XRET

Marine Biological Laboratory
Woods Hole, MA
October 2-5, 2026

Organizers

Jennifer Landino
Coral Zhou
Shinuo Weng
James Dewar

Sponsors

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innovation through passion

xenopus model organism welfare

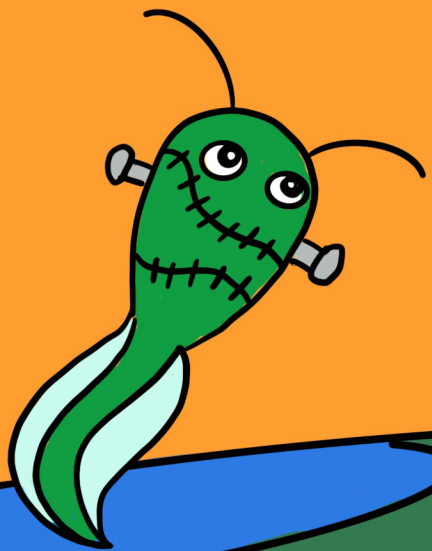

Illustration: James Parente
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FRIDAY OCTOBER 2, 2026

Arrival **Check-in at Swope**

6:00-7:30 pm **Dinner at Swope**

SATURDAY OCTOBER 3, 2026

7:30-8:40 **BREAKFAST**

8:40-8:45 **Introduction and Welcoming Remarks (Speck Auditorium)**
Jennifer Landino, Coral Zhou, James Dewar, Shinuo Weng

Session 1

8:45-9:05 Aaron Zorn (Cincinnati Children's Hospital)
Xenbase

9:05-9:25 Dominique Alfandari (University of Massachusetts, Amherst)
Production and Validation of Monoclonal Antibodies to Xenopus, Axolotl, and Mouse Targets

9:25-9:45 Gary Gorbsky (Oklahoma Medical Research Foundation)
Hitchhiker's Guide to Xenopus Cell Lines

9:45-10:05 Clayton Speed (Marine Biological Laboratory)
Genome-wide mapping of transgene integration sites in Xenopus laevis transgenic lines by SMRT-Sequencing

10:05-10:35 **COFFEE BREAK**

Session 2

10:35-10:55 Thomas Naert (Ghent University)
Pythia enables precise and predictable CRISPR-Cas9 genome integration and protein tagging through deep-learning-assisted design of microhomology-based templates

10:55-11:15 Ben Stinson (Dana-Farber Cancer Institute)
Elucidating mechanisms of DNA double-strand break repair using Xenopus egg extracts

11:15-11:35 James Dewar (Vanderbilt University)
Efficient Generation of Polyclonal Antibodies for Immunodepletion and Functional Inhibition

11:35-11:55 Jade Konsler (California Institute of Technology)
Investigating Transcription-Replication Conflicts in Xenopus Egg Extract System

12:00-1:30 LUNCH

Session 3

1:45-2:05 Amy Sater (University of Houston)
Modeling Traumatic Brain Injury in Xenopus: Yap and the Mitochondrial Response to Injury

2:05-2:25 Bianca Graziano (University of California, San Francisco)
Autism Protein STXBP1 at Cilia

2:25-2:45 Swetha Godavarthi (Rochester Institute of Technology)
Receptor-Driven Retrograde Control of Neurotransmitter Identity: From Xenopus Synapse to Synaptic Reprogramming

2:45-3:05 Taiyo Yamamoto (University of Zurich)
Things we built while modeling ADPKD in Xenopus

3:05-3:35 COFFEE BREAK

Session 4

3:35-3:55 Anne Carlson (University of Pittsburgh)
Sperm Factor Lost: PLCZ1 Absence and Egg-Autonomous PLC Activation in Xenopus Fertilization

3:55-4:15 James Ferrell (Stanford University)
TRIGGER WAVES

4:15-4:35 Helena Cantwell (University of California, Berkeley)
Probing subcellular changes at the egg-to-zygote transition

4:35-4:55 Gregory Schwarz (Dartmouth College)
A Novel Tool for Actin Organization in an Artificial Cortex

4:55-5:15 SHORT BREAK

Session 5

5:15-5:35 Tim Mitchison (Harvard Medical School)
Quantitative Proteomics of Native Mitotic Spindles Reveals Biochemical Scaling and Size-Dependent Exclusion by Microtubule Bundles

5:35-5:55 Xianrui Cheng (University of Southern California)
Cytoskeletal History-Dependent Spindle Length Scaling

6:00-7:30 DINNER

KEYNOTE

8:00-9:00 Hiro Funabiki (Rockefeller University)
The Xenopus Egg Extract As a Chromatin Biochemistry Discovery Tool

9:00-11:00 MIXER @ Captain Kidd

SUNDAY OCTOBER 4, 2026 ——————————————————————————

7:30-8:45 BREAKFAST

Session 6

8:45-9:05 Matt Good (University of Pennsylvania)
Spatially ordered zygotic genome activation fulfills embryo quality control

9:05-9:25 Coral Zhou (University of Kansas)
Illuminating the dark genome with a X. laevis telomere-to-telomere (T2T) assembly

9:25-9:45 Asako Shindo (University of Osaka)
Cellular competence underlying nutrient-dependent organ morphogenesis

9:45-10:05 Juyeon Hong (University of Texas at Austin)
The Extreme Distal Tip (EDT): A Distinct Domain in Motile Cilia

10:05-10:35 COFFEE BREAK

Session 7

10:35-10:55 Michael McKinney (Vanderbilt Health)
More Than a Model: Veterinary Perspective on Xenopus Health

10:55-11:15 Peter Walentek (University Freiburg Medical Center)
Models and resources for mucociliary research in Xenopus

11:15-12:15 Junior Strategic Planning

12:15-1:30 LUNCH

1:30-2:30 Strategic Planning (including relay of junior requests)

Session 8

2:30-2:50 Carole LaBonne (Northwestern University)
Evolution of Pou5 function and the origins of neural crest potential

2:50-3:10 Adam Session (Binghamton University)
Considerations for Xenopus Comparative Genomics Analyses

3:10-3:30 Ben Evans (McMaster University)
The genetic trigger for female sex determination in Marsabit clawed frogs (Xenopus borealis)

3:30-3:50 Kelly Tseng (University of Nevada Las Vegas)
Deciphering Mechanisms of Eye Regeneration

3:50-4:20 COFFEE BREAK

Session 9

4:20-4:40 John Young (Simmons University)
Leveraging Xenopus for undergraduate discovery based laboratory instruction

4:40-5:00 Christa Merzdorf (Montana State University)
Aquaporins in noncanonical Wnt signaling and calcium imaging, and gonadectomies

5:00-5:20 Jacques Robert (University of Rochester Medical Center)
Evaluating the Effects of Heatwaves and Micro- and Nanoplastics on Antiviral Immunity and Immune–Brain Interactions

5:20-5:40 Catherine Redmond (University of Michigan)
The Xenopus Keratin Atlas: Defining Functional Counterparts to the Mammalian "Keratin Code"

6:00-8:00 **Lobster Dinner**

KEYNOTE

8:00-9:00 Mustafa Khokha (Cedars-Sinai Medical Center)
Can we possibly learn something new about beta-catenin? Surprises from CP genetics

9:00-11:00 **MIXER @ Captain Kidd**

MONDAY OCTOBER 5, 2026

7:30-9:00 **BREAKFAST**

9:00-10:30 **Animal Health and Welfare Workshop**
Led by Coral Zhou & Michael McKinney

10:30-12:00 **Artificial Intelligence in Biomedical Research Workshop**
Led by James Dewar

**materials will be available asynchronously for all conference attendees to ensure access to those who may need to depart early*

12:00 **LUNCH AND DEPARTURE**

Xenbase

Stanley Chu[1], Andrew J Bell[2], Vaneet Lotay[1], Ngoc Ly[2], Troy J Pells[1], Taejoon Kwon[3], Sergei Agalakov[1], Virgilio Ponferrada[2], Courtney Lenz[1], Christina James-Zorn[2], Brad Arshinoff[1], Erik Segerdell[2], DongZhuo Wang[1], Konrad Thorner[2], Malcolm Fisher[2], Kamran Karimi[1], Aaron M Zorn[2], Peter D Vize[1]

[1]Department of Biological Sciences, University of Calgary, Calgary, Alberta T2N1N4, Canada. [2]Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, United States. [3]Department of Biomedical Engineering, Ulsan National Institute of Science and Technology, Ulsan 44919, Republic of Korea.

We are pleased to report that Xenbase has secured NICHD funding until 2031. We will review Xenbase's status and highlight recent additions to functionality and content, including genomic synteny tools and improvements to genome annotation. We will solicit community input on planned new features such as single-cell genomics.

Production and Validation of Monoclonal Antibodies to Xenopus, Axolotl, and Mouse Targets

Allen French and Dominique Alfandari

University of Massachusetts Amherst, Department of Veterinary and Animal Sciences.

Antibodies are critical tools for studying protein localization, function, and assembly into functional complexes. While antibodies to most human proteins are commercially available, few exist for other model organisms. Our group generates hybridomas producing monoclonal antibodies to targets selected by investigators across three key research areas: *Xenopus* development, axolotl regeneration, and mouse immunology and reproduction. We design and produce proteins for use as antigens, immunize mice, generate hybridomas, and screen for clones that specifically recognize the target protein. We then collaborate with requesting investigators to validate antibody function in vivo using assays tailored to their specific research questions. Here we present our current approaches to antibody production and validation, along with a progress report on the project.

The Hitchhiker's Guide to Xenopus Cell Lines

Gary J. Gorbsky
Cell Cycle & Cancer Biology Research Program, Oklahoma Medical Research Foundation and Department of Cell Biology, University of Oklahoma Health Campus

For nearly a century, *Xenopus* has been a fundamental system for cell and developmental biology. A variety of immortal cell lines derived from adult and larval tissues have been developed and now constitute an additional research tool to complement the strong advantages of *Xenopus* embryology. Their large cell size and natural compatibility with the *Xenopus* egg extract system make them particularly powerful for mechanistic biology. Cell lines are particularly efficient for dissection of signaling pathways and tracking cell cycle events. Compared to mammalian cell lines, *Xenopus* cell lines are significantly simpler to maintain. They can thrive at room temperature in a desk drawer, no CO_2 incubator required, and importantly carry little risk of harboring human pathogens, easing biosafety and regulatory issues. These attributes render *Xenopus* cultured cells ideal tools for student laboratory instruction and for research projects in laboratories and institutions without extensive cell culture infrastructure. Their room temperature and CO_2-free culture make them ideal for live cell microscopy. *Xenopus* cell lines can accelerate discovery in many ways. Hypotheses can be screened in cells in days rather than months, then taken back to the embryo when physiological context matters. Expanded use of these lines also stands to reduce reliance on live animals while lowering the costs of whole-animal husbandry and experimentation. To illustrate their utility, we will highlight how *Xenopus* cell lines have been critical in pioneering studies of cell cycle control and cell division.

Genome-wide mapping of transgene integration sites in *Xenopus laevis* transgenic lines by SMRT-Sequencing

Clayton Speed[1] Nikko-Ideen Shaidani[1], David Mark Welsh[2], Marko E Horb[1]
[1]Eugene Bell Center for Regenerative Biology and Tissue Engineering and National Xenopus Resource, Marine Biological Laboratory, Woods Hole, MA. [2]Bay Pall Center, Marine Biological Laboratory, Woods Hole, MA

The National *Xenopus* Resource (NXR) serves as the central repository for *Xenopus* transgenic animals, curating more than 130 community-derived lines (83 *X. laevis*, 47 *X. tropicalis*). These lines have been used experimentally for decades. Yet the chromosomal integration site, transgene copy number, and higher-order architecture of their transgene arrays remain undefined. Targeted-locus studies in mice have shown that roughly half of transgenic lines carry transgene-induced disruptions of endogenous genes, alongside deletions, inversions, and stray foreign DNA. These structural hazards can confound phenotype interpretation. That risk only grows as *Xenopus* lines are increasingly paired with CRISPR-based gene editing. To close this gap, we sequenced ten *Xenopus laevis* lines using PacBio SMRT-Seq long-read sequencing. The *de novo* assembly of the transgene cassette allowed us to resolve the transgene locus in each line. Six lines carried transgenes integrated within genes (disrupting *neurl1b.L, znf534.L, ints3.L*, *rap1* and *gap2.S*), while four were intergenic. Most transgenes formed tandem head-to-tail arrays. Notably, L0009, carries a single-copy insertion. Several lines also harbored unexpected sequences, including transgene fragments and residual vector DNA, echoing the pattern seen in mice. Copy number for these will need orthogonal confirmation via droplet digital PCR. Together these results establish a validated path toward fully characterizing the NXR's transgenic collection.

Pythia enables precise and predictable CRISPR-Cas9 genome integration and protein tagging through deep-learning-assisted design of microhomology-based templates

Taiyo Yamamoto[1,2], Munevver Burcu Cicekdal[3,4,5], Koen Vromant[5], Ruth Röck[1], Kris Vleminckx[5], Soeren S. Lienkamp[1,6,7], Thomas Naert[5]

[1]Institute of Anatomy, University of Zurich, Zurich, Switzerland, [2]PhD Program in Molecular Life Sciences, Life Science Zurich Graduate School, Zurich, Switzerland, [3]Department of Biomolecular Medicine, Ghent University, Ghent, Belgium, [4]Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium, [5]Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium [6]Zurich Kidney Center, Zurich, Switzerland, [7]Department of Health Sciences and Technology, ETH Zurich, Zurich, Switzerland

Generating transgenic *Xenopus* lines has long relied on REMI and I-SceI-based methods, which yield random, multicopy integrations subject to position effects and generational silencing. Routine endogenous protein tagging has also remained largely out of reach. Last year, we introduced Pythia, a deep-learning-assisted framework that designs short tandem microhomology repair arms to channel CRISPR-Cas9 repair toward predictable, frame-retentive, single-copy transgene integration. In *X. tropicalis*, this enabled targeted integration at the *hipp11* (h11) safe harbor with stable germline transmission, and endogenous protein tagging at physiological expression levels, including fluorescent fusions of Myh9, Acta2 and Ncam1, imaged live and recovered by immunoprecipitation. Here I will focus on what has evolved since. By consolidating the workflow into a protocol with several practical and logistical improvements, we have further raised integration efficiencies. Our detailed bench-side workflow, covering embryo handling, injection-day timing, and screening, is described at a level of operational detail that our original research paper did not provide, addressing the practical bottlenecks that determine whether the method works reliably in a given lab. On the computational side, the updated Pythia web tool now offers genome-wide precomputed designs for protein tagging in *X. tropicalis*, mouse and human, including both direct exon tagging and intron-targeting exon replacement strategies, plus a custom design module for any locus or species. I will share concrete success stories, troubleshooting lessons, and remaining bottlenecks, with the aim of lowering the barrier for any *Xenopus* lab to adopt targeted single-copy transgenesis and endogenous tagging as routine techniques. Finally, I will outline how these tools open further avenues for cancer modeling in *Xenopus*, where precise integration enables faithful reconstruction of fusion oncoproteins at endogenous loci, a class of driver lesions that random transgenesis cannot accurately model.

Elucidating mechanisms of DNA double-strand break repair using *Xenopus* egg extracts

Benjamin M. Stinson

Department of Radiation Oncology, Dana-Farber Cancer Institute and Harvard Medical School

Nonhomologous end joining (NHEJ), the primary pathway of vertebrate DNA double-strand-break (DSB) repair, directly re-ligates broken DNA ends. Damaged DSB ends that cannot be immediately re-ligated are modified by NHEJ processing enzymes, including potentially mutagenic polymerases and nucleases, to enable joining by DNA Ligase IV (Lig4). However, DSB ends that are initially compatible for re-ligation are typically joined without end processing. Using single-molecule imaging and *Xenopus* egg extracts that recapitulate NHEJ, we show that end processing occurs within a "short-range synaptic complex" in which DNA ends are closely aligned.1 Furthermore, we demonstrate that a single Lig4 binds both DNA ends to drive initial short-range complex formation.2 Thus, Lig4 is poised to ligate compatible ends upon initial formation of the short-range complex before error-prone processing. Moreover, confinement of end processing to the short-range complex ensures that DNA ends undergo ligation as soon as they become compatible, thereby minimizing mutagenesis. Our results provide a molecular basis for the fidelity of NHEJ and highlight the utility of *Xenopus* egg extracts for biophysical studies of DNA repair.

Efficient Generation of Polyclonal Antibodies for Immunodepletion and Functional Inhibition

James M Dewar
Vanderbilt University School of Medicine

Immunodepletion allows protein function to be dissected in *Xenopus* egg extracts but requires antibodies that efficiently recognize and remove endogenous targets. Over several years, my laboratory has developed a workflow for generating polyclonal antibodies that now achieves a success rate approaching 100% for immunodepletion applications. Commercial vendors can perform antigen generation, antiserum production, and affinity purification end-to-end, allowing laboratories to obtain purified antibodies through a fully outsourced production process. We will describe this workflow, including antigen selection, assessment of depletion efficiency, and functional validation, and present use cases from our studies of DNA replication and repair. Our two most recently generated antibodies, targeting exonuclease 1 (EXO1) and topoisomerase IIIα (TOP3A), also inhibit their targets when added directly to extracts. The EXO1 antibody blocks nascent strand degradation, enabling analysis of how EXO1 restrains progression of uncoupled replication forks (Grogan *et al*, 2026, *bioRxiv*). The TOP3A antibody provides a means to investigate the resolution of DNA intertwines during replication (Van Ravenstein *et al*, 2026, *bioRxiv*). These findings extend the utility of antibodies generated for immunodepletion to direct functional inhibition, allowing protein activity to be perturbed without prior removal from extracts. This workflow provides a practical route to generating reagents for mechanistic studies in *Xenopus* and highlights inhibitory activity as an additional property to assess during antibody characterization.

Investigating Transcription-Replication Conflicts in *Xenopus* Egg Extract System

Shay He, Omar Salah, Jade Konsler, Maria Altshuller, and Daniel Semlow
Division of Chemistry and Chemical Engineering, California Institute of Technology

Collisions between transcription machinery and the replication fork are a major source of endogenous replication stress, yet how replication forks tolerate these conflicts remains poorly understood. Here, we use *Xenopus* egg extracts to model replication fork collisions with a stabilized RNA polymerase II (Pol II) complex covalently attached to the non-template (coding) DNA strand. We find that head-on collisions with the Pol II roadblock result in pronounced replication fork stalling and activate the ATR-dependent replication stress checkpoint. Pol II is subsequently degraded in a ubiquitin-, p97-, and proteasome-dependent manner that permits the fork to bypass the roadblock. In contrast, co-directional collisions do not measurably stall fork progression but do impair lagging-strand synthesis. This suggests that the replisome can displace Pol II from the leading-strand template while Pol II remains associated with the lagging-strand template. Together, our findings provide a mechanistic framework for how replication forks resolve orientation-specific collisions with Pol II.

Modeling Traumatic Brain Injury in Xenopus

Alexander Ferrer, Donavaughn Baucom, Mahmoud Alhomouz, Brendan Singletary, Basabdatta Adhikari, and Amy K. Sater
Department of Biology and Biochemistry, University of Houston, Houston TX, USA

Traumatic Brain Injury (TBI) is a global public health problem, and the “secondary injury” phase of TBI constitutes a chronic disease that can persist for months or years. We have developed the tadpole as a model for large-scale studies of TBI and have found that Tamoxifen (TMX) is highly neuroprotective, reducing apoptosis and promoting behavioral and cellular recovery. Transcriptome profiling suggests that TMX alters the Interleukin-6 (IL6) family signaling network, upregulating expression of specific ligands, receptors, and pathway components. In parallel, we are currently investigating dynamics and function of the mechanoresponsive transcription factor yap following TBI. While yap is largely cytoplasmic in sham-treated tadpole brains, it enters neuronal nuclei within 3 hours following injury returning to the cytoplasm within 24 hours. Treatment of injured tadpoles with Tamoxifen (TMX) leads to persistence of yap in neuronal nuclei; yap may mediate some of the neuroprotective activities of TMX. Yap also contributes to mitochondrial regulation: we observe an accumulation of abnormal mitochondria in injured brains, along with an upregulation of several nuclear-encoded mitochondrial genes that are known targets of yap in mammalian cells. Treatment with the yap/tead inhibitor verteporfin immediately after injury results in decreased expression of these mitochondrial genes, as well as a reduction in *aqp4* and the anti-apoptotic factor *birc2*. Our findings implicate yap in cell survival and the recovery of mitochondrial homeostasis following traumatic brain injury, and studies in progress investigate possible regulatory links between yap, mitochondrial homeostasis, and the IL6 network.

Autism Protein STXBP1 at Cilia

Bianca Graziano
University of California, San Francisco

Syntaxin-binding protein 1 (STXBP1) is an essential regulator of the SNARE protein complex and neurotransmitter release. Pathogenic variants in *STXBP1* cause severe developmental and epileptic encephalopathies, often accompanied by autism, intellectual disability, and movement disorders. Intriguingly, individuals with STXBP1-related disorders also frequently experience respiratory complications, including recurrent pulmonary infections. These symptoms are generally considered secondary to neurological dysfunction. However, we recently found STXBP1 at the base of cilia, raising the possibility that this canonical synaptic protein has an unexpected role in ciliary biology. Using *Xenopus* airway multiciliated cells as an *in vivo* model, we find that STXBP1 is expressed during multiciliated cell differentiation, localizes to the base of motile cilia, and is required for normal multiciliogenesis. Because STXBP1 regulates membrane fusion by controlling SNARE proteins at synapses, we asked whether it might use similar machinery at cilia. We identify the SNARE proteins STX2, STX3, and SNAP29 as candidate components of this ciliary trafficking machinery. This function is not restricted to motile cilia. STXBP1 and these SNARE proteins also localize to the base of primary cilia in multiple human cell types, including cortical neural progenitors and induced neurons. Depleting STXBP1 in neural progenitors disrupts cilia-dependent Hedgehog signaling, revealing a requirement for STXBP1 during neural development before synapses have formed. Together, our results identify cilia as a previously unrecognized site of STXBP1 function. We propose that STXBP1 regulates membrane trafficking at both synapses and cilia by deploying distinct combinations of SNARE proteins at these specialized cellular compartments. This shared trafficking mechanism provides a potential link between the neurological and respiratory features of STXBP1-related disorders and raises the broader possibility that ciliary dysfunction contributes to diseases caused by canonical synaptic genes.

Receptor-Driven Retrograde Control of Neurotransmitter Identity: From *Xenopus* Synapse to Synaptic Reprogramming

Swetha Godavarthi
Integrated Sciences Academy, Rochester Institute of Technology

How neurons establish and maintain their neurotransmitter identity remains a central question in neuroscience. At the *Xenopus laevis* neuromuscular junction, we have found that this process is not determined solely by the presynaptic neuron. Instead, postsynaptic neurotransmitter receptors can signal retrogradely through transsynaptic bridges to regulate the stability of presynaptic neurotransmitter identity. Different postsynaptic receptors engage distinct transsynaptic bridges, providing a molecular link between postsynaptic receptor organization and presynaptic neurotransmitter identity. The accessibility of the *Xenopus* tadpole allows this mechanism to be examined across levels of organization, from receptor complexes and neurotransmitter phenotype to synaptic transmission and whole-animal behavior. Using targeted manipulation of the neuromuscular junction, we find that disruption of the canonical cholinergic transsynaptic bridge alters motor output, while recruitment of a noncanonical GABAergic bridge can restore neuromuscular function. Thus, receptor-dependent retrograde signaling is not simply a mechanism for maintaining neurotransmitter identity but also impacts motor function. These findings illustrate the power of *Xenopus* to connect molecular mechanisms at developing synapses with circuit function and behavior in the intact animal. More broadly, they suggest that receptor-driven transsynaptic bridge complexes serve as instructive signals that can be redirected to alter neuronal phenotype and restore function. This receptor-driven plasticity may provide a mechanism for understanding and manipulating synaptic dysfunction in neuromuscular disorders.

Things we built while modeling ADPKD in *Xenopus*

Taiyo Yamamoto[1], Marcela Camenzind[1], Ronja Podlaszewski[1], Sara Zorawski[1], Thomas Naert[2], Soeren Lienkamp[1,3]
[1]University of Zurich, Switzerland. [2]Ghent University, Belgium. [3]ETH Zurich, Switzerland

Studying Autosomal Dominant Polycystic Kidney Disease in *Xenopus*, we have developed new methods and tools that we hope will also be useful to the community. Using our *pkd1* knockout as an example, this talk will contain three parts. **Part 1.** Finding the perfect CRISPR gRNA can be quite a bit of work, usually involving lots of tabs and tools. We have developed a new webtool that aggregates scores, which simplifies the search for the perfect guide and takes some of the guesswork out of it. Using this workflow on *pkd1*, we get highly efficient editing and a clear cystic phenotype in both *tropicalis* and *laevis*. **Part 2.** Tailbuds don't tend to like being embedded in agarose and time-lapse imaged. We have systematically investigated reasons for death during imaging and now have a setup where our embryos are still perfectly happy after 24 hours of confocal imaging. Time-lapse imaging our *pkd1* knockouts, we observed something unexpected. Cysts were not initially driven by excess proliferation or an abnormal dilatory signal. Rather, tubular dilation is a physiologically occurring mechanism, and our *pkd1* knockouts lacked the ability to re-constrict the kidney tubule after this dilation. This completely shifted the point of view: instead of a dilatory signal, we were now investigating a failed contractile mechanism. **Part 3.** Rapid cellular dynamics like this are often controlled by signaling acting on existing machinery rather than by transcriptional changes, which is why we wanted to perform a phosphoproteomic screen. We have therefore developed a protocol for tissue- and time-specific phosphoproteomics of the pronephros, which should be translatable to other tissue types. This approach has revealed dysregulated cellular signals during cyst initiation. We are now further investigating these sites as potential therapeutic targets.

A Sperm Factor Lost: PLCZ1 Absence and Egg-Autonomous PLC Activation in Xenopus Fertilization

Rachel E. Bainbridge, Joel C. Rosenbaum, Kayla M. Komondor, and Anne E. Carlson
Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA

In eggs from sexually reproducing animals, fertilization triggers a rapid rise in intracellular Ca^{2+} that activates the processes required for embryonic development. In externally fertilizing species such as the African clawed frog, *Xenopus laevis*, this Ca^{2+} signal drives a fertilization-triggered depolarization of the egg membrane, the fast block to polyspermy, during which sperm can bind but cannot enter the egg. We have shown that fertilization opens the Ca^{2+}-activated Cl^- channel TMEM16A, producing a Cl^- efflux that depolarizes the membrane. As in mammals, TMEM16A activation depends on a phospholipase C (PLC) isozyme that triggers Ca^{2+} release from the endoplasmic reticulum. Our goal has been to determine how fertilization activates PLC in *X. laevis*. In mammals and birds, the sperm-derived enzyme PLCζ is transferred into the egg at fertilization and is thought to trigger this Ca^{2+} release. Because *X. laevis* sperm extracts can activate mouse eggs, we asked whether *X. laevis* sperm carry an unannotated PLCZ1 ortholog. Using comparative genomics, transcriptomics, and synteny analysis, we identified PLCZ1 orthologs in 25 amphibian species, including 14 previously uncharacterized orthologs, but found none in *X. laevis* or its close relative *X. tropicalis*. Synteny analysis of the PLCZ1 locus revealed a shared gene deletion across the Pipidae family, indicating this loss occurred in their common ancestor rather than being specific to Xenopus. RNA sequencing of *X. laevis* testes likewise found no evidence of an unannotated PLCZ1 transcript, and unmapped reads that superficially resembled PLCZ1 were instead traced to incompletely assembled PLCD1-4 transcripts, the most abundant PLC isoform in *Xenopus* testis. Given the apparent absence of a PLCζ-like sperm factor, we tested whether sperm contribute any PLC activity to the egg at fertilization. Inhibiting sperm-derived PLC did not alter Ca^{2+}-dependent activation of TMEM16A, whereas inhibiting egg-derived PLC abolished channel activation entirely. Analysis of egg transcriptomic and proteomic data identified PLCγ1 and PLCβ1/3 as the PLC isoforms present in fertilization-competent eggs, but disrupting the canonical tyrosine kinase and G-protein-coupled receptor pathways that activate these isoforms did not block PLC-dependent Ca^{2+} release at fertilization. Together, these results indicate that *X. laevis* relies on an egg-autonomous, non-canonical PLC activation mechanism rather than a PLCζ-anchored sperm factor pathway, revealing that the calcium-release mechanisms driving egg activation are more evolutionarily diverse across vertebrates than previously appreciated.

TRIGGER WAVES

Zhengda Li and James Ferrell
Department of Biochemistry, Stanford University

In *Xenopus* eggs and egg extracts, apoptosis spreads through the cytoplasm as a trigger wave, a self-regenerating front of caspase activity the can propagate without slowing down or petering out. In a convex-everywhere cell like a frog egg, this allows caspase activity to act on every part of the cell in a timely fashion. On the other hand, if apoptosis were to begin in a thin tube like a filopodium or dendritic spine and then spreads towards a bigger volume like a cell body or dendrite, the apoptotic wave might get arrested at the junction between the thin tube and the bigger volume, because the apoptotic mediators get more effectively diluted by diffusion right at that point. We have derived a scaling law for how thin a finger-like cell protrusion must be to compartmentalize a trigger wave of a given speed and tested this law through experiments on Xenopus egg extracts in a microfluidics device.

Probing subcellular changes at the egg-to-zygote transition

Helena Cantwell
Postdoc, Heald Lab, Department of Molecular and Cell Biology, University of California, Berkeley

One of the first steps of animal development is the remarkable transformation, at fertilisation, of a sperm cell and an egg cell into a totipotent zygote capable of embryogenesis. At this transition from egg-to-zygote, there is little change to overall cell size or shape but subcellular structures are rapidly, dramatically remodelled to facilitate a switch in cell division programme from meiosis to mitosis. Highly asymmetric meiotic divisions of the oocyte and egg are followed, just one cell cycle later, by the symmetric first embryonic mitosis of the zygote. The small, barrel-shaped meiotic spindle is replaced by a large zygotic spindle that nucleates abundant astral microtubules at spindle poles. To uncover underlying mechanisms we combined drug treatment of Ciona robusta (sea squirt) eggs with perturbation experiments and phosphoproteomics in *Xenopus laevis* egg and embryo cytoplasmic extracts. Our data support a model in which Casein Kinase 2 activity attenuation at the egg-to-zygote transition leads to activation of RanGTP-regulated microtubule effectors and drives mitotic spindle morphology. The molecular mechanisms underlying other subcellular changes at the egg-to-zygote transition remain exciting avenues for future research.

A Novel Tool for Actin Organization in an Artificial Cortex

Gregory Schwarz, Roger Calvas Gonzales, Jennifer Landino
Department of Biochemistry and Cellular Biology, Geisel School of Medicine, Dartmouth College

Actin is essential for a variety of cell processes, and its subcellular organization underlies specific functions. Importantly, actin is thought to generate force through the assembly of organized networks, rather than single filaments. For example, during cytokinesis, actin is organized into parallel bundles within the cytokinetic furrow, which generates the force to pull the membrane inward, whereas actin outside of the furrow is branched and more disordered. While actin organization has been widely studied in cells and using purified proteins, few tools exist for investigating actin network organization and rearrangements *in vitro*. Here, we used an artificial cell cortex comprised of *Xenopus laevis* egg extract and supported lipid bilayers to investigate actin network assembly in 2-dimensions and to track reorganization of the network over time. We developed a novel analysis workflow to determine bulk actin organization, and classify 2D networks as disordered, ordered, or swirled conformations. Using the ImageJ plugin, OrientationJ, to quantify local anisotropy across the bilayer. We found that each network classification occupies a unique phase space as correlated by the vector angle standard deviation and dominant angle in the field of view. Additionally, we used this approach to track changes in the network conformations (ordered, disordered, and swirled) over time and quantified the displacement of the actin network in organizational phase space. We found that actin network reorganization from disordered to swirled is directed, suggesting it is mediated by external force. Preliminary results show that active Rho proceeds directed actin re-organization, suggesting a model wherein Rho signaling, *via* Formins or non-muscle Myosin II, generates forces that drive reorganization of the actin network. In the future, we will investigate if bilayer composition regulates the actin network organization, and changes in organization over time. We will also further investigate the molecular mechanisms that underlie 2D directed actin reorganization.

Quantitative Proteomics of Native Mitotic Spindles Reveals Biochemical Scaling and Size-Dependent Exclusion by Microtubule bundles

Matt Sonnet, Christine Field and Tim Mitchison
Harvard Medical School

Mitotic spindles are a specialized organizational state of cytoplasm built from dynamic microtubules and chromatin. We lack a quantitative description of their biochemical composition and scaling behavior, in part because weakly bound proteins tend to dissociate during isolation procedures. We assembled spindles in *Xenopus* egg extract, isolated them by rapid filtration with no wash step, then measured the spindle:cytoplasm partition coefficient for thousands of proteins. The spindle-enriched proteome is dominated by microtubule and DNA binding proteins with no evidence for a matrix compartment. The total concentration of microtube binding proteins is similar to that of tubulin, showing the lattice is very crowded. To determine how spindle biochemistry changes during cleavage division we titrated the DNA:cytoplasm ratio in extract. Microtubule composition was relatively constant, with a few outliers, while chromatin composition changed markedly. These data help explain the relatively constant dynamics of spindle microtubule during early cleavage divisions compared to large changes in chromatin biology. Our analysis revealed a major role for steric exclusion in determining the character of cytoplasm near microtubule bundles. Organelles and 200nm latex beads were excluded from the entire spindle while ribosomes were locally excluded within bundles. Steric exclusion by microtubule bundles may be the cause of the “polar ejection” force which pushes chromosome arms out of spindles and shapes the metaphase plate.

Cytoskeletal History-Dependent Spindle Length Scaling

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The mitotic spindle is a dynamic microtubule structure that segregates duplicated chromosomes during cell division. Its length scales with cell size, but the underlying mechanisms remain incompletely understood. Previous work has linked spindle length to cell volume, cytoplasmic properties, spindle assembly factors, and microtubule dynamics during M phase. Because spindle assembly occurs as part of a continuous cell cycle, we asked whether cytoplasmic organization during interphase influences spindle length in the following mitosis. Using cycling Xenopus laevis egg extracts, we found that spindle length decreased over consecutive cycles and correlated positively with the size of cell-like cytoplasmic compartments formed during the preceding interphase. Transient perturbation of interphase microtubules reduced aster size, and spindles subsequently measured after mitotic entry were shorter than those in untreated controls. Together, these findings establish a link between cytoskeletal organization in interphase and spindle length in the following mitosis, suggesting a temporal aspect of spindle scaling.

The *Xenopus* Egg Extract As a Chromatin Biochemistry Discovery Tool

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Since its introduction by Yoshio Masui, the *Xenopus* egg extract has remained a unique cell-free system capable of recapitulating diverse chromatin and intracellular events under precise cell cycle control. Despite rapid advances in genome engineering in mammalian cells, introducing specific epigenetic modifications or chromatin structural features at defined cell cycle stages within a native cellular context remains technically challenging. Exploiting the unique experimental capacity of *Xenopus* egg extracts to bypass these difficulties, our laboratory has been investigating the roles of nucleosomes in chromosome inheritance, identity, and integrity. In this presentation, I will share our experience using *Xenopus* egg extracts as a discovery tool, along with our recent studies on the regulation of DNA methylation and nucleosome assembly.

Spatially ordered zygotic genome activation fulfills embryo quality control

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Early embryo development features autonomous, maternally driven cell divisions that self-organize the multicellular blastula or blastocyst tissue. Maternal control cedes to the zygote starting with the onset of widespread zygotic genome activation (ZGA), which is essential for subsequent cell fate determination and morphogenesis. Intriguingly, although ZGA onset is highly regulated at the level of the whole embryo, it can be non-homogenous and precisely patterned at the single-cell level. We previously demonstrated a stereotyped spatial and temporal ordering of ZGA in a model vertebrate embryo. Unknown, however, was whether this precise ZGA patterning was required for development. To address this fundamental question, we devised a strategy to spatially control cell divisions that perturb blastula embryo organization. We demonstrate the feasibility of spatially inverting the cell size pattern of embryos and find that these inverted embryos exhibit a flipped pattern of ZGA. Mispatterned ZGA along the animal-vegetal axis triggers embryo apoptosis, revealing that gastrula embryos have a built-in quality control system to sense inappropriate ZGA patterning, including regionalized defects in transcriptional onset. The quality control response is non-autonomous, dependent on an anti-apoptotic signal that suppresses cell death outside the animal hemisphere. These results reveal the requirement of properly patterned ZGA for normal development and the existence of a surveillance system of embryo quality control exquisitely tuned to the spatial and temporal ordering of genome activation and zygotic gene expression.

Illuminating the dark genome with a *X. laevis* telomere-to-telomere (T2T) assembly

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Roughly half of the vertebrate genome consists of repetitive elements, including transposable elements, that are typically epigenetically silenced. Once dismissed as "junk" DNA, certain classes of repetitive sequences — such as LINE-1 elements — have recently gained attention as potential drivers of speciation, development, and disease. Other repetitive elements, including rDNA, telomeres, and centromeres, play essential roles in genome stability and are frequently implicated in speciation due to their rapid evolution. Despite their biological importance, repetitive regions have been notoriously difficult to assemble using short-read sequencing approaches, limiting progress in the field. Long-read sequencing technologies have now made near-complete, telomere-to-telomere (T2T) genome assemblies possible, including resolution of these previously intractable regions. Here we report recent progress on a T2T assembly of a female J-strain *Xenopus laevis* — the first known T2T genome assembly of a polyploid species. Using a combination of Oxford Nanopore, Omni-C, and PacBio deep sequencing, we applied methods analogous to those used for the human T2T genome. Compared to the v10 assembly, the T2T assembly adds approximately 165 million bp of additional sequence, bringing the total significantly closer to the estimated *X. laevis* genome size of ~3 Gbp. The newly resolved regions are predominantly repetitive arrays — centromeres, rDNA clusters, subtelomeres, and telomeres — along with some coding regions that were previously unassembled. We are currently validating the assembly using experimentally derived sequences for rDNA and centromeres, as well as complex coding loci such as the Hox gene clusters. Upon release, we anticipate that the T2T assembly will open new avenues for studying how the repetitive genome contributes to genome stability in the context of the cell cycle, development, and evolution in *X. laevis*.

Cellular competence underlying nutrient-dependent organ morphogenesis

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Nutrients and metabolism are important regulators of development and tissue morphogenesis, but how cells within individual organs respond to nutrients remains incompletely understood. *Xenopus* tadpoles begin feeding during organogenesis, providing a system to directly manipulate dietary nutrient availability. We developed nutrient-restricted feeding and whole-body 3D imaging approaches to examine the role of specific nutrients in individual organ morphogenesis. In the thyroid, glucose reduces cell adhesion molecules and promotes follicle morphogenesis, whereas amino acids may promote tubule formation in the pronephros. These organs remain in developmental stasis before feeding, indicating that their morphogenesis is coupled to the nutritional environment. We have recently explored whether the rate of development before feeding influences cellular responses to nutrients in the thyroid. As is well known, environmental temperature substantially alters the time required for *Xenopus* embryos to reach the feeding stage. Our recent observations suggest that tadpoles undergoing prolonged pre-feeding development at lower temperatures show delayed or impaired thyroid morphogenesis after feeding. This suggests that nutrient-dependent morphogenesis depends on a state established during embryonic development, potentially involving metabolism. In this presentation, I will discuss the nutrient-dependent flexibility of organ morphogenesis and the cellular competence to respond to nutrients during organogenesis.

The Extreme Distal Tip (EDT): A Distinct Domain in Motile Cilia

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Motile cilia on multiciliated cells (MCCs) generate synchronized fluid flow, essential for tissue homeostasis and normal development. Despite the high conservation of core structural components such as basal bodies and axonemes, the extreme distal region in motile cilia remains poorly understood. Here, we define and characterize a molecular domain at the very distal end of 9+2 motile cilia, the extreme distal tip (EDT), and investigate its role in MCC development and function. Through complementary live imaging, proteomic, functional, and biochemical approaches across *Xenopus*, mouse, and human systems, we identified Ccdc78, Ccdc33, and Jhc1 as evolutionary conserved components that define the EDT. The distinct functions of EDT proteins showed collective support for normal MCC development and function. This work provides a unique framework for understanding distal ciliary organization and opens new avenues for investigating how specialized sub-ciliary domains contribute to cilia structure and function.

More Than a Model: Veterinary Perspective on *Xenopus* Health

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Although widely used as a research model, studies on the health and wellbeing of South African Clawed Frogs (*Xenopus laevis*) remain lacking. While there may be numerous reasons for this, it frustrates *Xenopus* investigators and veterinarians alike. To address this dearth of information, we underwent a collaborative study aimed at characterizing the health implications of gonadotropin treatment in laboratory-reared female X. *laevis*[1]. Frogs underwent a standard gonadotropin protocol, PMSG and hCG, followed by manual egg collection to assess egg quantity as well as quality. To obtain blood for serum biochemistry and white blood cell differential, a terminal cardiocentesis was then performed at 48 hours, 72 hours, and 7 days post-injection. These three experimental groups were compared to a pre-injection control group (n=5 per group). Compared to controls, treated frogs displayed significantly higher lipase, blood urea nitrogen, and lactate dehydrogenase. Regarding egg production, 9 of 15 frogs were found to be good quality layers. Out of the remaining 6 bad quality layers, 2 had free coelomic eggs of significant volumes (15 mL and 3 mL) during postmortem collection. Injection of female *X. laevis* with gonadotropins, or sex hormones, is a well-established procedure for egg production. However, despite widespread use, this will be the first published diagnostic evaluation of treatment on frogs themselves. This study is an example of the collaboration needed between investigators and veterinarians responsible for *Xenopus* care. As the regulatory landscape and accreditation criteria shift, it will take a cooperative effort to ensure *Xenopus* health standards evolve, enabling the continued use of this species and the valuable data they provide.

Models and resources for mucociliary research in *Xenopus*

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Xenopus embryos are an excellent model to study cilia and mucociliary epithelia. Particularly, the embryonic mucociliary epidermis serves as model to study multiciliated cell biology as well as development and patterning of mucociliary tissues in the context of human disease. Similarly, the gastrocoel roof plate (GRP, the left-right organizer in amphibians) and the neural tube/brain of *Xenopus* embryos are popular models to study motile and primary cilia-mediated processes. Over the past years, we have invested efforts to broaden the toolkit and resources to study cilia and mucociliary epithelia in *Xenopus* that we want to share with the *Xenopus* community. I will present updates on the state of datasets and tools soon-to-be-shared with the community: 1. Updated re-analysis of scRNA-seq data and a temporal atlas of genome accessibility on epidermis development. 2. New datasets and methods to investigate the *Xenopus* tadpole foregut epithelium, a new model for mucociliary research. 3. A new computational image analysis tool to study ciliation and cell morphology changes in the GRP. Furthermore, I will give a brief update on the CSHL *Xenopus* course (currently co-lead by Rachel Miller and me) and would like to gather feedback from the community on how we can ensure to keep the course most relevant and useful for the community in the coming years.

Evolution of Pou5 function and the origins of neural crest potential

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The emergence of neural crest was a defining innovation in vertebrate evolution, but how these cells acquired their broad developmental potential remains unresolved. Comparisons between *Xenopus* and lamprey provide an opportunity to distinguish ancestral features of neural crest regulation from changes that arose during vertebrate diversification. Our studies reveal both conserved activity and divergent expression of Pou5, a central regulator of pluripotency. Lamprey pou5 is expressed in blastula animal-pole cells but is absent from neural crest. Nevertheless, both lamprey and Xenopus Pou5 enhance neural crest formation when expressed in *Xenopus*, whereas *Xenopus* Pou3 proteins do not. These findings suggest that neural crest-promoting activity arose following the Pou3/Pou5 duplication and was retained in lamprey despite changes in its developmental deployment. A Pou3 chimera with the Pou5 POU-S, linker, and homeodomain gained neural crest promoting activity, indicating that changes in this region of the protein drove the acquisition of novel functions after duplication. To investigate the sequence changes underlying Pou5 functional divergence, we reconstructed ancestral POU domains before the vertebrate Pou3/Pou5 duplication and early in Pou5 evolution. These analyses identify two strongly supported substitutions within the first helix of the POU-specific domain that are retained in lamprey Pou5 and *Xenopus* Oct91. These candidate changes provide a focused route to testing how protein evolution altered developmental activity. Together, comparative embryology and ancestral reconstruction connect the evolution of Pou5 sequence and expression to the origins of neural crest developmental potential.

Considerations for Xenopus Comparative Genomics Analyses

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In the long-read sequencing era, the number of “high-quality” Xenopus genomes has increased dramatically. While the amount of Xenopus genomic sequences has increased and these assemblies are lauded for having more DNA than previous, little effort has been made to ask about the quality of these assemblies. In particular, many long-read assembly pipelines are optimized for the human genome, which differs from Xenopus in the organization of the sex-determination region, ribosomal DNA, and subtelomeres. Assembly algorithms that do not take into account these differences in genome organization often incorrectly assemble the Xenopus genomes. We present comparative analysis between a number of published and unpublished Xenopus genomes to illustrate how these assembly errors may affect different genomic comparisons and offer suggestions on how to both identify and fix these differences for common experiments. In addition, we present analysis on how allopolyploid Xenopus genomes offer unique opportunities to study the evolution of diploid genomes.

The genetic trigger for female sex determination in Marsabit clawed frogs (*Xenopus borealis*)

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A central question in biology asks why the genetic basis of sexual differentiation evolves quickly in some groups even though disruption of this process carries severe risks such as sterility. A challenge to understanding mechanisms of this rapid evolution is a lack of functionally verified genetic triggers for sex determination. To begin to remedy this, we set out to identify, characterize, and functionally test the genetic trigger for female sex determination in Marsabit clawed frogs (*Xenopus borealis*), which has a species-specific and probably newly evolved W chromosome. Genomic and transcriptomic data identify a candidate that was expressed specifically in female gonads during sexual differentiation. We knocked out this gene, which is a female-specific duplicate of wnt3.L – hereafter wnt3-W – and induced complete female-to-male sex reversal in all of 28 individuals. These individuals have male-typical external and internal morphology, mating behavior, mating calls, and testis histology, and are fertile. This demonstrates that wnt3-W is the genetic trigger for feminization in *X. borealis* and adds to only one other functionally verified trigger for sex determination in amphibians. Phylogenetic and cytogenetic analysis indicates that wnt3-W originated recently on the tip of the *X. borealis* W chromosome, after this species diverged from all other extant *Xenopus*. Wnt3-W is usual being the first known trigger for sex determination in the Wnt gene family, and because wnt3 is more generally involved with vital, but not sex-specific functions. The discovery of wnt3-W contributes to a growing number of secreted peptides that are known to trigger sexual differentiation in animals.

Deciphering Mechanisms of Eye Regeneration

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A main challenge in the regeneration field has been to identify the mechanisms and suitable cell types needed to enable tissue repair. Although the process of vertebrate eye development is well-studied and characterized, the mechanisms that can induce eye stem cell proliferation following injury or disease remain challenging to identify. The highly regenerative *Xenopus laevis* is an established model for both development and organ regeneration. Tailbud embryos readily regrow functional eyes after ablation. Successful eye regrowth requires increased and extended retinal stem cell proliferation with a concurrent delay in new eye formation. Eye differentiation largely recapitulated the developmental process. This model facilitates the use of interdisciplinary approaches to compare the molecular and cellular mechanisms underlying development and regrowth. Our studies highlight the eye regrowth model as a robust platform for systematically defining the mechanisms required for regeneration and building a blueprint for effective ocular repair.

Leveraging *Xenopus* for undergraduate discovery based laboratory instruction

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Xenopus continues to be an important model system for understanding key aspects of cell and developmental biology. Furthermore, their large embryos and ease of care make *Xenopus laevis* an excellent organism to employ at institutions of all sizes for instructional laboratory courses. Including discovery-based, independent research activities in general biology lab instruction provides students with foundational experience for future careers in science. Using *Xenopus* to introduce vertebrate research has the benefit of recruiting scientists into our field and ensuring a longevity for the model system. Here, I will describe efforts we have made to use *Xenopus* in an introductory laboratory course aimed at first year college students. I will describe the types of questions that students are able to ask in this setting and provide the findings from one such experiment that tests the UV protective effects of embryo pigment. Overall, these efforts have proven to be effective in recruiting students to research labs and preparing them for graduate school and careers in biomedical fields.

Aquaporins in noncanonical Wnt signaling and calcium imaging, and gonadectomies

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In the *Xenopus* gastrula, the dorsal mesoderm undergoes convergent extension, which we have shown requires the aquaporin Aqp3b. Using Keller explants, we demonstrated that Aqp3b acts through the Wnt/PCP pathway, specifically through RhoA, but not Rac1. Explant and cell migration assays suggest that Aqp3b may act as a switch, biasing cells away from a migratory (Rac1) state and towards the intercalative (RhoA) state required for convergent extension. Aqp3b is also required for neural tube closure, which involves apical constriction and convergent extension. Aqp3b is expressed only in the neural folds during neural tube closure, yet interfering with its expression results in general failure of neural ectoderm cells to undergo apical constriction. Since neural ectoderm cells are linked by gap junctions (Warner, 1973) and our Keller explant experiments showed that Aqp3b acts through the Wnt/Ca2+ pathway as well, we are testing whether Aqp3b acts on neural ectoderm cells from a distance by calcium signaling via gap junctions. We developed a program to capture calcium transients (using the calcium indicator GCaMP6s) in fluorescence time lapse recordings. Existing programs are two-dimensional, leaving time to be evaluated manually. Our program includes analysis across time; in addition to background subtraction and normalization over the injected area, revealing area-normalized transients' characteristics. Finally, we developed a gonadectomy technique that allows sequential use of male frogs' gonads, thus reducing the number of male frogs by half.

Evaluating the Effects of Heatwaves and Micro- and Nanoplastics on Antiviral Immunity and Immune–Brain Interactions

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Micro- and nanoplastics (MNPs) are ubiquitous environmental pollutants that vary widely in size, shape, and polymer composition. While the effects of uniform plastic microspheres at high concentrations are well studied, much less is known about the immunotoxicity of environmentally relevant MNPs with irregular shapes and heterogeneous sizes (I-MNPs), particularly during early development. How MNP exposure interacts with environmental stressors such as heatwaves is also poorly understood. To address these areas of concern, we are leveraging *Xenopus laevis* tadpoles, whose post-embryonic development, including immune system maturation, occurs externally, resulting in direct exposure to waterborne pollutants and temperature changes. Using polyethylene terephthalate (PET), a widely used industrial plastic, we generated standardized I-MNPs with heterogeneous sizes and irregular shapes below 20 μm. We found that exposure to PET I-MNPs through water or oral ingestion at doses as low as 1 μg (~5,800 particles) impaired macrophage function, weakened antiviral immunity, and reduced resistance to viral infection. PET I-MNPs were efficiently internalized by macrophages *in vivo* and persisted for at least one week. We further examined how PET I-MNP exposure interacts with repeated heatwave events. Multivariate gene-expression analyses revealed that heatwave exposure altered integrated stress and endocrine responses in the brain, while increasing TLR, NF-κB, and pro-inflammatory signatures. PET I-MNP exposure did not simply exacerbate these effects but selectively reshaped the physiological response to heat stress. Collectively, our findings identify macrophages as a potential critical target of environmentally relevant I-MNP exposure and establish *Xenopus* as a powerful comparative model for investigating the combined effects of plastic pollution and climate-related heat stress on immune function and immune–brain interactions.

The Xenopus Keratin Atlas: Defining Functional Counterparts to the Mammalian "Keratin Code"

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Keratins are a large, biochemically diverse paralogous gene family (54 genes in human, 74 in *Xenopus laevis*, 44 in *Xenopus tropicalis*) and are the defining intermediate filament proteins of vertebrate epithelia. Keratins, as strong, flexible cytoskeletal polymers, provide essential mechanical integrity to epithelia through their coupling to cell-cell and cell-matrix adhesion complexes. In addition to this well-defined structural role, keratins have also been discovered to play direct roles in cell fate modulating signaling pathways. The patterned expression of keratins across development, tissue type, differentiation state, and disease constitutes a well-characterized "keratin code" in mammals. Although *Xenopus* has a rich history in developmental, epithelial, and cytoskeletal research, no comprehensive functional map onto this mammalian code exists for *Xenopus* keratins. Because the keratin gene family expanded and diversified rapidly with the transition to terrestrial life and the evolution of hair, few 1:1 sequence orthologs exist between amphibian and mammalian keratins. Nevertheless, frogs and mammals share similarly diversified simple- and complex-epithelial keratins expressed in homologous tissues, and the biochemical and mechanical pressures that drive keratin diversification are conserved across vertebrates. This diversification is concentrated in the intrinsically disordered head and tail domains rather than in the sequence-constrained, coiled-coil–forming rod domain. To identify functional counterparts for keratins across this evolutionary distance, we therefore combined sequence-independent analysis of head- and tail-domain amino acid composition with standard analyses of sequence identity, phylogeny, synteny, and expression pattern. Here we present an integrated resource that characterizes the complete *Xenopus* keratin family and assigns functional counterparts to human and mouse keratins. Alignment-free clustering of 225 frog (*krt*), human (*KRT*), and mouse keratins (*Krt*) by head- and tail-domain composition recovers the conserved simple-epithelial groups (*krt7/8, krt18, krt19*) and isolates mammalian hair keratins. Additionally, we show that the frog *krt12* paralogs occupy both crosslinking and non-crosslinking barrier-epithelial niches in embryonic and adult frog tissues, including an appendageal epithelial cluster (*KRT17, krt12.2*), an adult skin cluster (*KRT14, KRT16, krt12.6*), and a mucosal epithelial cluster (*KRT24, krt12.4, krt12.5*), rather than 1:1 orthologs with mammalian corneal *KRT12*. We integrate these compositional groupings with reprocessed public datasets: bulk RNA-seq across development and adult tissues in *X. laevis* and *X. tropicalis*, single-cell RNA-seq from frog, mouse, and human, and developmental proteomics and phosphoproteomics. Together, with HCR RNA FISH and live imaging of fluorescently tagged keratins in embryos, these analyses define the evolution of the keratin gene family between amphibians and mammals, the functional composition of *Xenopus* keratins, and their expression and post-translational modification across development and adult tissues. This atlas establishes a shared vocabulary for keratin biology in *Xenopus* with the mammalian "keratin code" and identifies the frog genes best suited for modeling specific mammalian keratins in embryonic and adult tissues.

Can we possibly learn something new about beta-catenin? Surprises from CP genetics

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Cerebral palsy (CP) is the most common childhood motor disability and a major cause of lifelong neurologic morbidity. Although CP has traditionally been attributed to prenatal or perinatal brain injury, recent genomic studies show that about 1:4 individuals with CP have an underlying monogenic condition. Of these, variants in *CTNNB1*, which encodes β-catenin, have emerged as an important cause of CP-spectrum neurodevelopmental disease. However, over 400 distinct *CTNNB1* variants have been reported and the mechanisms by which patient-associated *CTNNB1* mutations disrupt brain development are unclear. β-catenin is a multifunctional protein that regulates canonical Wnt-dependent transcription and cadherin-based cell adhesion at adherens junctions that has been heavily studied using the *Xenopus* system. While Xenopus has been critical for elucidating the mechanism of β-catenin signaling and regulation, we propose that CP genetics still provides surprises to the function of this protein.

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