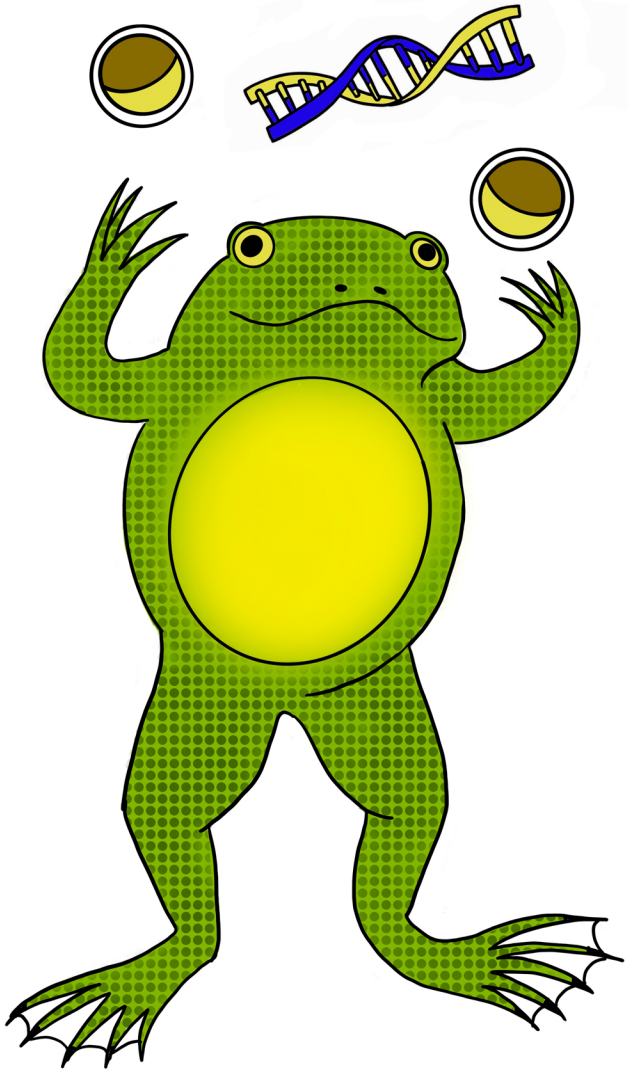
2024 *Xenopus* Resources and Emerging Technologies Meeting

Marine Biological Laboratory
Woods Hole, MA
October 11-14, 2024



Organizers

Helen Willsey
Jennifer Landino
Coral Zhou

Sponsored by:

Iwaki Aquatic
Aquaneering
Tecniplast
Xenopus 1
Xenopus Express

Friday October 11, 2024

Arrival Check-in at Swope

6:00-9:00 pm **BBQ Dinner** (MBL club)

Sponsors

Iwaki, Aquaneering, Tecniplast, *Xenopus* One and *Xenopus* Express

Saturday October 12, 2024

7:00-8:40 BREAKFAST

8:40-8:45 **Introduction and Welcoming Remarks (Speck Auditorium)**
Jennifer Landino, Coral Zhou, Helen Willsey

Session 1. Resources

8:45-9:00 Nikko-Ideen Shaidani (Marine Biological Laboratory)
Xenopus Mutant Resource

9:00-9:15 Annie Godwin (University of Portsmouth)
European Xenopus Resource Centre (EXRC) updates and priorities

9:15-9:30 Aaron Zorn (University of Cincinnati)
Xenbase: genome annotation improvements

9:30-9:45 Katie Stein (NIH)
NIH Funding and Resources for Xenopus Research

9:45-10:00 Jacques Robert (University of Rochester)
Xenopus Research Resource for Immunobiology (XRRI): new genetic tools and leading effort on biological impact of microplastic water contaminants

10:00-10:15 Gary Gorbsky (Oklahoma Medical Research Foundation)
Expanding the Toolset: Creation of Novel Xenopus laevis and Xenopus tropicalis Cell Lines and Their Applications in Gene Editing

10:15-10:30 Dominique Alfandari (University of Massachusetts, Amherst)
Producing novel monoclonal antibody to Xenopus, Axolotl and Mouse to improve rigor and reproducibility

10:30-10:45 Doug Houston (University of Iowa)
DSHB: Sharing monoclonal antibodies through open science

10:45-11:00 COFFEE BREAK

Session 2. Development & Disease Modeling I

11:00-11:15 Jakub (Kuba) Sedzinski (University of Copenhagen)
Phenotypic profiling of developing mucociliary epithelium

11:15-11:30 Rachel Miller (University of Texas Health, McGovern Medical School)
Advancing our understanding of kidney development and birth defects using innovative technologies

11:30-11:45 Casey Griffin (New York University)
Deciphering the mechanisms of Nager syndrome using Xenopus tropicalis

11:45-12:00 Andrea Wills (University of Washington)
Deciphering the contributions of carbohydrate and nucleotide metabolism to Xenopus regeneration

12:00-1:45 LUNCH

Session 3. Reconstitution Using Extracts

1:45-2:00 Yasuhiro Arimura (Fred Hutch Cancer Center)
Applying cryo-EM to Xenopus egg extract system to elucidate the structural basis of biological events on chromosomes

2:00-2:15 Susannah Rankin (Oklahoma Medical Research Foundation)
Protein tricks in extracts and embryos

2:15-2:30 Jesse (Jay) Gatlin (University of Wyoming)
The Shape of Extract: Creating Discrete Volumes of Cell-Free Xenopus Extracts to Study Fundamental Cellular Processes

2:30-2:45 Gembu Maryu (Yang Lab, University of Michigan)
Visualization and manipulation of Cdk1 oscillations in water-in-oil droplets

2:45-3:00 Martin Wühr (Princeton University)
Protein turnover measurements in embryogenesis

3:00-3:30 COFFEE BREAK

Session 4. Biophysical and Mathematical Modeling

3:30-3:45 Sarah Woolner (University of Manchester)
Measuring and applying tension in Xenopus: adventures with Flipper-TR and Lego

3:45-4:00 Lance Davidson (University of Pittsburgh)
New and improved! Tools to explore extreme mechanics of morphogenesis

4:00-4:15 Kristian Franze (University of Cambridge)
Measuring and manipulating tissue mechanics to understand brain development in Xenopus laevis

4:15-4:30 Shinuo Weng (Johns Hopkins University)
TFlux and Flex: Building Insights into Cell and Tissue Mechanics with Useful Fluctuation

Session 5. Development & Disease Modeling II

4:30-4:45 Adrian Thompson (Brown University)
Novel approaches for modeling neurodevelopmental effects of sodium channel dysregulation in the developing brain of Xenopus laevis tadpoles

4:45-5:00 Richard Behringer (University of Texas, MD Anderson Cancer Center)
Role of anti-Müllerian hormone in Xenopus tropicalis

5:00-5:15 David Vijatovic (Sweeney lab, Institute of Science and Technology Austria)
Adeno-Associated Viral Tools to Trace Neural Development and Connectivity across Amphibians

5:15-5:30 Vanja Stankic (Chen Lab, University of Texas, MD Anderson Cancer Center)
Cell at a Time: Unveiling the Cellular Landscape of Xenopus Lungs During Metamorphosis

5:30-5:45 Pat Kearns (University of Massachusetts, Boston)
There's what on my Xenopus? Manipulating the Xenopus laevis microbiome to understand immunological function and amphibian microbial ecology

5:45-6:00 Bruno Reversade (A*STAR)
Of Frogs & Men: modeling Mendelian disease to better understand disease pathogenesis

6:00-8:00 DINNER

KEYNOTE

8:00-9:00 Thomas Naert (University of Ghent)
Non-random DNA repair allows predictable genome engineering

9:00-11:00 MIXER @ Captain Kidd

Sunday October 13, 2024

7:00-8:45 BREAKFAST

Session 6. Omics

8:45-9:00 Leonid Peshkin (Harvard University)
XePA: Xenopus Protein Atlas

9:00-9:15 Jose Abreu (Harvard University)
Xenopus Embryo Atlas: every single cell?

9:15-9:30 Hui Chen (University of South Carolina)
Quantifying Nascent Transcription in Early Embryogenesis

9:30-9:45 Shinhyeok Chae (Ulsan National Institute of Science and Technology (UNIST)
Cross-species integration of Xenopus single-cell sequencing data

9:45-10:00 Nayeli Reyes-Nava (Wallingford lab, University of Texas at Austin)
Proteomics, AlphaFold, and disease modeling in Xenopus

10:00-10:15 Karel Dorey (University of Manchester)
Uncovering the mechanisms underpinning regenerative neurogenesis using single-cell transcriptomics

10:15-10:30 Can Aztekin (Swiss Federal Institute of Technology Lausanne)
Xenopus regeneration and new sequencing approaches

10:30-11:00 COFFEE BREAK

11:00-12:00 Junior Strategic Planning

12:00-1:30 LUNCH

1:30-2:30 STRATEGIC PLANNING (including relay of junior requests)

Session 7. Development & Disease Modeling III

2:30-2:45 Shiri Kult Perry (Shubin lab, University of Chicago)
The Xenopus respiratory system reveals common tetrapod mechanisms for growth, regeneration, and healing.

2:45-3:00 Nicole Edwards (Zorn Lab, Cincinnati Children's Hospital)
Discovering the developmental basis of endosome trafficking disorders and congenital anomalies using Xenopus

3:00-3:15 Lydia Youmans (University of Texas, Houston)
As the Frog Folds: Exploring Human Genetic Variants of Neural Tube Defects in Xenopus

3:15-3:30 Mustafa Khokha (Yale University)
Are ion channels morphogens? Surprising results inspired by patient based gene discovery.

3:30-3:45 Kris Vlemnickx (University of Ghent)
Modeling human cancer and inherited disease, expanding the experimental landscape to oncogenes and the non-coding genome

3:45-4:00 COFFEE BREAK

Continuation of Disease Modeling

4:00-4:15 Adrian Romero (Miller Lab, UTHealth Houston)
Frogs, Bone, and Kidney: Exploring Developmental Processes in Xenopus

4:15-4:30 Jack Govaerts (Schoborg Lab, University of Wyoming)
Building a 3-D Developmental Atlas of X. laevis with Microcomputed tomography

4:30-4:45 Engin Deniz (Yale University)
Unveiling the complexities of CSF circulation using Xenopus and OCT imaging

4:45-5:00 Andrea Vu (Robert Lab, University of Rochester)
T cell gene expression profiling in Xenopus laevis during Mycobacteria infection

Session 8. Cell Biology

5:00-5:15 Saurabh Kulkarni (University of Virginia)
Understanding multiciliogenesis in Xenopus

5:15-5:30 Enzo Bresteau (Northwestern University)
Apical Size Reduction by Macropinocytosis Alleviates Tissue Crowding

5:30-5:45 Jaeho Yoon (National Cancer Institute)
Limitations of Existing Proximity Labeling Methods and the Development of a New Approach

5:45-6:00 Leslie Sepaniac (Bement Lab, University of Wisconsin)
Synthetic constitution of traveling Rho GTPase waves at the cell cortex

6:00-8:00 DINNER

KEYNOTE

8:00-9:00 Rebecca Heald (University of California, Berkeley)
Using Xenopus to investigate the effects of ploidy on cell and developmental biology

9:00-11:00 MIXER @ Captain Kidd

Monday October 14, 2024

7:00-9:00 BREAKFAST

9:00-10:30 XRET GO Jamboree Workshop

10:30-12:00 Cryopreservation Workshop and NXR Tour

12:00 LUNCH AND DEPARTURE

Session 1. Resources

Xenopus Mutant Resource

Nikko-Ideen Shaidani, Kelsey Coppenrath, and Marko Horb
Eugene Bell Center for Regenerative Biology and Tissue Engineering and National Xenopus Resource, Marine Biological Laboratory, Woods Hole, MA USA

Over the last 10 years, the National *Xenopus* Resource has generated nearly 300 mutants to model human disease in *X. laevis* and *X. tropicalis*. To better engage the community and promote these mutants, we have established the NIH funded *Xenopus* Mutant Resource. Visitors can stay at an MBL cottage at no cost and utilize any of the mutant and transgenic lines at the NXR. Additionally, we can train scientists in husbandry, cryopreservation, genome editing, and work with visitors to establish new mutant and transgenic lines. We hope to provide incentive for labs to come to the NXR and collaborate across multiple disciplines. Here I present our progress with expansion of the NXR and opportunities for scientists to take advantage and advance their research goals.

European *Xenopus* Resource Centre (EXRC) updates and priorities

Annie Godwin
University of Portsmouth

The EXRC continues to make, curate, quality assure and distribute genetically altered *Xenopus*, wild-type animals, *Xenopus* “byproducts” and molecular reagents alongside acting as a research hotel for those needing to use its resources and expertise on site. The use of the centre has increased markedly, with a shift from live animal transport to sending of testes, frozen sperm, oocytes and embryos continuing. A series of new community requested transgenic animals, including inducible cas9 expression and multiple signalling reporter lines are in build or growing up. The mutant project to support clinical geneticists is going from strength to strength and these lines are becoming available to researchers wishing to undertake studies on the diseases they recapitulate. We are already beginning to plan for the next funding round and your input into what resources and activities should be prioritised is needed.

Xenbase Update and NIH Renewal Priorities

Malcolm Fisher[1], Andrew Bell[1], Vaneet Lotay[2], Ngoc Ly[1], Brad Arshinoff[2], Stanley Chu[2], Christina James-Zorn[1], Boluwatife Osifalujo[1], Troy Pells[2], Virgilio (VG) Ponferrada[1], Kamran Karimi[2], Dong Zhou (Joe) Wang[2], Taejoon Kwon[3], Peter Vize[2], Aaron Zorn[1]
[1]Xenbase, Cincinnati Children’s Hospital Division of Developmental Biology; [2]Xenbase, University of Calgary; [3]Ulsan National Institute of Science and Technology

We will share recent updates and ongoing work at Xenbase, including:
1. **Advances in Genome Annotation**: Thousands of new gene names and the continued maintenance of a Xenbase GFF to support bioinformatic analysis.
2. **Integration with External Resources**: Improved interoperability with other online biomedical knowledgebases, including NCBI, UniProt, Ensembl, and the Alliance of Genome Resources.
3. **Genome Browsers and Synteny**: JBrowse2 and tools for genomic synteny analysis.
4. **New *Xenopus* Media**: Expanded collection of *Xenopus* images and videos.

We seek community feedback as we prepare for the Xenbase NIH renewal application, due in January 2025. Your input will help prioritize improvements and new initiatives to accelerate your research.

NIH Funding and Resources for Xenopus Research

Kathryn Stein
Eunice Kennedy Shriver National Institute of Child Health and Human. Development, National Institutes of Health

This talk will cover NIH research priorities, current funding opportunities, and resources available for the Xenopus community.

***Xenopus* Research Resource for Immunobiology (XRRI): new genetic tools and leading effort on biological impact of microplastic water contaminants**

Jacques Robert
Department of Microbiology and Immunology University of Rochester Medical Center, Rochester, NY 14642, USA

Over the last few years, the XRRI has distributed MHC-defined inbred animals and reagents (e.g., antibodies, cell lines, recombinant ranavirus) to laboratories worldwide, as well as provided technical assistance, training and hosting multiple investigators. In collaboration with Xenbase and multiple investigators, we have continued to validate, define, improve the annotation and Xenbase pages of immune genes in the two *Xenopus* genomes. New genetic tools developed at XRRI include (1) the generation of a chytrid (Bd) stable tranfectant expressing tdTomato fluorescent reporter, which will facilitate future genetic modification of this devastating amphibian fungal pathogen; and (2) the implementation of a very efficient lentiviral transduction system to express recombinant proteins in *Xenopus* cell lines. Finally, the XRRI is now part of a new Rochester NY-based research center co-funded by NSF and NIH that aims to unravel health impacts of exposure to microplastics (MPs) in changing climate. We have leveraged *Xenopus* as a sensitive and reliable comparative experimental systems for investigating the bidistribution and effect of ingested MPs on the development of anti-microbial immunity. Notably, we found a rapid accumulation of MPs upon exposure within tadpole intestine, liver, and kidneys, persisting over a week. This accumulation leads to compromised antiviral immunity and diminished resistance against viral infections. These findings carry substantial significance, raising concerns not only for aquatic vertebrates but also for human health.

Expanding the Toolset: Creation of Novel *Xenopus laevis* and *Xenopus tropicalis* Cell Lines and Their Applications in Gene Editing

Gary Gorbsky
Oklahoma Medical Research Foundation

Xenopus tropicalis and *Xenopus laevis* are key research models in cell and developmental biology. To enhance the usefulness of these species, we have developed methods for generating spontaneous immortal cell lines from neurula stage embryos. The major obstacles to overcome in initiating cell cultures include bacterial and fungal contamination and promoting the adhesion of starter cells to culture surfaces. The use of alcohol washes and inclusion of antibiotics minimizes contamination. Coating of tissue culture surfaces with laminin-511 E8 fragment potentiates the adhesion of certain cell types. Cells are propagated using conventional culture methods as adherent cultures at a simple, diluted, low calcium L-15 medium containing fetal bovine serum without the use of a CO2 incubator. Individual cell lines were derived from cloning of starter cultures and the resultant cell lines have been propagated through many generations. Cloning of single cells was enhanced by inclusion of 50% medium conditioned by growth of the X. laevis S3 cell line. Most cell lines we have derived exhibit euploid karyotypes, though some display structural and numerical chromosome abnormalities. Various lines display distinct morphologies and are likely derived from distinct precursor cells from the embryos. Where completed, transcriptome analyses support the conclusion that lines are of distinct lineages. Electroporation has proven superior to lipid-based transfection reagents in exogene expression in *Xenopus* cell lines. CRISPR-Cas9 methods have been used for fluorescent tagging of endogenous genes. These approaches will be useful in the generation and manipulation of cell lines from normal and mutant strains of *X. tropicalis* and *X. laevis*. Cell lines can provide rapid and cost-effective sources of data in mapping physiological pathways and probing consequences of gene modification. Initial studies done with *Xenopus* cell lines can also aid in optimizing gene editing approaches that can be subsequently applied to modifications of embryos, increasing efficiency and reducing use of whole animals.

Producing novel monoclonal antibody to *Xenopus*, Axolotl and Mouse to improve rigor and reproducibility

Dominique Alfandari
University of Massachusetts, Amherst

Animal models are critical for biomedical research. They allow us to rapidly test the importance of any gene for development or diseases. Advances in technology now allow us to reduce or eliminate protein expression in the entire animal or in selected tissue. Similarly, mutations in the coding sequence of a gene associated with a disease can be introduce in animal model to test if they are indeed responsible for the disease state. Antibodies are critical tools to study protein localization and function. They can be used to block a protein function, send it for degradation, immunoprecipitate partners associated with the protein to identify functional complexes. While companies have focused on developing antibodies to human proteins, and to a lesser extend to mouse proteins, there is a need for antibodies that recognize proteins in diverse animal models. Despite the close similarity of protein sequences between human and mouse, many antibodies do not cross react. In addition, some proteins that have been recently identified or studied have not been targeted. Here we propose to produce antibodies against selected proteins in mouse, and amphibian (Xenopus and Axolotl) to facilitate the work in these animal models.

DSHB: Sharing monoclonal antibodies through open science

Doug Houston
University of Iowa

The Developmental Studies Hybridoma Bank (DSHB; https://dshb.biology.uiowa.edu) is an international resource dedicated to the sharing of monoclonal antibodies (mAbs) for research. Started in 1986 and housed at the University of Iowa since 1998, the DSHB's mission is threefold: 1) to bank and maintain the availability of important research monoclonal antibodies and hybridomas - without regard to commercial profitability; 2) to distribute these reagents at the lowest possible cost for non-profit research and teaching; and 3) to disseminate information on the 'art' of hybridoma technology and on the use and applicability of these antibodies. In addition to its growing collection of over 600 recombinant mAbs, the DSHB maintains over 5000 different hybridoma cell lines, many of which have been faithfully propagated and shared for over 40 years. This presentation is an overview of DSHB, our monoclonal antibody and web resources, and some research activities of DSHB.

Session 2. Development & Disease Modeling I

Phenotypic profiling of developing mucociliary epithelium

Jakub (Kuba) Sedzinski
University of Copenhagen

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 the tissue from initially pluripotent cells remains uncharacterized. The morphogenetic shaping and cell fate choices in the mucociliary epithelium 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. Similarly, with fate choices, neighboring cells end up in differing cell types as intercalating cell types become evenly spaced out, but at the same time cells also signal to each other to regulate their differentiation. 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 Xenopus mucociliary epithelium. Using explanted prospective epidermis, aim to 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. Simultaneously, we trace the positioning of different cell types throughout the development by visualizing the cell type specific marker genes in developmentally critical stages of the explant to link the cell trajectories to cell

types. The detailed backtracking of the cell's histories allows us to connect individual cell behaviors to cell fate. 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.

Advancing our understanding of kidney development and birth defects using innovative technologies

Amaya Craft[1,2], Brandy L. Walker[1,2], Vanja Krneta-Stankic[1,2], Mark E. Corkins[1,2], Ulrich Rothbauer[3,4] Rachel K. Miller[1,2,5,6]

[1]Department of Pediatrics, Pediatric Research Center, UTHealth McGovern Medical School, Houston, Texas 77030; [2]MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences, Program in Genetics and Epigenetics, Houston, Texas 77030; [3]Pharmaceutical Biotechnology, Eberhard-Karls University; [4]Tuebingen, Germany; §Natural and Medical Sciences Institute at the University of Tuebingen, Reutlingen, Germany; [5]Department of Genetics, University of Texas MD Anderson Cancer Center, Houston, Texas 77030; [6]MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences, Program in Biochemistry and Cell Biology, Houston, Texas 77030

Kidneys are vital organs that consist of epithelial tubes called nephrons, which act as filtration units to filter blood and concentrate metabolic waste into urine. Our group uses the frog (*Xenopus laevis*) embryonic kidney, called the pronephros, to understand the fundamental processes underlying nephrogenesis and to model human congenital anomalies of the kidney and urinary tract (CAKUT) resulting in defects in the formation of nephrons. The development of nephrons requires the condensation of mesenchymal nephron progenitor cells, followed by subsequent epithelialization and tubulogenesis. During this process, Wnt/beta-catenin signaling, which is required for condensation of nephron progenitors, must be attenuated for nephron differentiation and epithelialization to proceed. Prior studies suggest the planar cell polarity (PCP) Wnt trajectory may diminish beta-catenin-mediated signaling during this transition. However, a mechanism by which PCP components regulate Wnt-mediated gene expression in the nephron has not been identified. Our preliminary data indicate that loss of the PCP effector, Daam1, disrupts cadherin-mediated junctions resulting in the accumulation of beta-catenin in nephric nuclei, indicating a shift in beta-catenin activity from the junctional to the Wnt signaling pool. However, current tools to evaluate the subcellular localization of beta-catenin during development are difficult to visualize and evaluate. Furthermore, existing strategies do not enable the study of beta-catenin protein dynamics via timelapse imaging in vivo. Therefore, we have adopted a beta-catenin chromobody, which has been used in cell lines and recently in zebrafish, to visualize the beta-catenin interchange between the nuclear, cytoplasmic, and junctional pools during *Xenopus* development via time-lapse imaging. Our preliminary data indicate that the nuclear localization of beta-catenin is consistent with regions of active canonical Wnt signaling in the dorsal axis of late gastrula and the skin of tailbud embryos. Furthermore, junctional beta-catenin is observed in adhesive cells in the dorsal lip of the blastopore and the developing skin. While our goal is to understand the timing of the Wnt/beta-catenin and Wnt/PCP trajectories during nephron formation and the interplay between the junctional versus signaling pools of beta-catenin, we believe that this tool will be useful to the broader *Xenopus* community and has the potential to transform our understanding of beta-catenin dynamics during numerous developmental processes.

Deciphering the mechanisms of Nager syndrome using *Xenopus tropicalis*

Casey Griffin
New York University

Nager syndrome is a rare craniofacial and limb disorder characterized by midface retrusion, micrognathia, absent thumbs, and radial hypoplasia. This disorder results from haploinsufficiency of SF3B4 (splicing factor 3b, subunit 4), a component of the pre-mRNA spliceosomal machinery. The spliceosome is a complex of RNA and proteins that function together to remove introns and join exons from transcribed pre-mRNA. While the spliceosome is present and functions in all cells of the body, most spliceosomopathies – including Nager syndrome – are cell/tissue-specific in their pathology. In Nager syndrome patients, it is the neural crest (NC)-derived craniofacial skeletal structures that are primarily affected. To understand the pathomechanism underlying this condition, we generation a *Xenopus tropicalis* sf3b4 mutant line using the CRISPR/Cas9 gene editing technology. We have described the sf3b4 mutant phenotype at neurula, tail bud, and tadpole stages, and performed temporal RNA-sequencing analysis to characterize the splicing events and transcriptional changes underlying this phenotype. Our data show that while loss of one copy of sf3b4 is largely inconsequential in *Xenopus tropicalis*, homozygous deletion of sf3b4 causes splicing defects and massive gene dysregulation, which disrupt craniofacial development and survival.

Deciphering the contributions of carbohydrate and nucleotide metabolism to *Xenopus* regeneration

Andrea Wills
University of Washington

Session 3. Reconstitution Using Extracts

Applying cryo-EM to *Xenopus* egg extract system to elucidate the structural basis of biological events on chromosomes

Yasuhiro Arimura
Fred Hutch Cancer Center

Since my postdoctoral research in the Funabiki lab at the Rockefeller University, I have been focused on developing methods to visualize Cryo-EM structures of chromatin-associated complexes within cellular environments. By combining the *Xenopus* egg extract system and cryo-EM, I have established a method for isolating nucleosomes suitable for cryo-EM from interphase and metaphase chromosomes. This method facilitated the determination of high-resolution structures of nucleosomes as well as the metaphase specific linker histone H1.8-bound nucleosomes. However, although I have detected > 100 chromatin-associated complexes in my sample using mass spectrometry, determining the cryo-EM structure of these less-abundant

complexes was challenging due to their heterogeneity and the high sample concentrations (0.05-5 mg/mL) required for cryo-EM. To address this, I developed MagIC-cryo-EM, a technique that enables the structural analysis of target protein complexes by locally concentrating them on nanomagnetic beads. This method allows for the structural determination of target protein complexes in heterogeneous samples at concentrations as low as < 0.0005 mg/mL. The technique requires only 5 ng of sample per grid (including 2 ng of DNA), representing a 100- to 1,000-fold reduction in the amount of sample needed compared to traditional cryo-EM, making it feasible to determine the structures of numerous native chromatin-associated complexes. These works open the door for scientists in the *Xenopus* community to elucidate the structural basis of biological events in the egg extract.

Protein tricks in extracts and embryos

Jingrong Chen, Courtney Sansam, Chris Sansam, and Susannah Rankin
Oklahoma Medical Research Foundation, Oklahoma City, OK

My lab is interested in how nuclear events are coordinated with cell cycle progression, particularly during early development. We have been investigating the interaction between the chromosome cohesion machinery and a DNA replication protein called TICRR/Treslin. Our goal is to perform depletion and rescue experiments in which we assay specific mutations in TICRR and how they impact cohesin function. This is challenging because TICRR is large (~2000 amino acids) and may in fact form an obligate heterodimer with another large protein called MTBP. To circumvent these technical hurdles we have been exploring the use of protein assembly through use of the Spycatcher-Spytag system. In this system, appropriately tagged proteins spontaneously form covalent bonds, allowing assembly of large proteins by engineering smaller domains. We are currently testing whether proteins assembled through this method can be used to rescue replication in the *Xenopus* egg extract system. We have successfully assembled TICRR *in vitro* from its subdomains, and are currently testing these constructs in extracts from which TICRR has been depleted using Trim-Away. I will discuss current conclusions about the feasibility of using this approach in extracts and embryos.

The Shape of Extract: Creating Discrete Volumes of Cell-Free *Xenopus* Extracts to Study Fundamental Cellular Processes

Jay Gatlin
University of Wyoming

Cell-free extracts derived from *Xenopus* eggs are an established model system to study basic biological processes. The system's power stems largely from our ability to manipulate its progression through the cell cycle and to recapitulate fundamental cellular processes such as nuclear formation and mitotic spindle assembly. The open nature of extracts, i.e. the ability to simply add reagents via pipetting, is a major experimental advantage and contributes greatly to their experimental tractability. In this short talk, I hope to convince you that confining this open system into small and discrete volumes, effectively putting the genie back into the bottle, not only enables the pursuit of novel research questions, but also the exploration of unexpected emergent phenomena.

Visualization and manipulation of Cdk1 oscillations in water-in-oil droplets

Gembu Maryu
Yang Lab, University of Michigan

Xenopus egg extract has long served as a fundamental tool for studying cell cycle mechanisms. Building on this, we adopted a synthetic cell approach by encapsulating the extract into cell-sized water-in-oil droplets using a microfluidic device. This method enables single-cell analysis, overcoming ensemble averaging effects typically observed in bulk extract samples. To monitor real-time Cdk1 dynamics within this system, we developed a novel FRET biosensor, Cdk1-EV, which revealed autonomous, undamped oscillations. Our results show that the cell cycle periods in spatially homogeneous droplets (lacking a nucleus) are tunable by adjusting cyclin B1 mRNA concentrations, with clear pulsatile activation patterns. In contrast, droplets containing reconstituted nuclei demonstrated increased robustness, characterized by biphasic activation correlated with nuclear membrane breakdown. Current efforts focus on manipulating nuclear size and protein localization to investigate how spatial complexity influences the robustness and stability of these oscillatory periods.

Protein turnover measurements in embryogenesis

Martin Wühr
Princeton University

Session 4. Biophysical and Mathematical Modeling

Measuring and applying tension in *Xenopus*: adventures with Flipper-TR and Lego

Sarah Woolner
University of Manchester

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 coordinated fashion. Whilst we are beginning to understand the cellular mechanisms that link cell behaviour and force in single cells, a major gap 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 discuss some new work where we have developed an imaging pipeline to use Flipper-TR, a live cell membrane tension probe, in the animal cap to explore how membrane tension is impacted by the speed and magnitude of tissue stretch. I will also discuss a Lego stretch machine that we have built

which allows the user to adjust the speed of stretch application to better match strain rates seen in vivo during morphogenesis. By using Lego, we hope that this machine will allow more labs to try these experiments without the large financial commitment of buying a commercial system.

New and improved! Tools to explore extreme mechanics of morphogenesis

Lance Davidson
University of Pittsburgh

Rigorous biophysical and biochemical analysis of mechanobiology require the ability to quantify and control the kinematics of living tissues under native and experimentally generated strain. No where is this more evident than in studying epithelial morphogenesis in Xenopus where early development is accompanied by some of the highest tissue-scale strain rates observed in developmental models. We present two tools that aid these efforts. The first is a hardware toolkit, the TissueTractor that allows controlled application of high strain (> 100%) to organotypic explants of Xenopus embryos ranging from the animal cap to the neural plate while cells are visualized with high numerical aperture objectives. The TissueTractor is 3D printed and assembled in the lab, and allows rapid exchanges of samples, and can be used with a variety of mammalian cell types and primary cultures. The second is a software toolkit, StrainMapperJ that can be used to quickly visualize and quantify kinematic features such as strain and flow from time-lapse sequences. Quantitative kinematic values can be extracted from time-lapses collected from a range of imaging modalities such as stereoscope, brightfield, and confocal image pairs and sequences. These tools can be combined into a powerful analytic pipeline to expose and test mechanobiology feedback on the subcellular, cellular, and supracellular tissue scales

Measuring and manipulating tissue mechanics to understand brain development in *Xenopus laevis*

Kristian Franze
University of Cambridge

During the development of the brain, neuronal axons may grow over large distances before reaching their targets. In order to move, neurons have to exert forces on and thus mechanically interact with their environment. We here show that mechanical interactions of neurons with their environment are highly dynamic. Using time-lapse in vivo atomic force microscopy-based stiffness mapping, we identified stiffness gradients in the developing Xenopus laevis brain which contribute to regulating axon growth and pathfinding. Interfering with brain stiffness and mechanosensitive ion channels in vivo both led to similar aberrant neuronal growth patterns with reduced fasciculation and pathfinding errors. Ultimately, mechanical signals not only directly impacted neuronal growth but also indirectly by regulating the availability of and neuronal responses to chemical guidance cues, strongly suggesting that chemical and mechanical signaling pathways are intimately linked, and that their interaction is crucial for neuronal development.

TFlux and Flex: Gaining New Insights into Cell and Tissue Mechanics through Fluctuation Analysis

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Understanding the mechanics of biological systems is essential for dissecting complex processes such as tissue morphogenesis. However, current techniques for studying cellular mechanics in vivo often require specialized equipment or reagents and frequently lack sufficient spatiotemporal resolution. Here, we introduce you to an innovative technique, Tension by Transverse Fluctuation (TFlux). It is an image-based non-invasive method that offers time-resolved subcellular analysis using widely available fluorescent reporters and microscopes. Employing this technique, we have unveiled a planar cell polarity (PCP)-dependent mechanical difference in the cell cortex of individual cells engaged in convergent extension. Importantly, TFlux's non-invasive nature makes it highly adaptable to a wide array of biological contexts. Ongoing work focuses on integrating biophysical modeling and time-frequency analysis to uncover subcellular mechanical variations from various sources.

Session 5. Development & Disease Modeling II

Novel approaches for modeling neurodevelopmental effects of sodium channel dysregulation in the developing brain of *Xenopus laevis* tadpoles

Thompson, A, Toro C, Vaduva, C, Mendoza, D, Navarez, K, Thompson, R, Aizenman, C.
Brown University

$Na_v1.6$ channelopathies cause a devastating childhood disorder characterized by intellectual disability and infantile seizures. This epilepsy manifests as generalized recurrent seizures beginning in infancy that are often resistant to current medications. To develop effective treatment strategies, we must first understand the cellular mechanisms by which individual neurons and circuits transition and become abnormally excitable as a result of $Na_v1.6$ channel dysfunction. Answering these questions requires an experimentally tractable model system that has the necessary levels of analysis, ranging from single synapses to circuits, that can be used to study the molecular determinants of these events and serve as a high-throughput pipeline to help identify novel therapeutic targets. Our previous work revealed that modulation of $Na_v1.6$ channel expression is a key regulator of intrinsic excitability in neurons of the *Xenopus laevis* optic tectum during circuit development, and for homeostatic changes in excitability following a period of enhanced visual stimulation. Here, I will discuss how we have extended on these findings to describe a model of developmental $Na_v1.6$ channel dysfunction induced by rearing tadpoles in the specific $Na_v1.6$ channel inhibitor MV1312 and characterizing the molecular, biophysical, circuit and behavioral changes. I will show how chronic developmental inhibition of $Na_v1.6$ channels causes increased excitability of tectal neurons and hyperexcitability of the visual circuit, and that this abnormally high excitability is driven by a maladaptive increase in the amplitude of sodium currents. Additionally, I will discuss a new method for the automated quantification of behaviors performed

by *Xenopus laevis* tadpoles, and how this was used to demonstrate a spontaneous seizure phenotype in our model. Our findings reveal cellular and molecular mechanisms for how chronic $Na_v1.6$ channel dysfunction affects neuronal and circuit development and function during a disease-relevant window of embryonic development.

Anti-Müllerian hormone in *Xenopus tropicalis* sex development

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Anti-Müllerian hormone (AMH) induces the regression of the Müllerian ducts, an essential step for male differentiation in mammals. Recessive mutations in *Amh* or *Amh* receptor (*Amhr2*) genes in male mice, dogs, and humans result in the formation of a uterus and oviducts. A lack of AMH in female mice leads to accelerated ovarian follicle recruitment and premature depletion of the oocyte reserve. Teleost fishes do not have Müllerian ducts but do have *amh* and *amhr2* genes. *amh* and *amhr2* mutant teleosts that are genetic males can develop as fully sex-reversed phenotypic females. In some teleost species, *amh* on the Y chromosome functions as the testis-determining gene. *amh* and *amhr2* mutant teleosts that are genetic females have enlarged ovaries caused by unregulated germ cell proliferation. Thus, the AMH signaling pathway regulates a range of reproductive organ phenotypes across vertebrate species. Mammals, teleost fishes, and amphibians share a common ancestor. To investigate the role of AMH signaling during amphibian reproductive organ development, we used CRISPR-Cas gene editing to generate *amh* and *amhr2* mutant *Xenopus tropicalis*. A line of frogs with a 5-bp deletion in exon 2 of the *amh* gene was isolated. ~50% of genetic males homozygous for this allele developed as fully sex-reversed females. The sex-reversed females formed ovaries with oocytes and oviducts that were identical to control females. These findings indicate that *amh* is required for male development in *Xenopus tropicalis*.

Adeno-Associated Viral Tools to Trace Neural Development and Connectivity Across Amphibians

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The development, evolution, and function of the vertebrate central nervous system (CNS) can be best studied using diverse model organisms. Amphibians, with their unique phylogenetic position at the transition between aquatic and terrestrial lifestyles, are valuable for understanding the origin and evolution of the tetrapod brain and spinal cord. Their metamorphic developmental transitions and unique regenerative abilities also facilitate the discovery of mechanisms for neural circuit remodeling and replacement. The genetic toolkit for amphibians, however, remains limited, with only a few species having sequenced genomes and a small number of transgenic lines available. In mammals, recombinant adeno-associated viral vectors (AAVs) have become a powerful alternative to genome modification for visualizing and perturbing the nervous system. AAVs are DNA viruses that enable neuronal transduction in both developing and adult animals with low toxicity and spatial, temporal, and cell-type specificity. However, AAVs have never been shown to transduce amphibian cells efficiently. To bridge this gap, we established a simple, scalable, and robust strategy to screen AAV serotypes in three distantly related amphibian species: the frogs *Xenopus laevis* and *Pelophylax bedriagae,* and the salamander *Pleurodeles waltl,* in both developing and post-metamorphic animals. For each species, we successfully identified at least two AAV serotypes capable of infecting the CNS. In addition, we developed an AAV-based strategy that targets isochronic cohorts of developing neurons – a critical tool for parsing neural circuit assembly. Finally, to enable visualization and manipulation of neural circuits, we identified AAV variants for retrograde tracing of neuronal projections in post-metamorphic animals. Our findings expand the toolkit for amphibians to include AAVs, establish a generalizable workflow for AAV screening in non-canonical research organisms, generate testable hypotheses for the evolution of AAV tropism, and lay the foundation for modern cross-species comparisons of vertebrate CNS development, function, and evolution.

One Cell at a Time: Unveiling the Cellular Landscape of *Xenopus* Lungs During Metamorphosis

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Evolution intricately shapes organ structures to meet the unique functional demands of each species. Traditionally, the molecular mechanisms behind these adaptations have been explored at the DNA level, often overlooking the critical cellular context in which they operate. Advances in single-cell genomics now enable more precise links between phenotypes and genotypes within relevant cell types. The overall lung morphology between mammals and amphibians differs significantly, with alveologenesis—the formation of gas exchange sites—evolving through distinct processes. In mammals, terminal alveologenesis results in alveoli forming at the ends of the respiratory tree. In contrast, during metamorphosis, frogs undergo a process we termed radial alveologenesis, where alveoli form around an air-conducting canal. By integrating single-cell RNA

sequencing, multiscale high-resolution imaging, and CRISPR-based gene editing, we are mapping the morphological and transcriptional landscapes that drive radial alveologenesis. Focusing on key gene networks, such as those regulated by SOX2, we are investigating how conserved molecular pathways shape radial alveologenesis and contribute to the development of divergent lung structures. Uncovering these species-specific designs promises not only to highlight nature's ingenuity but also to reveal previously unknown regulatory nodes, opening new avenues for bioengineering and therapeutic innovations.

There's what on my *Xenopus*? Manipulating the *Xenopus laevis* microbiome to understand immunological function and amphibian microbial ecology

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The microbiome, or the collection of microorganisms associated with a specific habitat, plays a key role in host function and well-being. In particular, the skin microbiome of amphibians plays a key role in protection from fungal pathogens including chytrid and filamentous fungi. Here, we discuss techniques to create gnotobiotic *Xenopus laevis* tadpoles and their interaction and protective role against the amphibian pathogen, Fusarium keratoplasticum. In addition, we explore microbiome and peptide differences between wild caught *X. laevis* from South Africa and lab-raised animals.

Of Frogs & Men: modeling Mendelian disease to better understand disease pathogenesis

Bruno Reversade
A*STAR

KEYNOTE

Pythia: Non-random DNA repair allows predictable CRISPR/Cas9 integration and gene editing

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CRISPR-based genome engineering holds enormous promise for basic science and therapeutic applications. Integrating and editing DNA sequences is still challenging in many cellular contexts, largely due to insufficient control of the repair process. We find that repair at the genome-cargo interface is predictable by deep-learning models and adheres to sequence context specific rules. Based on in silico predictions, we devised a strategy of triplet base-pair repeat repair arms that correspond to microhomologies at double-strand breaks (trimologies), which facilitated integration of large cargo (>2 kb) and protected the targeted locus and transgene from excessive damage. Successful integrations occurred in >30 loci in human cells and in in vivo models. Germline transmissible transgene integration in Xenopus, and endogenous tagging of tubulin in adult mice brains demonstrated integration during early embryonic cleavage and in non-dividing differentiated cells. Further, optimal repair arms for single- or double nucleotide edits were predictable and facilitated small edits in vitro and in vivo using oligonucleotide templates.
We provide a design-tool (Pythia-editing.org) to optimize custom integration, tagging or editing strategies. Pythia will facilitate genomic integration and editing for experimental and therapeutic purposes for a wider range of target cell types and applications.

BioRxiv: https://doi.org/10.1101/2024.09.23.614424
Competing Interest Statement
T.N. and S.S.L. have filed a patent application (EP23192134.7) in relationship to this work.

Session 6. Omics

XePA: *Xenopus* Protein Atlas

Leonid Peshkin
Harvard University

Understanding the protein expression across tissues and organisms is crucial for elucidating functional similarities and differences in physiological processes and thus selecting the right model organism in biomedical studies. Here, we present a comprehensive analysis of protein repertoires across 25 tissues in *Xenopus* frogs, utilizing advanced quantitative mass spectrometry to measure the levels of over 15,000 proteins. By comparing these profiles, we reveal tissue-specific expression patterns and identify conserved proteins shared across diverse organs. https://xenopus.hms.harvard.edu/proteomics/

Xenopus Embryo Atlas: every single cell?

Jose Abreu
Harvard University

When the original single-cell atlas of *Xenopus* embryo was published in 2018, we did not know what fraction of cells and cell types were reflected and missed the localization. We have developed workflows that allow us to count every single cell and potentially profile every single cell. In this talk, I will present our advances and discuss the tradeoffs as well as exciting questions from developmental and evolutionary perspectives that we ask in our group. I will describe and share resources we created that are available immediately and outline forthcoming updates to these resources.

Quantifying Nascent Transcription in Early Embryogenesis

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Early embryogenesis requires a transition of developmental control from maternal factors to zygotic genes that are newly transcribed at the early stages of development. Zygotic genome activation (ZGA), conserved in metazoans, is essential for early cell fate specification and germ layer formation, and dysregulation of ZGA can lead to severe consequences ranging from developmental defects to embryonic death. Therefore, study how ZGA is regulated is important for our understanding of not only genome regulation and cell fate decisions in early development but also the basis of developmental disorders. However, due to the presence of abundant maternal transcripts in the early embryos, a major challenge has been to accurately characterize the genes newly transcribed during ZGA. To overcome this, using *Xenopus* early embryos as a model, we have developed novel methodologies to metabolically label the nascent transcripts with 5-ethynyl uridine (5-EU) followed by click reaction to conjugate fluorophores for in situ imaging or biotin for EU-RNA isolation and sequencing. The highly sensitive and selective method allowed us to

specifically track and quantify the accumulated nascent transcripts during ZGA. Confocal imaging of nascent transcription in single cells of wholemount embryos revealed a new gradual spatiotemporal pattern of ZGA in the blastula where the animal pole initiates ZGA earlier than the vegetal pole, primarily dictated by a cell size threshold. Nascent transcriptome analysis on whole embryos and dissected regions uncovered spatially distinct patterns of gene activation and a temporally sequential activation of germ layer-specific genes at the mid-blastula stages - most ectodermal genes are activated earlier than the endodermal genes - that is linked to the single-cell view of ZGA. Importantly, our method has been demonstrated to be applicable in other embryonic systems, including zebrafish and mouse early embryos. We conclude that quantification of the nascent transcription using imaging and sequencing methods represents a powerful to study the regulatory mechanisms of ZGA, cell fate specification and germ layer initiation in early embryogenesis.

Cross-species integration of *Xenopus* single-cell sequencing data

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Single-cell RNA sequencing has revolutionized our understanding of the role of heterogeneous cell population and their gene regulation dynamics at the individual cell level, becoming an essential tool in cell and developmental biology. Furthermore, it allows us to analyze conserved and divergent functions of various cells across multiple taxa with the guidance of orthologous genes. However, due to the duplicated L and S genes, the normalization and the cross-species integration are challenging in *Xenopus laevis*, even against its close relative *Xenopus tropicalis*. In this talk, I will discuss the *Xenopus laevi*s single-cell data normalization and integration methods I have investigated to compare to other species, including *X. tropicalis*, mice, and humans. Together with canonical correlation analysis (CCA), this approach can successfully identify conserved cell clusters in different species and their shared molecular signatures. I will also show how each method can affect the result of cell clustering and differentially expressed gene identification with the example of mucociliary epithelium and spinal cord single-cell data. Additionally, I will discuss the SPLiT-seq, which allows smaller-scale single-cell analysis without the instrument and may be suitable for less complex tissues in *Xenopus* development.

Proteomics, AlphaFold, and disease modeling in *Xenopus*

Nayeli G. Reyes-Nava

The University of Texas at Austin

Cilia are complex microtubule-based projections with both sensory and motor functions. Axonemal dyneins drive the coordinated beating of motile cilia, which play critical roles in human airway biology and disease. Mutation of the human LRRC56 gene has been linked to motile ciliopathies,

and while LRRC56 is essential for cilia beating in both humans and model organisms, the underlying mechanisms remain unclear. We have used the power of *Xenopus* multiciliated cells for live imaging and proteomics to investigate the cellular and molecular dynamics of Lrrc56. We found that Lrrc56 localizes within cytosolic foci known as DynAPs, basal bodies, and mature axonemes. We observed specific loss of outer dynein arms (ODAs) upon knockdown of lrrc56, suggesting that Lrrc56 is a crucial player on axonemal dynein transport. ODAs are pre-assembled in the cytoplasm and transported through the cytoplasm to the base of cilia, and our laboratory has shown that this is a dynamic and multiprotein process. We have used affinity purification mass-spectrometry (AP-MS) of in vivo interaction partners, combined with AlphaFold modeling to identify and validate candidate interacting proteins of Lrrc56. Our findings will provide new depth and breadth to our understanding of axonemal dynein transport in vertebrates and its contribution to ciliopathies. This project was funded by the NIH (R01HD085901) and the UT Austin Provost's Early Career Fellows program.

Uncovering the mechanisms underpinning regenerative neurogenesis using single-cell transcriptomics

Moussira Alameddine, Lauren Phipps, Shinhyeok Chae, Taejoon Kwon and Karel Dorey
University of Manchester

Mammals have limited regenerative capabilities of their central nervous system. In contrast, amphibians such as Xenopus tadpoles can regenerate a fully functional spinal cord, following amputation. A hallmark of spinal cord regeneration is the re-activation of Sox2+ neural progenitor cells (NPCs) to promote the generation of new neurons. However, how is neuronal differentiation controlled during regeneration and whether the transcriptional programme differ during developmental and regenerative neurogenesis are still unresolved issues. To answer these questions, we have established techniques to perform bulk and single cell RNA sequencing on isolated spinal cord during regeneration in Xenopus tropicalis. These experiments have identified all the main cell types present in the mammalian spinal cord, establishing Xenopus as a relevant model to study spinal cord regeneration. Comparison of the transcriptome before and 3 days post injury has uncovered a population or regeneration-specific NPCs characterised by changes in cell cycle dynamics. Furthermore, Monocle analyses of the dataset suggest that the differentiation programme of NPCs differ significantly in the uninjured and regenerating spinal cord. To understand the transcriptional trajectory driving neurogenesis after injury, we are developing techniques to perform multiplex single cell / single nuclei RNAseq on regenerating spinal cords at different timepoints post-injury. Altogether, these data highlight the power of transcriptomics approaches to complex biological problems, by allowing us to identify new factors required for neurogenesis after injury, leading to successful spinal cord regeneration.

Xenopus regeneration and new sequencing approaches

Can Aztekin
Swiss Federal Institute of Technology Lausanne

Unlike mammals, *Xenopus* exhibits remarkable regenerative capabilities. However, the complexity and heterogeneity of regenerating tissues present challenges in identifying key factors necessary for regeneration. Recent advancements, particularly through the use of various single-cell sequencing methodologies, have begun to address these challenges. In this presentation, I will discuss our progress in applying multiple -omics approaches to uncover the molecular and cellular mechanisms driving regeneration, with an emphasis on the technical aspects and limitations of these methodologies.

Session 7. Development & Disease Modeling III

The *Xenopus* respiratory system reveals common tetrapod mechanisms for growth, regeneration and healing

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In recent years, we have seen a significant increase in our understanding of the mechanisms of development, regeneration, and healing of the respiratory system. However, most of these studies have been limited by their focus on mammalian systems. Here, we aimed to identify the underlying molecular mechanisms that are active during lung growth and tissue repair in amphibians, specifically *Xenopus tropicalis* (*X. tropicalis*). First, we analyzed the stem cell composition and signaling pathways that are active in epithelial and mesenchymal cells during lung growth. Then, we established a protocol for lung injury to assess the types of stem cells underlying tissue repair. In mammals, Sftpc+ (AT2) cells are alveolar stem cells that can differentiate to Krt8+ cells during lung homeostasis and post-injury repair. In this study, we identified Sftpc+ cells, along with the activity of key developmental signaling pathways, Hippo and Wnt, during lung maturation at post-metamorphosis stages. We then established a protocol for lung injury using chemically induced injury with bleomycin, which damages the lung through oxidative stress. The results show an elevation in collagen post-injury, indicating bleomycin's effect in causing lung fibrosis. *X. tropicalis* froglets survived 42 days post-injury, with a continuous decrease in fibrosis. To explore this effect, we analyzed the distribution of lung stem cells; Sox9 protein levels and Sftp gene expression were downregulated at the alveoli 42 days post-injury. In parallel, we identified loci with increased distribution of Krt8+ cells. The decrease in stem cell marker expression 42 days post-injury suggests they are differentiating as part of the healing process. Nevertheless, we could still detect them after a few weeks of healing. These results suggest that X. tropicalis has a regenerative capacity for lung tissue repair and that some of the developmental signaling pathways and stem cell markers are conserved between amphibians and mammals during lung growth, regeneration,

and healing. These findings show for the first time the physiological similarities between the anuran and the mammalian lung during growth and tissue repair processes, suggesting *X. tropicalis* as a potential animal model to study lung regeneration.

Discovering the developmental basis of endosome trafficking disorders and congenital anomalies using *Xenopus*

Nicole Edwards, Matthew Kofron, Aaron Zorn
Zorn Lab, Cincinnati Children's Hospital Medical Center

Endosome trafficking moves proteins between different cellular compartments. In adults, this pathway is important for cell physiology and homeostasis, while in development it can regulate cell shape changes, migration, and polarity. We have recently discovered that endosome trafficking is critical for organ morphogenesis using a Xenopus model of human congenital trachea-esophageal separation anomalies. We speculate that disruptions to endosome trafficking pathways underlie the complex comorbidities often seen in human patients with trachea-esophageal separation anomalies. We will highlight the advantages of Xenopus as a disease model to investigate endosome trafficking-related congenital anomalies using recent gene editing and imaging technologies. Using F0 CRISPR-Cas9 gene editing, we can titrate levels of protein loss of function between heterozygote (no phenotype) and complete null (embryonic lethal) to functionally screen patient risk alleles for disease causality in Xenopus. We observe that gRNA design significantly influences gene editing outcomes rather than the dose of CRISPR reagents. We will also demonstrate different microscopy imaging modalities to phenotype the trachea and esophagus and visualize changes to subcellular processes caused by disrupted endosomal trafficking. Moving forward we will use Xenopus to investigate the mechanisms underlying the comorbidities associated with endosomeopathies and determine how developmental bottlenecks are sensitive to disruptions in pleiotropic cellular pathways.

As the frog folds: exploring human genetic variants of neural tube defects in *Xenopus*

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Myelomeningocele (MMC), the most severe form of spina bifida, is a congenital defect characterized by the failure of the neural tube to close during early gestation. MMC is associated with several factors, including folic acid bioavailability, genetic predispositions, and environmental influences. Affected individuals often experience lifelong complications, such as impaired lower extremity motor function and compromised bowel and bladder control. Neural tube defects (NTDs) like MMC occur during early embryonic development, and several genes are believed to play critical

roles in this process. In our whole exome sequencing study of 511 Mexican American and European American MMC patients, we identified several genes with significant mutational burdens that may contribute to the pathogenesis of MMC. We are currently focusing on variants in the ALX1 and FAK genes, which showed high deleteriousness scores, particularly in the European American patient group. Evidence from murine models indicates that homozygous mutations in Alx1 lead to lethal embryonic acrania phenotypes, which can be rescued by intraperitoneal folic acid administration. Furthermore, our preliminary data from cultured Fak-/- mouse embryonic fibroblasts (MEFs) transfected with plasmid constructs containing FAK variants demonstrate that these patient-derived variants significantly impair cell migration. Building on these findings, we have initiated a comparative study in *Xenopus*, using morpholinos and CRISPR/Cas9 techniques. Our initial experiments with Alx1 and Fak morpholinos in *Xenopus* have led to an increase in NTD phenotypes, emphasizing the need for a more comprehensive analysis of gene function. We hypothesize that ALX1 and FAK are crucial regulators of neural tube formation, and that deleterious mutations in these genes may disrupt neural tube closure, resulting in NTDs. To further validate our hypothesis, we are developing a CRISPR/Cas9-mediated alx1 knockout model and conducting FAK patient variant analysis in *Xenopus*. These studies aim to clarify the functional roles of deleterious gene variants in neural tube development and to determine how their disruption may contribute to the occurrence of MMC.

Are ion channels morphogens? Surprising results inspired by patient based gene discovery

Mustafa Khokha
Yale University

Emerging evidence indicates that ion channels are critical for specific aspects of embryonic development. In the case of mechanosensation, certain channels can act as force sensors altering cell fate or cell behavior. However, ion channels are also critical for establishing membrane potential (Vm), a property of all cells; therefore, whether Vm can have regional instructive roles is less clear. If Vm is more than a permissive signal, then one would expect it to vary across a field of cells that will adopt different fates, and that manipulating ion channels would affect Vm regionally with corresponding local effects on cell fate. Our recent work indicated that Vm depolarization altered the fates of the ectoderm and lateral mesoderm, but whether this was a product of differential Vm across the germ layers or a permissive effect of a global change in Vm was uncertain. By measuring Vm in specific germ layers using sharp electrodes, we present data that indicate that the electrical properties of the germ layers are indeed different. The Vm of the endoderm varies both in its temporal development and in its response to extracellular ion concentrations and ion channel inhibitors. Indeed, based on our findings, we speculated that chloride channels may play a critical role, and blockage of chloride channels alters cell fate and LR patterning. Our results indicate that temporal and tissue specific expression of ion channels may have regional effects on Vm that translate to changes in cell fate. We propose that ion channels may therefore have morphogen-like properties worthy of further exploration which may be fruitful for understanding morphogenesis.

Modeling human cancer and inherited disease, expanding the experimental landscape to oncogenes and the non-coding genome

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In the past decade our lab has pioneered the use of TALEN- and CRISPR-mediated gene disruption to model human cancer and Mendelian disorders in Xenopus tropicalis. In the context of cancer, the possibility of gRNA multiplexing has allowed straightforward identification of cooperating driver mutations in tumor suppressor genes, as well as the mapping of dependency genes. To expand the experimental models and further their relevance to human cancer, we are now looking into strategies that complement the loss-of-function experiments with the activation of proto-oncogenes. For this we will be using CRISPR base editors to introduce activating missense mutations and are generating a transgenic line harboring a versatile exchange cassette in the hip11 'safe harbor' locus to mediate tissue-specific overexpression of oncogenes. Next to allowing Cre-mediated insertion of oncogenes, this line will also be useful for the introduction of reporters driven by cis-regulatory elements (CREs) of known disease genes that are present in the non-coding genome. This will make it possible to directly assess possible clinical variants in these CREs for identifying quantitative and qualitative changes in reporter activity. As such we want to further propagate Xenopus tropicalis as a powerful model for human disease.

Continuation of Disease Modelling

Frogs, Bone, and Kidney: Exploring Developmental Processes in *Xenopus*

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This work comprises two separate studies that utilize innovative technologies in *Xenopus* to investigate the developmental processes of bone and kidney tissues. The first study employs the chromatin conformation capture technique to explore how chromatin structure influences osteoblast gene expression. By examining the interactions between enhancers and promoters associated with bone-related genes, our goal is to uncover the transcriptional mechanisms involved in the matrix mineralization process of bone. The second study shifts our focus to the renal system, using *Xenopus* as a model organism to study genetic influences on kidney development and associated disorders. Through imaging and molecular techniques, we aim to enhance our understanding of renal abnormalities. These studies collectively aim to deepen our knowledge of the biological processes underlying bone and kidney development, ultimately providing insights that could inform strategies for addressing related developmental disorders.

Building a 3-D Developmental Atlas of *X. laevis* with microcomputed tomography

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One of the challenges associated with studying development is the fact that the embryo is perpetually changing across 3-dimensional space and time. Developmental atlases are key resources for cataloging these dynamic changes and serve to enhance our understanding of developmental mechanisms. To provide an updated resource that allows for visualization of the developing embryo in 3-D, we are using microcomputed tomography (μ-CT) to create a developmental atlas of *X. laevis* across all major developmental stages outlined by Nieuwkoop and Faber's Normal Table (St.1-66). The scans will be manually segmented such that a given organ or structure can be readily tracked over development – across all axes (dorsal-ventral, rostral-caudal, lateralmedial) and serve as training data for the development of deep learning AI algorithms that can automate this process, saving researchers the laborious task of image segmentation from tomography datasets. These resources will be freely accessible to the entire *Xenopus* community upon completion. In this talk, I will provide an update on our progress and discuss optimization of the scanning methods, including a hydrogel embedding procedure to better stabilize delicate tissue architecture, examples of segmentation, and the development of AI-based approaches for automatically quantifying the size and architecture of different structures.

Unveiling the complexities of CSF circulation using *Xenopus* and OCT imaging

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Congenital hydrocephalus (CH), characterized by abnormal ventricle expansion, affects approximately 1 in 1000 births. To date, there are no medical management options; the only known treatment is surgical, which has a failure rate of 50%. While genetic factors contribute to around 40% of cases, many remain undefined, hindering our understanding of the pathogenesis and genetic counseling efforts. Understanding the genetic underpinnings of congenital hydrocephalus remains essential for diagnosing, managing, and potentially preventing CH. We developed *Xenopus* as a model system for analyzing CH candidate genes. For hydrocephalus studies specifically, CNS can be accessed via the transparent skin during development, allowing optical coherent tomography (OCT) imaging to examine the developing brain, ventricles, and CSF circulation in real time. Using the CRISPR/CAS9 system, we depleted the candidate genes in *Xenopus,* and using OCT imaging, we analyzed the CNS development, ventricular development, and CSF circulation. Our research demonstrated the effectiveness of integrating whole exome sequencing for identifying candidate genes, followed by functional analysis using the *Xenopus* model system, providing a robust platform for analyzing variants in congenital hydrocephalus. Exploring the intricacies of CH is pivotal for pioneering preventative strategies for preventive and supportive strategies that can be developed for our patients. Our results underscore these genes' significant roles in brain development and their potential linkage to the hydrocephalus phenotype, paving the way for targeted medical therapeutic strategies to replace surgical options.

T cell gene expression profiling in *Xenopus laevis* during Mycobacteria infection

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Expanded nonclassical MHC-Ib gene lineages that are distinct from classical polymorphic MHC-Ia genes are found across jawed vertebrate genomes, although their significance in immunity is unclear. To date, the amphibian *Xenopus laevis* is the only species outside mice and human where nonpolymorphic MHC-Ib molecules directing the development and function of antimicrobial innate (i)T cells with limited TCR repertoire have been characterized. Notably, *X. laevis* provides a unique comparative model for investigating the critical roles of iT cells during mycobacteria infections caused by non-tuberculosis mycobacteria (NTM), such as Mycobacterium marinum. NTM are increasing in prevalence and are challenging to treat in humans. Using Illumina sequencing, we profiled the gene expression of splenic CD8+ and CD4-like T cells from M. marinum-infected (12 days) and mock-infected adult *X. laevis*. We also profiled gene expression of CD8+ peritoneal leukocytes of these animals. Results indicated that splenic CD4-like T-cells do not exhibit significant gene expression changes upon challenge with M. marinum. Surprisingly, CD8+ T cells exhibited far more down-regulated genes during M. marinum infection than up-regulated genes. We are currently examining potential changes in T cell receptor (TCR) repertoires during M. marinum infection, particularly over-represented TCRs indicative of iT cell populations.

Session 8. Cell Biology

Understanding multiciliogenesis in *Xenopus*

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Cilia regeneration is a physiological event, and while studied extensively in unicellular organisms, it remains poorly understood in vertebrates. In this study, using Xenopus multiciliated cells (MCCs) as a model, we demonstrate that, unlike unicellular organisms, deciliation removes the transition zone (TZ) and the ciliary axoneme. While MCCs immediately begin the regeneration of the ciliary axoneme, surprisingly, the assembly of TZ is delayed. However, ciliary tip proteins, Sentan and Clamp, localize to regenerating cilia without delay. Using cycloheximide (CHX) to block new protein synthesis, we show that the TZ protein B9d1 is not a component of the cilia precursor pool and requires new transcription/translation, providing insights into the delayed repair of TZ. Moreover, MCCs in CHX treatment assemble fewer (~10 vs. ~150 in controls) but near wild-type length (ranging between 60 to 90%) cilia by gradually concentrating ciliogenesis proteins like IFTs at a select few basal bodies. Using mathematical modeling, we show that cilia length compared to cilia number influences the force generated by MCCs more. In summary, our results question the requirement of TZ in motile cilia assembly and provide insights into how cells determine organelle size and number.

Apical Size Reduction by Macropinocytosis Alleviates Tissue Crowding

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Tissue crowding represents a critical challenge to epithelial tissues, which often respond via the irreversible process of live cell extrusion. We report cell size reduction via macropinocytosis as an alternative mechanism. Macropinocytosis is triggered by tissue crowding via mechanosensory signaling, leading to substantial internalization of apical membrane and driving a reduction in apical cell size that remodels the epithelium to alleviate crowding. We report that this mechanism regulates the long-term organization of developing epithelium in response to proliferation-induced crowding but also serves as an immediate response to acute external compression. In both cases, inhibiting macropinocytosis induces a dramatic increase in cell extrusion suggesting cooperation between cell extrusion and macropinocytosis in response to compression. Our findings implicate macropinocytosis as an important regulator of dynamic epithelial remodeling.

Limitations of Existing Proximity Labeling Methods and the Development of a New Approach

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Traditional approaches for identifying protein-protein interactions, such as the Co-immunoprecipitation assay, isolate protein complexes in their natural state but have several limitations. These include the possibility that interactions may not be direct, the loss of low-affinity and transient interactions, the necessity for appropriate antibodies, and the alteration of protein interactions due to membrane destruction. To overcome these limitations, proximity biotin labeling (PL) was developed as a complementary approach for mapping molecular interactions in living cells. PL employs engineered enzymes, such as peroxidases or biotin ligases, genetically fused to a protein of interest to covalently tag neighboring endogenous species. PL can investigate spatial proteomes across organelles, subcellular compartments, and macromolecular complexes but also has limitations that include; the need for ectopic expression of PL enzyme fusion protein, biotin permeability issues in animal tissue, numerous endogenous biotinylated proteins causing non-specific heavy background signals, and the labeling of non-specific neighboring proteins due to the dynamic nature of protein transport. An alternative technique, pupylation-based interaction tagging (PUP-IT), was initially developed by Dr. Min Zhuang's group. PUP-IT uses Prokaryotic ubiquitin-like protein (Pup) to label proteins interacting with a Pup-ligase fused bait, enabling the enrichment and detection of transient and weak interactions via mass spectrometry. However, PUP-IT shares similar limitations with previous biotin ligase-based proximity labeling techniques. Our goal is to develop and improve a method that overcomes the limitations of previous PL and PUP-IT techniques by utilizing Protein A fused with Pup-ligase. Formaldehyde-fixed cells or tissues will be incubated with a specific antibody targeting the bait protein of interest. Subsequently, the ProtA-Pup ligase will be added, binding to the specific antibody. Adding Pup peptides will label the neighboring proteins of the bait, enabling the identification of proximal endogenous proteins via

mass spectrometry. Our next-generation PL method relies on the specificity of antibodies and the abundance of targeted endogenous proteins or modifications. While the "classical" proximity labeling approach may negatively affect the bait's function, our method is performed in cross-linked tissue to avoid such limitations. Additionally, Pup can be conjugated with various tags to avoid non-specific background and increase specificity. A substantial advantage lies in being able to use fixed tissue, where one can choose the appropriate spatial and temporal expression of a protein. This innovative approach will offer a highly versatile toolkit for performing proximity labeling assays in any cell type and tissue of interest quickly and efficiently.

Synthetic constitution of traveling Rho GTPase waves at the cell cortex

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Remodeling of the cell cortex is largely controlled by actin regulators, including the Rho family of small GTPases. These small GTPases are often themselves regulated via coupled (fast) positive and (delayed) negative feedback. The fast positive feedback arises via direct or indirect stimulation of Rho activators (GEFs) by active Rho (i.e. Rho-GTP), while the delayed negative feedback has been proposed to arise via recruitment of Rho inactivators (GAPs) to actin filaments (F-actin). This basic regulatory scheme can produce propagating waves in computational models, and is believed to underlie the propagating Rho waves observed during cytokinesis. We have used synthetic biology to test this scheme and to better understand how basic features of cortical Rho waves – such as period, amplitude, velocity, and temporal width – are modulated. Specifically, we have devised a synthetic GEF for positive feedback by fusing the catalytic domain of a Rho GEF with a domain that binds specifically to active Rho. Likewise, we have devised an array of synthetic inhibitors for negative feedback by fusing the catalytic domain of a Rho GAP with domains that bind specifically to actin filaments. Co-expression of the synthetic Rho activator with each of these four synthetic Rho inhibitors generates cortical waves. Strikingly, however, the wave features produced in each case differ from each other and from the naturally occurring Rho waves: synthetic effectors dramatically lengthen the wave period (a 4-30-fold increase, compared to the natural Rho waves) and alter overall morphology (i.e. barbed and shortened waves, netted waves, rounded and cloudlike waves, or elongated waves with greater contiguous wavefronts). Interestingly, each of these synthetic inhibitors (with a varied F-actin binding region) results in waves with unique characteristics when co-expressed with the same synthetic Rho activator, suggesting that the inhibitors control wave patterning and dictate differences in major wave features.

KEYNOTE

Leveraging *Xenopus* to do cool experiments

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The goal of my lab is to elucidate the molecular mechanisms of cell division and size control. We take advantage of *Xenopus* systems, particularly cytoplasmic extracts prepared from eggs of the frog *Xenopus laevis* that reconstitute mitotic chromosome condensation and spindle assembly in vitro. To study mechanisms of spindle and organelle size control, we have utilized a smaller, related frog, *Xenopus tropicalis*, to investigate interspecies scaling, and extracts prepared from fertilized eggs at different stages of embryogenesis to study developmental scaling. Our research has provided novel insight into cell division and morphogenesis, processes essential for viability and development, and defective in human diseases such as cancer. Current projects focus on two major areas of research. The first is to elucidate molecular mechanisms that define the architecture of the spindle and mitotic chromosomes. We are studying how these mechanisms generate the diversity of spindle morphologies observed across cell types, and how their alteration contributes to chromosome segregation defects observed in cancer cells and in inviable hybrid frog embryos. The second area is to examine size control mechanisms at the subcellular, cellular and organism levels. We are investigating how mitotic chromosome size scales with cell size, and leveraging ploidy manipulation and a variety of amphibian species to explore relationships among genome size, cell size, embryonic development, and metabolism.

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