality eventually declines with age. Reactive oxygen species (ROS) produced as by-products of mitochondrial activity are associated with lower rates of fertilization and embryo survival. Yet, how healthy oocytes balance essential mitochondrial activity with the production of ROS is unknown. We discovered that oocytes evade ROS by remodelling the mitochondrial electron transport chain through elimination of complex I. Combining live-cell imaging and proteomics in human and *Xenopus* oocytes, we find that early oocytes exhibit greatly reduced levels of complex I. This is accompanied by a highly active mitochondrial proteostatic response, which is indicative of an imbalanced electron transport chain. Biochemical and functional assays confirm that complex I is neither assembled nor active in early oocytes. Thus, we report a physiological cell type without complex I in animals. Our findings also clarify why patients with complex-I-related hereditary mitochondrial diseases do not experience subfertility. Complex I suppression represents an evolutionarily conserved strategy that allows longevity while maintaining biological activity in long-lived oocytes.

*Speaker

Xenopus Modelling Disease (XenMD): Modelling rare monogenic human disease in Xenopus.

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One in 17 people will have or develop a rare disease with a genetic component. For these people, the pathway to improved diagnostic, preventative and therapeutic strategies can be found in the abundance of individuals' DNA sequence data. As well as identifying the gene causing a disease about one time in three, this uncovers a wealth of variants of unknown significance (VUS). For many VUS determining disease causality *in silico* is not attainable, a problem that is now increasingly addressed through model organism studies. Here we show how a local collaboration (XenMD) between clinicians, clinical geneticists and biologists emerged and further, how CRISPR/Cas9 modelling of variants in *Xenopus* is contributing to the clinic. We outline the pipeline developed to screen VUS, including the generation of knock-out novel-disease-gene lines and our approach to base-editing the *Xenopus* genome to re-create point variants. Furthermore, we demonstrate how we have characterised novel phenotypes in these lines. This has included demonstrating a newly described search behaviour strategy in *Xenopus* tadpoles that is equivalent to observations in human behaviour; we have found this to be altered in multiple frog models of human neurodevelopmental disorders.

*Speaker

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A website to select guide RNAs for Cas13d-mediated gene inactivation in Xenopus laevis

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Centre National de la Recherche Scientifique : UMR6290, Structure Fédérative de Recherche en Biologie et Santé de Rennes, Université de Rennes - CNRS – France

Inactivation of gene expression is a common methological approach to analyse gene function. While gene edition, by CRISPR/Cas9 for example has proven to be highly effective, it is not always the method most adapted to peculiar developmental situations. In particular, the early development of Xenopus rely heavily of maternally stored mRNAs that provide the genetic material required for early development. The recently identified RNA-guided ribonucleases of the class 2 type VI CRISPR/Cas system offer the promising opportunities to target directly mRNAs to adress gene functions.
On most loci Xenopus laevis is tetraploid and therefore requires the identification of guide RNA that target both alloalleles. We propose herein a small R shiny application to rapidly pick and choose the most likely functioning Cas13d guide RNAs targeting both alloalleles of a gene of interest.

*Speaker

Different maturation processes in central vestibular neurons during frog metamorphosis

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Central vestibular neurons are responsible for sensory-motor reflexive commands involved in gaze and posture stabilization in response to head movements. Vestibulo-spinal (VS) neurons project to spinal networks whereas vestibulo-ocular (VO) neurons contact oculomotor circuits. The developmental plasticity occurring during anuran metamorphosis offers a unique opportunity to understand how vestibular neurons properties mature facing specific developmental pressures.
In adult frog, central vestibular neurons present 2 phenotypes according to their discharge dynamic: 1) Phasic neurons, with a transitory discharge, supported by the Kv1.1 channel and 2) tonic neurons firing continuously. However, such a characterization remains unrelated to a specific vestibular function and nothing is known about the maturation of these neuronal dynamics. This study aims to investigate this question comparing VS and VO neurons through the Xenopus metamorphosis. On brainstem slice preparations, patch-clamp recordings and Kv1.1 immuno-labeling revealed a reverse proportion between tonic and phasic VS neurons from larva (20% of phasic) to juvenile (70% of phasic). The switch occurred at stage 54-58. Inversely, VO neurons remains mainly tonic over the metamorphosis (more than 95% in larvae vs 85% in juvenile). Electrophysiological properties for both phenotypes in both VS and VO populations did not change significantly during this period. BrDU pulse-chase showed that all vestibular neurons in juvenile originated from mitotic cells older before stage 54. HIS revealed a strong expression of the NeuroD factor, a post-mitotic neuronal progenitor marker, between stage 53 and stage 60, suggesting an important neuronal differentiation activity in the brainstem during this period. Altogether these results revealed different developmental pattern between VS and VO neurons probably related to the functional specificity of vestibular-driven stabilization of posture and gaze during metamorphosis, respectively. However, all central vestibular neurons demonstrated a common neurogenesis established in early larval life but with distinct neuronal differentiation profiles at the metamorphosis start.

Keywords: vestibular, xenopus, metamorphosis, neurogenesis

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Elucidating the Molecular and Functional Features of a Human WNT11 Mutation using Xenopus Embryos

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The role of Wnt11/ Wnt11b in murine kidney and heart development and in *Xenopus* left-right symmetry breakage has been previously shown, but no human cases with biallelic mutations resulting in WNT11 dysfunction have been reported to date. We have identified a homozygous frameshift variant (c.814delG, p.E272Nfs*13) in a male infant with *situs inversus*, bilateral renal hypodysplasia and Fallot tetralogy of the heart. Interestingly, this mutation leads to a C-terminally truncated ligand with similar length as two described Wnt11b constructs in *Xenopus*, XdnWnt11b and XWnt11b-short, which both act in a dominant-negative manner on WNT signalling. While the heterozygous father is healthy, the heterozygous mother showed *situs inversus*. This prompted us to hypothesise that the human *WNT11* mutation could result in dominant-negative effects. However, functional examinations using the *Xenopus laevis* embryo to investigate morphology and left-right symmetry breakage suggest a loss-of function-effect of the mutation. Western Blot analysis further confirmed absence of the ligand, suggesting the involvement of a control mechanism, such as unfolded protein degradation. By removing the 12 C-terminal frameshift amino acids of the human mutation and replacing them with the original amino acid sequence, dominant-negative activity of the ligands could be restored. We validated the findings by measuring WNT activity using the *Xenopus laevis* Wnt reporter line (*Xla.Tg(pbin7LEF-GFP)).* This study enhances our understanding of WNT structure/function relationships and serves as an example how *Xenopus* can be used to examine human mutations and phenotypes.

Keywords: Xenopus, Left Right Symmetry, WNT11, wnt11b, Human Genetics

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Function of the deutersosome associated protein Cspp1 in multiciliated cells

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Directional flow of biological fluids is generated at multiciliated epithelium surface thanks to coordinated beating of numerous cilia present at the apical surface of multiciliated cells (MCCs). To build their cilia, MCCs produce a large quantity of centrioles which are transformed into basal bodies and serve as a template for the elongation of the ciliary axoneme. Large scale centriole production is mainly ensured by specialized membrane-less organelles called deuterosomes. Recent work of the lab has characterized deuterosome associated proteome thereby identifying candidates for multiple centrioles production. Among them we focused on Cspp1, a centrosome and primary cilia-associated protein mutated in the neurodevelopmental ciliopathy Joubert syndrome. We used, Xenopus laevis model to address Cspp1 function in MCCs. Our observations revealed various sub-cellular localization of CSPP1 in differentiating and mature MCCs. At the cell scale morpholino based functional approaches revealed defects in centriologenesis and ciliogenesis. At tissue scale, a defect in the establishment of flow at the epidermal surface was observed in Cspp1 morphant. Altogether, these results reveal a role for Cspp1 in the large-scale production of centrioles, and provide a better understanding of its role during MCCs differentiation.

Keywords: Multiciliated cells (MCCs), Ciliogenesis, Basal Bodies, Centriologenesis, CSPP1

*Speaker

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TBC1D32 variants disrupt retinal ciliogenesis and cause retinitis pigmentosa

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Retinitis pigmentosa is the most common inherited retinal disease and is characterized by photoreceptor degeneration and progressive loss of vision. Four patients who diagnosed with RP from three unrelated families presented with variants in *TBC1D32*, which to date has never been associated with an IRD. To validate *TBC1D32* as a putative RP causative gene, we combined *Xenopus in vivo* approaches and human iPSC-derived retinal models. In *Xenopus*, our data showed that *TBC1D32* was expressed during retinal development and knockdown experiments demonstrated that it plays an important role in retinal pigment epithelium (RPE) differentiation. Furthermore, we identified a role for TBC1D32 in ciliogenesis of the RPE demonstrated by elongated ciliary defects associated with disrupted cell shape and adhesion. Similar defects were also found in RPE cells derived from the patient induced pluripotent stem (iPS) cells. Lastly, our results also suggested photoreceptor differentiation defects, including connecting cilium anomalies, as observed in TBC1D32 iPSC-derived retinal organoids. Overall, our data not only highlight a critical role for *TBC1D32* in the retina but also demonstrate that *TBC1D32* mutations lead to retinitis pigmentosa.

*Speaker

Gene expression from different species in Xenopus oocytes

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Probing compound effects in different species often remains a challenge especially when considering the needs of development of novel molecules that have high target specificities. This is well illustrated in the field of agriculture; with the urgent need of new insecticides that would be aiming for a specific pest while having minimal effects on insect pollinators and even less on humans, it becomes a priority to understand the mechanisms involved in neurotransmission and how it differs between species.
Thanks to the development of molecular biology and electrophysiological techniques, expression of ligand-gated ion channels (LGICs) from human and invertebrates in *Xenopus* oocytes now allows the study of effects at precise targets such as the neuronal nicotinic acetylcholine receptors (nAChRs). Moreover, the use of the *Xenopus* oocyte model offers additional advantages compared to traditional transfected cell lines and renders possible the investigation of LGICs function using automated approaches.

Considering that nicotine was one of the first natural insecticides, combined with the recent identification of indispensable factors for the expression of the *Apis melifera* nAChRs, it is tempting to compare in a head to head manner the effects of compounds at insect receptors against those observed at the major brain human a4b2 nAChRs.

Results presented herein illustrate first the feasibility of nAChRs expression form human and *Apis melifera* with their characteristic responses to the natural ligand acetylcholine (ACh), and data obtained for three molecules (nicotine, varenicline, and imidaclopride). The comparison reveals marked differences in the respective receptor properties.
Altogether, this work illustrates the ability of faithful expression of ligand-gated ion channels in *Xenopus* oocytes, and the opportunities provided by the combination of molecular biology with automated electrophysiology.

Keywords: xenopus, oocyte, electrophysiology, gene, gene expression, nAChR, receptor, insecticide

*Speaker

Genetic tools for making see-through fluorescent transgenics tadpoles

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Over the last few decades, a multitude of fluorescent transgenic *Xenopus* lines have been generated around the world. These animals are relatively transparent in the early larval stages, but as they develop, they acquire pigmentation, particularly in certain tissues such as the retina and central nervous system, which interferes with good fluorescence visualization. Albino lines have overcome this problem to some extent, but not completely, notably due to the presence of certain pigments such as iridescent and yellow ones. Chemical clearing techniques have been developed to make the animal transparent but they are time-consuming to perform, in addition they require fixed animals. We are currently developing pigment mutants with CRISPR-cas9 technology affecting the pigment producing cells such as melanophores, iridophores and xanthophores that allow us to monitor the fluorescence *in vivo* on transparent animals up to advanced stages of development.

*Speaker

Developing an adaptable visual behavior assay to functionally characterize brain regeneration in the axolotl (Ambystoma mexicanum)

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Axolotl, despite being a vertebrate, showcase an astonishing regenerative capacity even in highly complex tissues such as the central nervous system. Most studies in regenerative model systems concentrate solely on molecular or morphological aspects of regeneration, while the examination of functional outcomes *in vivo* is often overlooked. It is however crucial to assess if morphological regeneration also leads to restoration of function to its pre-injury state.
This project addresses axolotl visual behavior through the establishment of a novel assay. Weused a computer-controlled projection system, to present stimuli without direct water contact,to make sure the stimuli remain exclusively visual. Axolotls of varying sizes were subjected to different prey simulations to help us understand their size and shape preference.

Furthermore, the influence of various factors on prey hunting behavior, such as sex, ambient lighting, or the treatment of substances that are essential for brain injury studies, was evaluated. This allows a broad categorization of stimulus response events, which lays groundwork for future functional experiments.
In subsequent studies, we plan to disturb the axolotl's visuomotor system by injuring the main visual processing center in the brain, the optic tectum. During the course of regeneration of this brain area, we will explore the extent to which this reestablishes a functional visuomotor circuit by implementing the behavior assay described above.

Keywords: axolotl, behavior, regeneration, brain

*Speaker

Oocyte picker a revolution in recombinant expression

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Introduction of the possibility of recombinant gene expression in *Xenopus* oocytes opened new venues for functional testing. Sorting oocytes is done on the basis of their size and qualities using visual inspection carried out under a binocular microscope. The next step in the process is to inject into the cytoplasm, or in the nuclei, the plasmids containing the gene of interest. While the process of injection can be automatized using, for example, the "RoboInject" from Multichannel, sorting and dispensing the oocytes in a 96 microwell plate remains a tedious work that is conducted manually.
The aim of this work is to present a fully integrated system allowing sorting, injection and dispense of the oocytes in an automated manner. Based on image recognition coupled with a CoreXY table, the device allows efficient visualization and image recognition of the oocytes, injection using a precise pressure control. The final step of capturing the injected oocytes and dispensing into a 96 microwell plate with filling of physiological medium is also efficiently conducted.
Altogether, the work presented herein aims at reducing the stress associated with long time use of a binocular microscope for the selection of oocytes while allowing efficient injection and dispensing of the oocytes that can be successively used for recording in automated systems such as the "HiClamp".

Keywords: Intracellular injection, Automated sorting, Automated dispensing, Micro Injection, Technique

*Speaker

WNT/TCF-mediated transcriptional repression in embryonic development, the interaction with BARHL proteins

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The Wnt/β-catenin signaling pathway is a highly conserved system in animals playing a crucial role in embryonic development and stem-cell-mediated homeostasis (Hoppler and Moon, 2014). Its deregulation is tightly associated with cancer. At the molecular level, Wnt signalling influences the activity of transcription factors such as the members of the TCF/LEF family. More precisely, TCF/LEF proteins repress gene expression when Wnt is off, and activate it when Wnt is on. This bimodal functioning is mediated by physical interactions with the downstream component β-catenin. Not all TCF/LEF proteins are equal though. For instance, the TCF7L1 and TCF7L2 proteins function as stronger transcriptional repressors than the other family members, requiring stronger Wnt signalling for transcriptional activation.
We recently identified and characterized novel actors in the play, namely the BARHL homeodomain-containing transcription factors. BARHL proteins influence the bimodal function of TCF/LEF proteins downstream of Wnt/β-catenin signalling (Sena et al., 2019)(reviewed in (Bou-Rouphael and Durand, 2021)). In particular, BARHL2 physically binds to TCF7L1 thereby stabilizing its interaction with the transcriptional co-repressor Groucho/TLE (Sena et al., 2019). More recently, they further established that BARHL1, the closest paralog of BARHL2, interacts with both Groucho/TLE proteins and TCF7l1. BARHL1 inhibiting of TCF activity is strictly necessary for cerebellar stem/progenitor cells to exit their niche (Bou-Rouphael et al, 2024).

To shed light on the molecular determinants and functional implications of the interactions between BARHL and TCF/LEF proteins we implement a trans-disciplinary and multi-scale approach at the cross-talk of computational biology, artificial intelligence, quantitative biophysics, molecular biology, biochemistry, and developmental biology to assess and validate *in vitro* and *in vivo* bioinformatically predicted interactions. Our project articulates around three objectives: 1- to Identify the amino acid combinations responsible for modulating the specificity of the interactions between TCF and BARHL members, by leveraging the massive number of genomic sequences now available and the recent spectacular advances in protein and protein complex 3D structure prediction; 2- To directly assess the physical interaction between BARHL proteins and different TCF/LEF proteins through SPR (Biacore 3000); 3- To functionally validate the inferred and observed interactions and their biological consequences between the different TCF/LEF and BARHL molecules using classical embryological approaches.

*Speaker

U-ExM and TissUExM illuminate multiciliated cell architecture

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Multiciliated cells (MCCs) are epithelial cells specialized in the flow of physiological fluids through the beating of motile cilia on their surface. The various components of MCCs, such as deuterosome, centriole and cilia are nanometer-scale and require microscopy techniques with appropriate resolution for their observation. Recently, Ultrastructure Expansion Microscopy (U-ExM) and Tissue Ultrastructure Expansion Microscopy (TissUExM) has been introduced to observe biological structures at nanoscale using confocal microscopy. In the present work, we applied U-ExM on TissuExM on *Xenopus* cell line and embryos and revealed the architecture of MCCs centrioles and cilia with a resolution considerably higher than that of traditional confocal microscopy.

Keywords: expansion microscopy, superresolution, cilia, centrioles

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Influence of climate change on the Geographic Distribution of Mantellid Frogs (Genus Mantidactylus) in Madagascar

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Aim: This study aims to assess the impact of climate change on the geographic range of the Mantidactylus genus, which is endemic to the mountainous regions of Madagascar.
Location: The study focuses on Madagascar Biodiversity Hotspot.

Methods: Ecological niche models were employed to estimate the current and projected future distribution of the Mantidactylus genus under various climate scenarios. Utilizing occurrence data from 199 points encompassing 35 species, the models incorporated cloud cover averages due to the genus's reliance on misty forest conditions. Suitability values were correlated with altitude to investigate whether future suitable habitats would shift to higher elevations. Additionally, the study mapped regions where suitability loss or gain is expected, considering the presence of forest fragments. The persistence of individual species was assessed based on their respective suitability values.

Results: Our results indicate suitable areas for the occurrence of the genus Mantidactylus in Madagascar. Our models predicted significant losses of suitability in the future scenarios analyzed, mainly in the southern region of the areas of geographic distribution of the genus Mantidactylus and along the Madagascar biome. The results also indicate an increase in suitability for the Mantidactylus occurrence in the future, generally in regions above 1200 m in altitude. These increases in suitability at altitudes above 2000 m occur in non-forested areas. Analysis of the suitability for species occurrence revealed a pattern of loss of suitability, especially for species that occupied regions below 1500 m, which might be more affected by climate change.
Main conclusions: While future suitable habitats for the Mantidactylus genus are expected to shift to higher elevations, these new habitats are unlikely to contain forest remnants. Consequently, species within this genus face a high risk of population decline and potential extinction, especially those with restricted distributions.

Keywords: climate change, mountain species, range shifts, cloud forests, ecological niche modeling, Mantidactylus

*Speaker

Evaluation of CRISPR-Cas9/Cas13 approaches for knock-ins and knockdowns to study Neural Crest development in Xenopus laevis

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Neural Crest cells are a multipotent stem cell population of migratory cells specific to the vertebrates. They are specified at the neural plate border and undergo epithelial-mesenchymal transition (EMT) to initiate an extensive migration throughout the embryo before differentiating into numerous cell types such as cartilage and bone, neurons and glia cells as well pigment and endocrine cells. Our group focuses on the dynamics of EMT and cell migration of the cephalic neural crest cells in Xenopus laevis. We are currently developing CRISPR-based strategies to perform knock-in (Cas9) and knockdowns (Cas13) to study neural crest cell migration in vivo. In particular, we want to calibrate the use of Cas13-driven mRNA degradation as a complementary approach to Morpholinos®. As a proof of principle, we tested various concentrations and combinations of Cas13 mRNA and guide RNAs directed towards CXCR4 and Twist. Our preliminary results strongly suggest that CRISPR-Cas13 could be an easy-to-implement and cost-effective method to perform in vivo knockdowns in Xenopus laevis.

Keywords: Neural crest, EMT, Cas9, Cas13

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Using polarization microscopy for label-free visualization of axons in the Xenopus brain

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Polarization microscopy can be used to visualize anisotropic structures whose refractive index depends on the polarization and propagation direction of light – birefringent structures. As nerve fibers are birefringent, polarization microscopy is extensively used to visualize axons in humans and mammals. Here, we used a custom-built polarization microscopy setup to visualize axon tracts in the brain of the African clawed frog, *Xenopus laevis.* Adult *Xenopus laevis* brains were embedded in 4% agarose, and 70μm thick section prepared using a vibratome. Applying polarization imaging, we determined the location and orientation of axon fibers within the 2D-plane of each brain slice. In the future, we will use these date to generate 3D maps of axonal connections in the frog brain at different developmental stages, thus aiding studies of brain development and neuronal regeneration.

Keywords: Xenopus laevis, Polarization imaging, Axon visualisation

*Speaker

Signaling and morphogenetic contribution to pattern formation in the embryonic mucociliary epidermis of Xenopus laevis

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The combination of various specialized cell types and their individual molecular properties are indispensable for normal tissue functions, where multiple cell types must be kept in balance and arranged in stereotyped patterns for a correct organ function. Mucociliary epithelia are composed of basal cells (BCs) along with multiciliated cells (MCCs) and multiple secretory cell types (Hogan et al., 2014; Walentek & Quigley, 2017). The precise cell type proportions of multiciliated and secretory cell types is important to produce extracellular fluid flow in order to remove mucus and pathogens, as defective functions or an incorrect composition can lead to devastating diseases, such as ciliopathies, COPD or Asthma. Nevertheless, it remains enigmatic how differential cell type compositions are controlled to ensure mucociliary clearance.
To uncover conserved principles of vertebrate mucociliary tissue patterning we are using the embryonic *Xenopus laevis*epidermis. We hypothesize that a single standard unit of cells exists (micro-pattern), which can be either expanded to generate a homogenous pattern organ-wide or modified to generate pattern variations. First, we want to elucidate where the stereotypical pattern in the mucociliary epidermis of *Xenopus* is altered by generating a detailed morphological map using scanning electron microscopy. Ongoing experiments showed pattern variabilities along the anterior-posterior axis, similar to the proximal-distal mammalian airway. In addition, we found adjacent to the hatching and cement gland cells in the most anterior head epidermis a population of cells that are morphologically distinct but seem to have a similar morphology to goblet cells.

Moreover, we want to investigate the relationship between cell fate decisions, interactions and movements during patterning and morphogenesis. Hence, we have generated reporter constructs that fatefully mark the different mucociliary cell types. For that, we are using fragments upstream of the cell type specific genes and clone it upstream of a minimal promoter and fluorescent protein-coding sequence.

Next, we want to experimentally validate the effects of cell signaling on differential micro-pattern

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generation focusing on promising candidates contributing to pattern modification. Therefore, we want to use planar animal cap cultures that allow us to image cell type composition changes to evaluate if alterations can be induced in absence of interactions with adjacent tissues.
Collectively, this work should improve the understanding of vertebrate mucociliary cell type composition and adaption by analyzing signaling and morphogenetic contribution to pattern formation, which will lead to a better understanding of the basis of physiological adaptations to enhance the treatment of airway diseases.

P2X purinergic receptors functions during sensory nervous system development and nociception in Xenopus

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The purinergic signalling mainly concerns the extracellular effects of ATP, acting as a ligand for the purinergic P2 receptors, including the ionotropic ATP-dependent P2X and metabotropic ATP P2Y receptors. Functional P2X receptors are formed by the association of three subunits, named P2X1-7. These homotrimeric or heterotrimeric ATP dependant receptors are cation-permeable channels, whose activation leads to cell depolarization and calcium influx. This pathway plays a critical role in adult vertebrates, regulating the physiology of numerous organs but also in pathological conditions, especially in the central and peripheral nervous system, in which ATP is a major neuromodulator, regulating sensory transmission, nociception and chronic pain. Several lines of evidence demonstrated the contribution of homotrimeric P2X3, P2X4 and P2X7 and heterotrimeric P2X2/3 receptors to this disorder, placing them as promising therapeutic targets. We previously identified 9 *p2x X.laevis* genes showed that several P2X subunits are expressed in the developing sensory peripheral nervous system such as in the trigeminal ganglia and in lateral line nerves. However, surprisingly, no genomic sequence encoding the p2x3 subunit has been found in the *X.laevis* genome. The aim of this project is to decipher the roles of the different P2X receptors in the developing sensory systems and in the nociception and the potential functional compensation for p2x3 absence. Electrophysiological recordings in Xenopus oocytes were carried out to characterize the different receptors. Their expression domains in these different systems have also been analysed. Loss of function experiments of p2x subunits were carried out by targeted injections of oligonucleotides morpholino and behavioural tests were set up to study nociception in tadpoles.

Keywords: Neurogenesis, ATP, purinergic signaling, nociception

*Speaker

Modelling mitotic spindle dynamics in vivo and in silico: does stretching help you move?

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Cell division is vital for the growth and adaptation of tissues. The outcome of division, be it a contribution to tissue spreading or stratification, depends on division orientation. The orientation is determined by the mitotic spindle, which is dynamically positioned during metaphase by interaction of its astral microtubules with the cell periphery. The nuclear mitotic apparatus protein (NuMA) is localised dynamically to the cell cortex during cell division and has been implicated in spindle positioning due to its recruitment of dynein. NuMA-mediated spindle orientation is sensitive to tissue tension and is perturbed in cells experiencing an externally applied force, though NuMA's function in orienting divisions with external force still remains unclear. To determine how mechanosensitive spindle orientation is regulated, it is instructive to investigate the movements of the mitotic spindle as it is positioned. We use experimental and mathematical methods to investigate spindle movements in the Xenopus laevis animal cap tissue subject to an externally applied stretch. We utilise a mathematical model of attachment and detachment of cortical force generators (producing pulling forces) working antagonistically against centring forces of microtubules using stochastic simulations and Fokker-Planck (FP) equations, predicting stable oscillations of a spindle pole in 1D. Using asymptotic methods we reduce the FP system to a set of ordinary differential equations (ODEs). We probe the ODEs to determine the factors affecting the positioning of the stability threshold separating oscillatory and non-oscillatory solutions as well as the oscillatory period. This provides an extra tool with which to probe our experimental data.

Keywords: Mitosis, oscillations, cell division, division orientation, epithelia, odes, pdes, stochastic simulations, stochastic equations

*Speaker

Activation of neural stem cells: NOX signaling effects in the Xenopus retina

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Reactive oxygen species (ROS) influence the behavior of stem cells, such as their renewal, proliferation and differentiation, but the underlying mechanisms remain unclear. The objective of this project is to elucidate how stem cells regulate the production and elimination of ROS, and how these molecules contribute to maintaining these cells stemness. This work focuses on active retinal stem cells (RSCs) of *Xenopus*, an ideal model system for *in vivo* studies.
Our investigations reveal that RSCs exhibit higher ROS levels compared to neuronal progenitors or differentiated cells, and that they express a specific set of antioxidants. Furthermore, this work demonstrates that inhibition of NOX-dependent ROS signaling (NADPH oxidases) reduces RSC proliferation, and genetic analyses suggest that NOX influences Wnt/Hedgehog signaling, thereby regulating the transition of RSCs from quiescence to proliferation.
This project also aims to understand how RSCs maintain their redox balance and to explore the role of ROS in the regeneration of other cell types in the retina. The clarification of these biological mechanisms could shed light on regeneration processes in mammals, offering important therapeutic advances in Ophthalmology.

*Speaker

Micronucleus and nuclear abnormalities in Pelophylax ridibundus (Pallas, 1771); case study of industrially contaminated areas in Kosovo

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Industrial pollution caused by the anthropogenic factor constitutes one of the extremely large global problems, directly affecting living beings' populations. Due to their life cycle, limited mobility, skin permeability, life in water and land, amphibians represent the animal group with very high sensitivity and they can be used as bioindicators of environmental pollution. Pollution effects on erythrocyte nuclear morphology in *Pelophylax ridibundus* individuals were investigated. Animals were collected from four polluted industrial sites and one control site in Kosovo: Kishnica, Obiliq, Mitrovica, Drenas (polluted sites) and the Dragash (nonpolluted sites) in spring-summer and autumn-winter season. Blood was taken directly from the heart, by puncture in the ventricular apex; blood smears were prepared according to the standard protocol, stained with May-Grünwald - Giemsa method and observed under a light microscope. The observed changes were: micronuclei, binuclei, vacuolated nuclei and irregulary shape nuclei. Also, significant changes were found in the frequency of nuclear abnormalities between the research localities, as well as between the research seasons. The present study proves the practical usefulness of the frequency of micronucleation and nuclear abnormalities of *Pelophylax ridibundus* as important evaluative parameters for assessing the environmental health of ecosystems.

Keywords: Pelophylax ridibundus, Micronuclei, Nuclear abnormalities, Erythrocytes, Industrial pollution.

*Speaker

Tra2b regulates splicing of a coherent set of ciliary transcripts during ciliogenesis in Xenopus

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Cilia were one of the first cellular organelles discovered in 1675 and are extremely complex and diverse in function, ranging from motile cilia on multiciliated cells (MCCs), which beat in a synchronous fashion to remove mucus and debris in the mammalian airway, to the sensory primary cilia of developing neurons, which act as signaling antennae. Despite their diverse functions, most cilia share the same basic building blocks and similar steps in ciliogenesis. As a result, disturbed organization of ciliogenesis leads to diseases called ciliopathies, which are often systemic.

This raises the question of how this complexity can be achieved through the self-organized and conserved process of ciliogenesis. One possible mechanism is the post-transcriptional action of alternative splicing, performed by tissue-specific splicing factors. We have uncovered that Transformer 2β (Tra2b) regulates correct splicing of a coherent set of targets in MCCs of the *Xenopus* embryonic mucociliary epidermis as well as the primary ciliated neural tube. Loss of Tra2b or interference with correct splicing of its targets leads to defective cilia formation and function across tissues in *Xenopus*. These defects arise through mis-splicing leading to the formation of loss-of-function isoforms as well as through events causing expression of alternative isoforms with altered functions. In ongoing work, we are investigating how different isoforms contribute to ciliogenesis and cell type-specific cilia functions during vertebrate development. This work sheds light on post-transcriptional contributions to the generation of ciliary functional and morphological diversity in animals with implications for human ciliopathies.

Keywords: Xenopus, Cilia, Splicing, Tra2b, Ciliopathies

*Speaker

Improvements to the v10 Xenopus gene annotations via manual curation at Xenbase

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Xenbase, the *Xenopus* Model Organism Knowledgebase (www.xenbase.org) is the centralized resource for genomics and biological sciences supporting the international *Xenopus* research community. Xenbase plays a critical role by supporting discovery science in numerous biomedical fields including, developmental, cell and cancer biology and human disease modeling. Key questions are often first explored in the two major model frogs, *Xenopus tropicalis* and *X. laevis* . The *Xenopus* genomes, version 10 of which were released in 2019 for *X. tropicalis*, and 2021 for *X. laevis*, underpin all modern molecular research. Xenbase ingests the genome assemblies from several sources, primarily the JGI-UC Berkeley *Xenopus* genome group, the NCBI and EBI-EMBL, to produce Xenbase gene pages. We then integrate genomic data across Xenbase, including display of genomes on JBrowse, an interactive genome viewer, and we produce a general feature format (GFF) genome annotation file, which is needed for high throughput RNA-Seq and ChIP-Seq analyses. Unfortunately, the most recent genome builds for *X. laevis* and *X. tropicalis* had thousands of instances where previously named and/or well characterized genes lost key identifying metadata, including gene names and symbols. This presented a major obstacle for Xenopus researchers, reducing the impact and utility of the latest *X. laevis* and *X. tropicalis* genome annotations. We took on the challenge to improve the nomenclature and annotation issues for the v10 genomes, assessing as many genes and gene families as possible. Here, we outline the various approaches and strategies we have undertaken to address gene annotations for 1000s of genes and for several large gene families in the v10 genomes. Based on protein sequence identity, we recovered hundreds of annotations from v9, applying them to v10 gene models. Using a suite of synteny and homology tools we were able to characterize and provisionally name 1000s of genes that completely lacked proper names (i.e., anonymous 'LOC' number and 'uncharacterized' protein). Furthermore, using a combination of phylogenetic analysis and synteny patterns we assessed several large gene families, naming 100s of genes belonging to the Olfactory Receptors (OR), Major Histocompatibility Complex (MHCs), interleukin immune genes, heat shock proteins (HSPB), the FC receptors, and others. Importantly, we are sharing our genomics data with UniProt and the NCBI, as well as the Alliance of Genome Resources to facilitate future integration with the DIOPT pipeline, which will identify cross-species gene orthologs from protein sequences. We continue to collaborate with *Xenopus* researchers, the HGNC and domain experts to resolve gene nomenclature issues. Reach out if you have any questions about your genes! Email us at xenbase@cchmc.org. Xenbase is funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD/NIH).

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Keywords: Xenopus genome genes nomenclature orthology

Xenbase: latest support for genomics and disease models.

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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 our 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).

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The use of Xenopus oocytes for modelling neurological disease for novel drug discovery

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Given their rather large size and ease of manipulation, Xenopus oocytes, have allowed multiple applications ranging from studies of the embryo development up to intracellular or intranuclear injection of hexogen genetic material for recombinant expression.

Becoming a workhorse for electrophysiology, Xenopus oocytes are amenable to automated recordings using the two electrodes voltage clamp technique. Multiple examples spanning from ligand gated ion channels to transporters illustrate have shown the benefits offered from using Xenopus oocytes. In a broader scope, studies range from the characterization of gene mutations to the discovery of novel treatments for disorders of the central nervous system (CNS). As progress in genetics and molecular biology highlight large functional differences arising from a single to a few amino acid exchanges, the need for drug screening and functional testing against human proteins is increasing. The use of Xenopus oocytes to enable precise modelling and characterization of clinically relevant genetic variants constitutes a powerful model system that can be used to inform various aspects of CNS drug discovery. Data presented herein illustrate some data acquired at ligand gated, voltage gated, GPCR and transporters expressed at HiQScreen using recombinant methods.

Keywords: CNS, disease, model, genetic, pharmacology

*Speaker

Shaping the neuroinflammatory environment to promote retinal regeneration

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Teleost or amphibian Müller glia exhibit stem cell characteristics and are responsible for regenerating retinal neurons following injury, while their mammalian counterparts are unable to do so. Our lab interest is to dig into the molecular mechanisms that underlie such divergent regenerative properties. Indeed, we unexpectedly discovered that Müller cell-dependent retinal regeneration is hampered in young Xenopus tadpoles while being efficient in old pre-metamorphic ones. Interestingly, we found a remarkable correlation between these different Müller cell capacities and the status of microglia. Besides, our functional analysis revealed that young Müller cells could be forced to exit quiescence by providing immune stimulation, while the proliferative response of old Müller cells can be reduced when inflammatory signals are lowered. These data suggested that Müller glia cells' differential responses to injury may originate from differences in the neuroinflammatory niche. This work should contribute to a better understanding of the coupling between inflammation and regeneration.

*Speaker

The transmembrane receptor PTK7 is transferred between migrating Xenopus neural crest cells

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The neural crest (NC) is an excellent *in vivo* model to study questions of cell-cell communication in migrating cells. NC cells are a transient embryonic cell population that migrates extensively throughout the embryo contributing to various tissues. NC migration is controlled by a combination of chemotaxis, repellent guidance cues and dynamic cell-cell interactions, however, the precise molecular mechanisms as well as the means of signal integration are still a topic of ongoing research. Recently extracellular vesicles (EVs) have also been implicated in the regulation of NC migration. A well-known regulator of NC migration that is likely exchanged via EVs is the protein tyrosine kinase 7 (PTK7). PTK7 is a transmembrane protein required for contact inhibition of locomotion (CIL), a phenomenon by which NC cells change their directionality after cell-cell contact. PTK7 has been shown to function as a Wnt co-receptor and is endocytosed via a caveolin-1α-mediated pathway. Interestingly, we observed that *Xenopus* NC cells expressing PTK7-GFP were able to transfer this molecule to control NC cell. Therefore, we hypothesized that PTK7 may be exchanged via EVs in *Xenopus* NC cells. To test this, we co-expressed PTK7 and CD63, a member of the tetraspanin family, which are transmembrane proteins associated with plasma membrane and endosomes, and co-cultured these cells with control NC cells. Indeed, we observed an uptake of PTK7- and CD63-positive vesicles in control cells, further suggesting a transfer of PTK7 by EVs. Downregulation of CD63 did not affect the exchange of PTK7-positive EVs between NC cells. Currently, we are further analyzing the molecular mechanism regulating PTK7-positive vesicle secretion and their uptake in recipient cells.

Keywords: Neural crest (NC), extracellular vesicles (EVs), PTK7, CD63

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Comparative Analysis of Maternal Transcript Localization in selected vertebrate Oocytes

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The fusion of the oocyte and sperm to produce an intricate and complex organism remains a captivating subject within developmental biology. Spatially and temporally regulated molecules play a crucial role in tightly regulating embryogenesis. This research aims to fill the gap in our understanding of the initial animal-vegetal distribution in vertebrates. It does so by using high throughput RNA tomography sequencing to analyze and compare the maternal transcriptome and its sub-compartmentalization within the eggs of four distantly related models: African clawed frog (*Xenopus laevis*), axolotl (*Ambystoma mexicanum*), sturgeon (*Acipenser ruthenus*), and zebrafish (*Danio rerio*). Our findings reveal distinct animal-vegetal gradients containing sub-profiles of transcripts, with low conservation of vegetal transcripts across all models, while a higher degree of conservation was observed within the animal region. Despite this low conservation, we observed similar germ plasm-related vegetally localized transcripts, cell cycle-related animally localized genes, shared universal motifs, and localization pathways, which may point to some conservation with a common ancestor or functional convergence. Additionally, our study provides insights into the temporal dynamics of transcript localization during oocyte maturation, highlighting both the early and late vegetal pathways and, for the first time, early and late animal pathways. Overall, our results point to a dynamically altered maternal transcript landscape that is sublocalized within the egg, showing low conservation of localization across species. These findings have significant implications for our understanding of embryonic development and the evolutionary conservation of maternal transcript localization mechanisms across diverse vertebrate species.

Keywords: Tomo, Seq, maternal transcript localization, oocyte maturation, evolution

*Speaker

The role of the adaptive immune system during limb regeneration

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Salamanders are animals with a high regeneration ability, including the ability to regenerate limbs. After the loss of a limb the wound rapidly closes and a plethora of immune cells are present, their role still being defined. The immune system has been linked to both pro- and anti-regenerative roles and defining the impact of innate and adaptive immunity is critical to our
understanding of regeneration. The aim of this project is to elucidate phenotypically and functionally the cellular diversity of the salamander adaptive immune system, with the long-term
goal of uncovering the role of adaptive immune cells during limb regeneration. To be able to analyze the lymphocyte population, these cells need to be separated from erythrocytes. However, conventional methods have not been successful with salamander cells. Therefore, we are creating a targeted sorting approach for lymphocytes, we plan on harnessing the novel image enabled FACS-Sorter (BD FACSDiscover S8), which allows us to sort cells based on their morphology, and the use of antibodies. Subsequently we aim to establish the mixed lymphocyte reaction (MLR) assay. This method has been used in different species to investigate various B and T cell behavior (Salvadori & Tournefier, 1996). It stimulates lymphocytes isolated from one individual against irradiated allogeneic tissue from another. This will allow for a system to parse apart adaptive immune responses and different immunomodulatory tissue environments. The image enabled FACS will further allow us to sort the stimulated population of interest based on image-based data and morphology. This ongoing research will further illuminate the characteristics of the adaptive immune system of salamanders and its effects on regenerating tissue.

*Speaker

Transcriptional control of early nephrogenesis in Xenopus tropicalis

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Background: The transcription factors Pax8, Hnf1b, and Sall1 underpin early kidney development, and are associated with Congenital Anomalies of the Kidney and Urinary Tract (CAKUT). They are localised in distinct regions within the ureteric bud epithelium, and metanephric mesenchyme, which interact to drive kidney formation. Curiously, however, these factors are roughly co-expressed within the same developing pronephric region in *Xenopus*. Further understanding of their functions could uncover insights into the transcriptome underlying nephrogenesis.
Methods: We visualised cells in the developing pronephros, by confocal microscopy. Injections were combined with CRISPR/Cas9 knock-out (KO) of *pax8*, *hnf1b*, or *sall1* to analyse effects on kidney morphology. Termed single embryo transcriptomics (seRNA-seq), we performed CRISPR/Cas9 KO of *pax8*, *hnf1b* and *sall1* in individual embryos and bulk RNA-seq at an early stage. SeRNA-seq was intersected with a kidney-enriched *X. tropicalis* dataset to filter for renal genes. Localisation of seRNA-seq targets was performed using in situ hybridisation (ISH).

Results: We characterised pronephros development in molecular detail, highlighting key stages, from mesenchyme condensation to epithelialisation. KO of *pax8*, *hnf1b*, or *sall1* disrupted size, cell organisation and the basement membrane, underscoring their importance in proper pronephros formation. Considering this, we explored their roles in the transcriptional network using CRISPR/Cas9 and seRNA-seq. Renal and morphogenetic genes were identified (e.g. *slc12a1* up- and downregulated in *pax8 and hnf1b* KO respectively, *twist1* upregulated in *pax8* and *hnf1b* KO*).* Extracellular matrix genes (*lama1*, *col4a6*) were downregulated in the *sall1* KO. ISH validation of seRNA-seq revealed expression of several targets within the early and developed pronephros (e.g. *agpat3*, *traf4*).
Conclusions: Pax8, Hnf1b and Sall1 are key regulators nephrogenesis, and influence a network of renal and morphogenetic targets in *X. tropicalis*. Clarifying their roles will improve understanding of inherited kidney malformations and disease.

Keywords: kidney, kidney development, genetic kidney diseases, pronephros, RNAseq, xenopus

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Maturation of abducens motoneurons involved in the angular vestibulo-ocular reflex during larval development.

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During Gaze stabilization extraocular muscles produce reflexive compensatory eye movements to fix the image on the retina. Among such a complex sensorimotor transformation the vestibulo-ocular reflexes (VOR), generating compensatory eye movements in response to head motion, require vestibular inputs which are integrated by extraocular motoneurons.
In Xenopus larvae, the onset of the angular VOR (aVOR) is delayed compare to other visuo-vestibular reflexes. This delay could imply the existence of distinct parallel pathways in vestibulo-ocular networks, involving distinct motoneuron functional subpopulations.

Although relevant for understanding the establishment of these parallel pathways, the maturation processes of abducens motoneurons have not been elucidated. This led us to investigate how their properties differ during the establishment of the aVOR during pre-metamorphosis life.

Firstly, we performed electrophysiological recordings of unitary nerve discharges on *in vitro* isolated head preparations during passive head rotations. The abducens nerve discharge analysis, based on their spike amplitude, revealed different motor unit groups with distinct maturation patterns from the aVOR onset (stage 49-50) to a more mature stage (stage55-56). These results will be complemented with immunohistochemistry (Kv1.1, Kv3.1b, Cav3.3) and electron microscopy experiments, investigating the molecular phenotypes the axon myelination and neuronal size between these two stages.
In the future, we intend to perform the same approach on other extraocular motor nuclei, involved in ocular reflexes mature earlier, and in which we suspect different developmental patterns. These findings enhance our understanding of the development in neural networks controlling gaze-stabilizing ocular behavior in vertebrates.

Keywords: extraocular, abducens, motoneurons, ontogenesis, vestibulo ocular reflexes, xenopus

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Investigating the role of nuclear deformation in regulating cellular responses to strain rate

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Cells can sense and respond to mechanical forces in their environment, regulating various cell behaviours such as cell division, adhesion and migration. Forces are known to upregulate cell division by promoting cell cycle progression and mitotic entry. Recent discoveries revealed that this could occur through force-induced nuclear deformation. However, majority of these studies predispose the cells to fast strain rates. These strain rates do not recapitulate slower strain rates observed *in vivo*. Here, we investigate how strain rates affect nuclear deformation and cell division. By stretching *Xenopus laevis* animal cap tissues at varying rates, we showed that fast strain rate upregulates mitosis while slow strain does not. This is accompanied by a lack of nuclear deformation in slow-stretched tissue, unlike the nuclear flattening observed in fast-stretched tissues. Lastly, using morpholino-targeted knockdown of endogenous *Xenopus* Nesprin-2, we show that reduction in Nesprin-2 disrupts the nucleus' ability to deform accordingly to mechanical forces. Overall, our results indicate that strain rate differentially impacts nuclear deformation and cell division rate. This suggests that nuclear deformation may be vital for a cell's ability to regulate cell division in response to complex mechanical environment.

Keywords: cell division, strain rate, nuclear mechanic, cell shape, Nesprin, 2, nucleus

*Speaker

Investigating mechanisms of neuromuscular matching during nerve regeneration in Ambystoma mexicanum

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Tetrapods rely on the precise wiring of motor neuron axons to specific muscle fibers in the periphery to perform a wide range of behaviors. In mammals, damage to peripheral nerves often results in a complete loss of neuromuscular control from the affected nerve. In contrast, axolotls can fully restore function after the severing of all major nerves innervating a limb. Pioneering work from the mid-20th century revealed that the major motor nerves in axolotl limbs can re-innervate their target muscle territories and outcompete foreign nerves that had innervated in their absence. However, due to the technical limitations of the time, researchers could not determine the accuracy of nerve regeneration at the single axon level or probe the molecular mechanisms guiding the precise reattachment of nerves to muscles. I propose to use modern molecular and genetic techniques to answer these outstanding questions. To address the precision of re-targeting at the single axon level, I am generating novel brainbow animals that will allow me to trace single axons before and after regeneration in transparent axolotl limbs. Then, I will identify which cells within the muscle tissue are responsible for directing motor neurons to their targets. Finally, I will perform single-cell RNA sequencing and bulk RNA sequencing to screen for molecules involved in neuromuscular matching. Together, these experiments will elucidate how regenerating motor axons select specific muscle targets in the axolotl, which may one day lead to new pathways for treating peripheral nerve injuries in humans.

Keywords: Axolotl, regeneration, neurobiology

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Patterning and functionality of the regenerated nervous system

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The axolotl is known for its extraordinary regenerative capabilities, and the axolotl tail is particularly suitable to study regeneration of the spinal cord and locomotor-related spinal circuits. It is non-essential, yet possesses all the structures that are found further up the body axis. Few studies have assessed the precision of regeneration from sensory input to activity to motion, and I aim to access the patterning and functionality of the regenerated nervous system on multiple levels: (1) to visualize and track neuron projection patterns, connectivity, and muscle innervation; (2) to compare swimming behaviors and the activity of neurons within and across muscle segments in control vs. regenerated animals, and (3) to study whether sensory input into central pattern generators (CPGs) is affected by regeneration. First results showed that the muscle tissue is not well segmented after regeneration, however, a transgenic CAGGS-GCaMP reporter revealed that regenerated muscle fibers are highly active when induced with neurotransmitters. While no obvious difference in swimming behavior was detected in regenerated animals, regenerated tails were less reactive to touch-induced escape responses. These preliminary results revealed that even if patterning is not fully reestablished, the regenerated neuronal network is functional. In the future, this study will tell us how accurate organ regeneration needs to be, to be still functional.

*Speaker

Importance of the Xenopus oocytes in transporter studies

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Transporters are indispensable proteins which allow the passage of ions or other molecules across the plasma membrane of cells. Usually divided between electrogenic and electroneutral transporters are encoded by a large number of genes and widely expressed throughout our body. While electrogenic transporters are easily characterized using electrophysiological approaches, electroneutral are more difficult to study. However, this difficulty can be circumvented using the co-expression of a reporter protein.
This is exemplified in the case of chloride transporters also referenced as CCC's. These chloride transporters can be divided in NKCC's and KCC's which either transport chloride using the energy provided by sodium and potassium gradients or working exclusively on the potassium gradient. In neurons, chloride transporters are known to play an important role during the development and expression of KCC2's is associated with a shift of the chloride reversal potential below the excitability threshold. Tightly associated with this shift of chloride equilibrium, neurotransmission, mediated by GABAA or glycine receptors, was shown to be either excitatory during development or inhibitory during a more mature phase. In addition, it was shown that changes in chloride equilibrium can be observed during overload of activity such as that observed during epileptic ictus.
When co-expressed in the Xenopus oocytes, GABAA receptors which are exquisitely sensitive to the chloride gradient, offer a possibility to indirectly assess the activity of chloride transporters such as NKCC's or KCC's. This work illustrates the feasibility of this technique to analyze the activity of CCC's in different experimental conditions.

Keywords: Transporters, Electrophysiology, Reporter assay, Xenopus oocytes

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Germ cell gene editing: a dual approach

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This research aims to develop techniques that expedite the production of genetically modified *Xenopus tropicalis* lines through germline gene editing. These methods enable targeting of essential genes and eliminate mosaic effects commonly seen with embryo gene editing. Moreover, by bypassing a generation, we intend to reduce the number of animals required for the creation of mutant and transgenic lines.
At present, *in vitro* spermatogenesis has proven successful in various aquatic species but has remained a challenge in *Xenopus* frogs. In this study, germ cells were isolated from enzymatically dissociated *Xenopus tropicalis* testes and maintained in culture. Long-term cell survival and germ cell maturation were observed. Additionally, nucleofection experiments revealed that spermatogonia can be transfected without excessive cell loss.
Other strategies, such as spermatogonial transplantation, are being investigated. The combination of gene editing with innovative techniques holds the potential to streamline the generation of genetically modified *Xenopus tropicalis* lines, as shown by the promising outcomes observed thus far.

Keywords: germ cells, spermatogenesis, gene editing, genetically modified lines, Xenopus tropicalis

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Xenbase photo, drawing and video repository

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Xenbase serves as a central hub for data on *Xenopus* frogs, from genomes, genes and literature to mutants, phenotypes and reagents for fuelling research. What is less known is we also host a large corpus of image and video content, and serve as a data repository for data associated with papers. If you have photos and video (or other data) linked to a paper we can archive this content to ensure it is available and accessible in the long term. The Xenbase drawings, photographs and videos of *Xenopus* and *Xenopus* development are powerful adjuvants for teaching and research presentations. Most of these are open source and can be freely reused, though as with all data, citing the source is requested. Some content has specific reuse terms. Some wonderful, very high resolution and interesting options exist as alternatives for use in your work, and some examples are illustrated in this poster. These images also enrich the Xenopus Anatomical Ontology, which can be browsed at xenbase.org under the Anatomy and Development menu.

Keywords: development, embryogenesis, adult behaviour

*Speaker

Characterizing the roles of the transcriptional co-repressor Groucho in signal integration during early development

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The embryonic epidermis of Xenopus laevis is an informative model for the development of mucociliary tissues, such as the mammalian airway epithelium. Signaling plays an important role shaping the cell type composition of mucociliary epithelia during development, resulting in fine-tuned amounts of secretory and multiciliated cells, which provide a number of important biological functions, from protection against pathogens, maintaining pH and oxygenation. Alterations in signaling affect cell fate and differentiation and, therefore, influence future tissue function and maintenance. Thus, it is important to study the signaling pathways involved in developing mucociliary tissues to better understand how signaling integration facilitates self-organized patterning of mucociliary cell type compositions. Combinatorial input of Wnt and Notch signaling is necessary for specification and differentiation of the mucociliary cell types. Both pathways employ the transcriptional co-repressor Groucho (Tle, transducin-like enhancer of split), which inhibits target gene expression in the absence of signaling input. Thus, Groucho activity is important for integrating signaling pathway activities by inhibiting transcription during development of mucociliary cell lineages.
Here, we demonstrate that Groucho loss-of-function perturbs the expression of cell type-specific markers during the specification phase of the Xenopus mucociliary epidermis. Furthermore, the mature epidermis showed impaired mucus production and cell death indicating a role for Groucho in the function and homeostasis of the mature tissue. These insights, in parallel with epigenetic and transcriptomic data, will help us elucidate how the activity of co-repressors affects signaling integration during mucociliary development.

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Role of cilia-powered fluid flow on pathogen clearance

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Xenopus embryos naturally produce a ciliated epithelium on their skin around the time of hatching, which can create a flow in the surrounding fluid. The disappearance of multiciliated cells (MCCs) later on suggests a specific role of this epithelium at these stages. Possible roles could include clearance of irritants (pathogens/toxic compounds), oxygenation, etc. Another interesting aspect is that MCCs normally intercalate into the outer layer in a regularly spaced pattern over the entire surface of the embryo during its development. We wanted to study the importance of this regular pattern in the ability to generate a flow. We worked to develop a numerical model of flows generated by a surface with MCCs. The parameters of the model were informed by the MCC densities and distributions on wild-type embryos. We used particle tracking to visualize streamlines and measure fluid velocities on explants (with MCC distributions comparable to embryos) in order to validate our model. Our model suggests that the system is robust against changes in the MCC pattern and can still clear pathogens (unless there are large areas devoid of MCCs). In order to test this experimentally, we either perturbed MCC differentiation (Foxj1 Crispr) to produce embryos without functional MCCs (no flow), or MCC migration (Scf/Kit pathway inhibition) to produce embryos with abnormal MCC distributions (altered flow). We then incubated these embryos with *Aeromonas hydrophila* and checked their susceptibility to infection by measuring mortality or CFUs (Colony Forming Units in homogenates of embryos). We found no significant changes in infection rate in the case of altered flows, but increased infections in the case of no flows. This supports the view that cilia-powered flow is important for pathogen clearance, but that regular MCC distribution is not, in agreement with predictions from our numerical simulations. Further work is needed to elucidate the function of MCC patterning in ciliated epidermis physiology.

Keywords: Xenopus embryo, ciliated epithelium, development, flow, multiciliated cells

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Establishing the Xenopus tadpole foregut as a newmucociliary model

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Mucociliary epithelia can be formed from all the three germ layers and are found across different animal species, lining multiple organs such as the epidermis, the airways, the foregut or the oviducts. Mucociliary epithelium is composed of multiciliated cells and multiple types of secretory cells. Multiciliated cells beat in a coordinated manner to generate a directional flow which function depends on the tissue. In the human airways and Xenopus embryonic epidermis its function is mucociliary clearance. Imbalance on cell type composition leads to pulmonary disorders. Therefore, understanding the gene regulatory networks and molecular mechanisms that determine cell type composition will contribute to a better diagnosis and new treatment options for chronic airway diseases.

Xenopus embryonic mucociliary epidermis is an established model to study ciliogenesis and gene regulatory networks that regulate cell type composition. Even if it has already generated great knowledge that contributes to our understanding of ciliopathies and airway diseases, it also presents some limitations based on major differences when compared to the human airways mucociliary epithelia: different embryonic origin, different tissue structure, different MCC spacing. To bridge this gap between the simple embryonic mucociliary epithelia and the complex airway mucociliary epithelia, we propose to study the Xenopus tadpoles foregut. This so far non-studied mucociliary tissue is from endodermal origin, presents similar MCC spacing as in the human airways, and its cell type composition varies along the longitudinal axis, as it is the case in the human airways. Therefore, I want to characterize the tadpole's foregut mucociliary epithelia at the level of cell type composition, regulatory networks, chromatin accesibility and tissue structure, to establish it as a new model to study mucociliary epithelia, allowing us to discern which features of mucociliary tissues are species specific, and which ones are linked to the embryonic origin.

1-3 JULY 2024 - BORDEAUX, France

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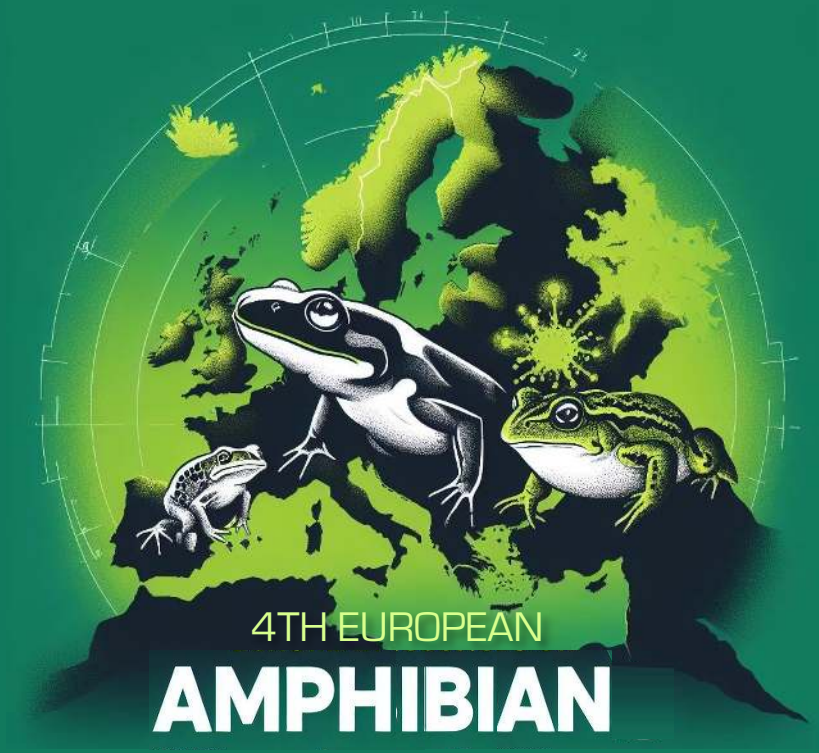

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David Dupasquier (France)
Aude Dupré (France)
Karen Echeverri (USA)
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