Specification of the primary embryonic axis in metazoan embryos: Insights from sea star development

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A critical early event during embryonic development in metazoans is the specification of the primary embryonic axis, the anterior-posterior (AP) axis in bilaterians. AP axis formation is strongly influenced by the maternally established animal-vegetal (AV) axis of the oocyte. A conserved mechanism for AP axis specification is through the localized activation of Wnt/β-catenin (cWnt) signaling in blastomeres derived from the vegetal pole. Studies in echinoderms have demonstrated that Dishevelled (Dvl), a central cWnt regulator, is required to activate this pathway in vegetal pole-derived blastomeres. Dvl protein localizes to puncta throughout the cortex in oocytes of the seastar *Patiria miniata,* and during maturation, these puncta vanish, and new puncta form at the vegetal pole. The maternal mechanisms that regulate Dvl activity in the early embryo are unknown. To identify the maternal factors that regulate Dvl and cWnt activation in vegetal blastomeres, I conducted RNA-seq and mass spectrometry on isolated *Patiria* oocyte animal and vegetal halves and found several mRNAs, long non-coding RNAs, and proteins that are differentially enriched at the two poles. After validating the asymmetric enrichment of these factors to either the vegetal or animal pole, I will experimentally test the hypothesis that these factors specify the AV axis and regulate AP axis formation by establishing cytoarchitectural polarity in the oocyte and/or activating cWnt signaling at the vegetal pole. This work will provide valuable insights into the maternal mechanisms that specify the AV axis in echinoderms and into the evolution of this fundamental polarity in animal eggs.

Chromatin accessibility differences between embryonic and differentiated cells of sea urchin

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We have detected accessibility at regulatory elements in 4-cell embryos long before their transcriptional activation. Also, many of the accessible sites in 4-cell embryos can be detected in other embryo stages and even in differentiated cells. It is not known what causes this early chromatin accessibility and whether early chromatin accessibility may represent the basic mechanism of transcriptional multipotency. We have proposed that early chromatin accessibility might be DNA sequence-dependent, triggering broad localization of labile nucleosomes at regulatory elements.

In this study, accessibility differences between early embryonic stages, larvae, and terminal differentiated immune cells are analyzed through DiffBind differential accessibility analysis and bedtools. This analysis may provide a basic understanding of how early chromatin accessibility relates to the accessibility of terminal differentiated cells. More specifically, the analysis focuses on answering whether some of the

accessibility sites are maintained from 4-Cell to later embryonic stages and perhaps to differentiated cells, whether there is a universal mechanism that shuts down some of the developmental accessibility sites in differentiated cells, which accessibility peaks are specific to differentiated immune cells, and whether differentiation-specific accessibility sites are enriched in pioneer transcription factor motifs.

Adapting to a rotating world: the molecular regulation of circadian rhythms in echinoderm larvae

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Most organisms inhabiting land and sea have evolved endogenous circadian clocks to synchronize their physiology and behavior to the daily light-dark alternations. Although widely studied in terrestrial organisms, the mechanistic bases of circadian rhythms are still poorly explored in marine zooplankton, including echinoderm larvae. The exploration of all available genomic and transcriptomic databases revealed the presence of all canonical animal circadian clock genes in at least 14 Ambulacraria species with the exception of Period, a "universal" component in protostome and deuterostome circadian oscillators. This gene loss hints at a divergent architecture of the circadian clock in this group of organisms. HCR-FISH experiments on sea urchin Paracentrotus lividus larvae showed that the main animal clock components (Clock, Bmal, Timeless, vCry, dCry and Hlf) are expressed in several larval territories, including neuronal populations (e.g., serotonergic neurons) and mesodermally derived cells distributed along the arms and the apex. Transcriptomic analysis over 48 hours of Paracentrotus lividus larvae exposed to light-dark cycles revealed that about 4% of the larval transcripts present a distinct peak of expressionduring the day, suggesting a sophisticated control of transcription according to the light-dark alternation. Among the most rhythmic transcripts, we found a set of circadian clock genes and genes encoding for proteins involved in fundamental larval activities such as skeletogenesis and pigment synthesis. Our study provides evidence of a complex regulation of the sea urchin larva transcriptional dynamics over the diel cycle, offering perspectives to elucidate the evolution and the diversification of salient time-keeping mechanisms in marine organisms.

Fluorescent pigmented cells in sea cucumbers: from development to function and beyond

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Most organisms inhabiting land and sea have evolved endogenous circadian clocks to synchronize their physiology and behavior to the daily light-dark alternations. Although widely studied in terrestrial organisms, the mechanistic bases of circadian rhythms are still poorly explored in marine zooplankton, including echinoderm larvae. The exploration of all available genomic and transcriptomic databases revealed the presence of all canonical animal circadian clock genes in at least 14 Ambulacraria species with the exception of Period, a “universal” component in protostome and deuterostome circadian oscillators. This gene loss hints at a divergent architecture of the circadian clock in this group of organisms. HCR-FISH experiments on sea urchin Paracentrotus lividus larvae showed that the main animal clock components (Clock, Bmal, Timeless, vCry, dCry and Hlf) are expressed in several larval territories, including neuronal populations (e.g., serotonergic neurons) and mesodermally derived cells distributed along the arms and the apex. Transcriptomic analysis over 48 hours of Paracentrotus lividus larvae exposed to light-dark cycles revealed that about 4% of the larval transcripts present a distinct peak of expressionduring the day, suggesting a sophisticated control of transcription according to the light-dark alternation. Among the most rhythmic transcripts, we found a set of circadian clock genes and genes encoding for proteins involved in fundamental larval activities such as skeletogenesis and pigment synthesis. Our study provides evidence of a complex regulation of the sea urchin larva transcriptional dynamics over the diel cycle, offering perspectives to elucidate the evolution and the diversification of salient time-keeping mechanisms in marine organisms.

Transcriptional potency in sea urchin embryos

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Developmental potency is controlled by accessibility to transcriptional regulatory elements (TREs), proximal promoters and distal enhancers. Our ATAC-seq analysis during sea urchin development and differentiation reveals surprisingly early and widespread TRE accessibility that is not explained by concurrent or subsequent transcriptional activation, or by pioneer transcription factor binding site enrichment. Our quantitative Micrococcal Nuclease and sequencing (qMNase-seq) analysis is testing if labile nucleosomes occupy early accessible TREs, possibly in association with multipotency histone variant H2A.Z. In addition, we identify transcriptionally engaged TREs by Precision Run-On and sequencing (PRO-seq), which sharply maps the location of paused or elongating RNA polymerase Pol II genome-wide, as well as the transcription start sites (TSSs) that define TRE functional boundaries. Our PRO-seq comparative analysis reveals that the majority of TRE accessibility changes during development are transcriptional, that numerous distal TREs are transcriptionally engaged despite their insignificant accessibility, and that many TREs become transcriptionally disengaged during embryogenesis. Some inducible genes are controlled at the transition from transcriptional pause to release into productive elongation. However, during embryogenesis the correlated pause and elongation changes in PRO-seq signals at promoters reveal that the majority of upregulated or downregulated genes are controlled by changes in transcription initiation rates. All of the above supports a model where distal TREs halted at pause during early embryogenesis relay their promoter regulation as transcription initiation inputs. This reconciles well with our previously

proposed evolution of distal enhancers from inducible-type promoters, which diversified the ancestral constitutive expression of regulatory genes during the dawn of metazoans.

Development and evolution of nervous system from a cell type perspective
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Stazione Zoologica Anton Dohrn, Napoli, Italy
One intriguing and still open fundamental question in biology is how different embryonic structures or distinct organs, originating from the same embryonic tissue, developed and evolved in different animals. Similarly, the great variation in body plans occurring during the life cycle of indirectly developing echinoderms, from their bilaterally symmetric larvae to their pentameral symmetric adults, has led to many inquiries about how such a change in developmental programs can be carried out by the same genome. To answer these questions, using single-nucleus transcriptomics we reconstructed the cell type atlas of post-metamorphic sea urchin juveniles and compared it to the single-cell atlas of the sea urchin larva providing insight into the conservation of genetic regulatory mechanisms in post-metamorphic cell types. Focusing on the nervous system, and integrating single cell transcriptomics, spatial gene expression analysis, and high-resolution electron imaging, we discovered that homologs of vertebrate neural genes and photoreceptive opsins appear to be expressed throughout the sea urchin body in a five-fold symmetric fashion. Discussing these results, in an animal that was once thought to have a "primitive" nervous system, I will propose that the echinoderm body plan is not only primarily head-like, but also displays an "all-brain" kind of organization.

Investigating conserved mechanisms of RNA transport to the mitotic spindle
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Mitosis is a critical and fundamental process in development, which requires tight spatial and temporal regulation. We have previously shown that localization of miR-31 and its validated targets, *Fascin, Rab35,* and *Gelsolin*, to the mitotic spindles of sea urchin embryos is critical for embryogenesis. Thus, understanding subcellular transport of RNAs to the mitotic spindle is important. We hypothesize that RNA-binding proteins mediate RNA transport by motor proteins along the cytoskeleton to the mitotic spindle. Using pharmacological inhibitors of cytoskeletal and motor proteins, we found RNA transport to be dependent on cytoskeletal actin and tubulin and the motor proteins kinesin and dynein. We bioinformatically identified a cytoplasmic polyadenylation element (CPE) within the *Fascin*, *Rab35,* and *Gelsolin* transcripts. Using reporter constructs and site-directed mutagenesis, we found CPE motif within the *Fascin* 3'UTR necessary for its spindle localization. Knockdown of the corresponding CPE-binding protein, CPEB, also disrupted *Fascin* localization, demonstrating that *cis*-CPE recognized by CPEB in part governs RNA spindle localization. To perform unbiased identification of proteins involved in RNA transport, we use Oligonucleotide-directed proximity-interactome mapping (O-MAP), a technique for RNA-targeted proximity biotinylation to pull-down proteins near spindle-localized transcripts. We will use O-MAP to identify RBPs important for subcellular transport of spindle-enriched RNAs in both sea urchin embryos and mammalian cells to identify conserved mechanisms of RNA trafficking. Since proper cell division is paramount for all cells, organismal development, and human health, understanding RNA transport during cell division is critical.
Echinoderm embryos to model epithelial morphogenesis: from cell biology to evo-devo

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Embryos of many echinoderm species develop freely in sea water with a fertilisation envelope as the only protection from environmental insults. In the sea star Patiria miniata the fertilisation envelope not only protects, but also helps shape the early embryo, as the embryonic cells form a blastula by lining the inner surface of the envelope. This raises the possibility that the spatial constraints imposed by the envelope may influence epithelial morphogenesis, e.g. compaction, cell density and cell connectivity.
We used live-imaging of the sea star embryo coupled with deep learning-based segmentation, to dissect the relative contributions of cell density, tissue compaction, and cell proliferation on epithelial architecture. We found that the 3D connectivity of cells within the tissue changes over time as epithelial compaction and cell density increase. Importantly, these changes in 3D packing are due to the combined effects of cell division within the embryo and of the spatial constraints acting on the embryos.
These results raise interesting hypotheses concerning the role of protective structures, such as the fertilization envelope, in key aspects of embryonic development and its evolution.

From transcription to deposition – genetic and cellular regulation of sea urchin skeletal growth

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Research on sea urchin larval skeletogenesis has greatly contributed to understanding the biological regulation of biomineralization, the process in which organisms use minerals to harden their tissues. The larval skeleton of the sea urchin is made of two spicules, which are frameworks of calcite rods generated by the skeletogenic lineage. The spicules are engulfed in a tubular cavity shared by the skeletogenic syncytium and elongate by the deposition of mineral and matrix proteins at their tips. In this talk I will describe our recent findings on the transcriptional and cellular regulation of this process. We discovered a positive feedback circuitry where VEGF signaling activates the ERK pathway in the skeletogenic cells near the tips of the skeletal rods and ERK drives the expression of VEGF receptor (VEGFR) in these cells. Furthermore, ERK activity is essential for the transcription of the key skeletogenic transcription factor, ETS and the spicule matrix protein, SM50, at the tips of the skeletal rods, and for SM50 clearance from the cells at the back. SM50 proteins accumulate near the skeletogenic nuclei and overlap with microtubules filaments that elongate from the Golgi to the spicule cavity, implying an active transport of vesicles bearing matrix proteins from the Golgi to the spicule cavity. Around the spicule cavity, microtubule filaments are found in the vicinity of active focal adhesion kinase (FAK), suggesting the assistance of focal adhesions in vesicular deposition into

the spicule cavity. Our findings illuminate the intricate genetic and cellular mechanisms that drive sea urchin skeletal growth.

Gene enrichment in the anterior half of *Heliocidaris erythrogramma* larvae: Why is it so neural?

Alejandro Berrio, Esther Miranda, David McClay, Gregory A. Wray

Recent advancements in single cell genomics have significantly enhanced our understanding of the gene regulatory networks involved in development. Despite this progress, the mechanisms underlying the development of neural cells and behavioral traits remain obscure. Larvae of *Heliocidaris erythrogramma* (*He*) navigate the water column without feeding while forming a posterior rudiment that eventually develops into a juvenile within three to four days. In this study, we investigated the molecular differences between the anterior and posterior halves of *He* larvae using RNA sequencing methods. Our findings revealed an enrichment of neural genes in the anterior half, while the posterior half displays, as expected, genes likely involved in rudiment assembly. Further investigation of these neural genes using our single-cell *He* atlas laid new hypotheses related to potential non-visual photobehaviors, which were subsequently evaluated in live larvae. These results provide novel insights into the molecular basis of neural gene expression in sea urchin larvae and highlight potential mechanisms underlying their developmental and behavioral traits.

PFAS Compounds PFOA and Gen X are Teratogenic to Sea Urchin Embryos

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Per-and polyfluorinated substances (PFAS) are synthetic chemicals that are used to make fluoropolymer coatings found in many products, such as non-stick pans, clothing, cosmetics, and food packaging. These highly persistent molecules are known as "forever chemicals" since they neither degrade environmentally nor break down enzymatically within biological systems. PFAS compounds readily contaminate water sources, and as a result, certain PFAS molecules have bioaccumulated in exposed species including humans. The purpose of this study was to define the effect of two PFAS molecules, the ostensibly more toxic perfluorooctanoic acid (PFOA) and the more recent, reportedly safer chemical hexafluoropropylene oxide dimer acid (Gen X), on the

development of *Lytechinus variegatus* sea urchin embryos. We examined the effects of PFOA and Gen X on development and patterning using morphological analysis, immunostaining, HCR-FISH, and Particle Image Velocimetry (PIV). The results show that both PFAS compounds are teratogenic to sea urchin embryos. PFOA and Gen X each function at different intervals during development and provoke distinct phenotypic and gene expression outcomes. Despite beliefs that Gen X would be a safer alternative, our findings indicate that Gen X has earlier and more severe effects on endomesoderm and dorsal-ventral axis specification, neural development and function, and pattern formation compared to PFOA. These results illustrate the dangerous teratogenic potential of environmentally accumulating PFAS like Gen X, underscoring the negative ecological implications that accompany continuing commercial and industrial use of PFAS in the absence of remediation strategies.

Alizarin Red Perturbs Skeletal Patterning and Biomineralization via Catalase Inhibition
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The sea urchin larval skeleton is secreted by PMCs, and its pattern is mediated by the reception of cues from adjacent tissues that direct the positioning and local differentiation of the PMCs. We developed polychrome labeling of PMC-generated biomineral to gain temporal insight into skeletal development, and we thereby discovered that alizarin red (AZ) exposure promotes skeletal patterning and neuronal defects; these findings are supported by transcriptomics analyses. Particle imaging velocitometry experiments show that normal cilia function directs ventral flows to the mouth. In contrast, AZ exposure leads to abnormal flow patterns, suggesting that AZ exposure impairs neural patterning and/or function along with disrupting skeletal patterning. Finally, we offer evidence that AZ inhibits catalase function and show that reactive oxygen species (ROS) exposure is sufficient to phenocopy AZ-mediated skeletal patterning defects. Together, these results reveal a mechanism for AZ-mediated teratogenic effects that disrupt normal developmental pattern formation.

Sea urchin (Lytechinus variegatus) population at the northern range-edge fatally vulnerable to current peak temperature
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Duke University

The rise of sea surface temperatures due to climate change poses a significant threat to marine ecosystems. Populations at the edges of a species' range are particularly vulnerable, as they often inhabit the extremes of their species' tolerance. These range-edge populations also may be useful for conservation efforts due to the adaptations for surviving more extreme conditions compared to core populations. Hence, understanding how range-edge populations respond to thermal stress is useful in studying species-wide

resilience. This study investigated the thermal resilience of a North Carolina northern range-edge population of the sea urchin Lytechinus variegatus, comparing it with a Gulf of Mexico core population. Through acute and chronic temperature exposure trials measuring survival and behavioral responses, I determined that the northern population's upper thermal threshold is 32°C, with chronic exposure causing complete mortality. While in the southern core population, 100% survived. The northern population also showed reduced behavioral performance in both acute and chronic treatments. These findings suggest that northern range-edge populations of L. variegatus have significantly lower thermal resilience than southern core populations. Both populations experience 32°C temperatures in their natural habitats; however, with rapid climate change, the northern population has only experienced peak sea surface temperatures over 31°C within the last two years. This suggests that magnitude, frequency, and duration of peak temperature may be a more accurate metric for predicting population resilience to climate-induced thermal stress compared to mean temperatures.

Technological upgrades to the MBL GERN course, and thoughts on the future of community-wide network models.

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Gene regulatory network models have played an important role in the study of sea urchin development, and serve as the cornerstone of the MBL GERN course. The 2023 offering of GERN highlighted opportunities to advance the tools and approaches for network creation, curation, modeling, and sharing using new experimental and computational technology. In particular, we are planning to expand discussion of single cell RNAseq in future offerings of GERN. In 2024, we also pioneered a new computational platform, dinkum, that provides a Jupyter Notebook interface to gene regulatory network modeling and supports execution and sharing of models as well as BioTapestry output. We are also convening discussions on how to better align the GERN course and educational software development with community maintenance of models like the Sea Urchin Endomesoderm GRN.

Refining functional capacities of immune cell populations in sea urchin larvae

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Immune systems orchestrate the complex relationships – ranging from pathogenesis to symbiosis – between hosts and the microbial world. As invertebrate deuterostomes, echinoderms can provide unique insights into the origins of the molecular machinery that underpins vertebrate adaptive immunity as well as the unique cell types that carry out immune-related functions. The larval stage of the purple sea urchin (*Strongylocentrotus purpuratus*) provides an experimentally tractable, transparent, morphologically simple system in which to study the complexity of the

system-wide immune response at the resolution of single cells. In response to seawater exposure to the marine bacterium *Vibrio diazotrophicus*, larvae undergo a synchronous, non-lethal inflammatory response that involves changes in gut morphology and immune cell recruitment. Using a novel strategy to genetically modify *Vibrio,* we have generated mutant strains that constitutively express acid-resistant fluorescent proteins. Exposing larvae to these strains reveals that, after penetrating the gut epithelium, *Vibrio* are rapidly phagocytosed by a subset of blastocoelar cells. Single-cell RNA sequencing data suggest that these cells express specialized transcripts that encode proteins involved in phagocytosing and degrading microbes. Further analysis of RNA-Seq data reveal transcriptional similarities in immune cell subsets between the larval and adult forms that may point to similar developmental trajectories or functions. Together this work continues to refine our understanding of the ontogeny, function and homology of these echinoderm immune cell lineages.

Impact of exposure to DAPT on development of secondary podia and skeleton in post metamorphic juvenile sea urchin, *Heliocidaris erythrogramma*

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Notch signalling is involved in many developmental processes including in endomesoderm specification and specification of non-skeletogenic mesoderm (Croce & McClay 2010) and tube foot and spine regeneration (Reinardy et al., 2015) in sea urchins. We investigated the role Notch signalling in juveniles of the direct developing sea urchin *Heliocidaris erythrogramma* a model species to investigate gene regulation in post-metamorphic stages. From 4.5 days post fertilisation the juveniles were treated with the γ-secretase inhibitor DAPT. At this age the juveniles had 5 primary podia and 3 adult spines, structures that are patterned in a repeated series along the five axes of the developing juvenile. DAPT treatments started before the secondary podia develop and extended for 15 days by which time control juveniles had two pairs of tube feet and prominent long pointed spines. Juveniles treated with DAPT did not have secondary tube feet on external view. Confocal microscopy revealed that while buds of these tube feet were present, they did not differentiate. The spines of treated juveniles had stunted or no growth. In situ hybridisation showed loss of expression of *Pax6* expression in the tube feet and perturbed expression of *msp130* in the spines. Our findings indicate a role for notch signalling in development of structures that develop in a repeated array along the ambulacral axes of the post metamorphic sea urchin.

Fertilization kinetics after marine heatwave exposure

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Extreme events such as marine heatwaves (MHWs) are increasing in frequency and intensity. These episodic disturbances are associated with mass mortality, shifts in community composition, and loss of ecosystem services. Among broadcast spawners with complex life

histories, such as sea urchins, exposure to MHWs during one life history stage could have a carryover effect on the next. Here, we combined experiments with numerical simulations to examine how exposure to MHWs during gametogenesis influenced gamete quality and fertilization success. Sensitivity to the MHW stress differed significantly between individuals in both sexes. Overall, there was a reduction in cAMP concentration in sperm, which was in turn associated with a reduction in swimming speed and percent motile sperm. Surprisingly, some females produced eggs with a much thinner jelly coat (>50% reduction) after MHW exposure, leading to a smaller target area for sperm. To assess the population consequences of these changes, we applied a modified fertilization kinetics model that allows for variations in traits between groups of gametes as well as time dependence in such traits. We predicted a reduction in fertilization success at environmentally relevant sperm concentrations, as well as changes to the risk of polyspermy and hybridization. Our work highlights the importance of considering the transgenerational impact of extreme events, such that recruitment failure could occur even if the event did not coincide with the pelagic larval stage.

Horizontal transfer of *msp130* genes and the evolution of metazoan biocalcification

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The formation of calcified skeletons is crucial for the development, physiology, and ecology of many marine metazoans. The evolutionary origins of the genetic toolkit required for biocalcification are widely debated. MSP130 proteins, originally identified through their expression specifically by sea urchin skeletal cells, have been hypothesized to have been acquired by metazoans from bacteria through horizontal gene transfer (HGT). Here, we provide support for an HGT-based origin of metazoan MSP130 proteins by conducting phylogenetic and in silico protein analyses utilizing high-quality genomes. We show that *msp130* genes underwent duplications within almost all biocalcifying bilaterian phyla and identify highly conserved intron-exon junctions specific to bilaterian *msp13*0 genes. The absence of MSP130 proteins in calcifying, non-bilaterian metazoans and other basal eukaryotes suggests that an ancestral msp130 gene underwent an HGT event that predates bilaterians, but not metazoans. We report striking structural similarities between bilaterian and bacterial MSP130 proteins, with each containing a seven-bladed, barrel-like motif that encompasses a choice-of-anchor domain, and identify highly conserved, predicted Ca^{2+}-binding sites associated with the barrels. These findings point to a conserved, ancient function for MSP130 proteins in biocalcification and support the view that lateral transfer of bacterial genes supported the appearance of calcified animal skeletons.

Enhancing Orthology with ECOP: A Pipeline for Echinoderm Comparative Genomics

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Echinoderms serve as powerful models for studying gene regulatory networks and genomic evolution, particularly in relation to developmental processes. Applying functional genetic discoveries from experimental echinoderm model systems to other taxa, including humans, requires robust classification of orthologous genes across distantly related species. The Echinoderm Comparative Orthology Pipeline (ECOP) integrates output from ten tree- and graph-based orthology tools—OrthoFinder, SonicParanoid, Hieranoid, OMA, Broccoli, FastOrtho, InParanoid, SwiftOrtho, ProteinOrtho, and OrthoDB—to assign weighted scores to identified orthologous and co-orthologous pairs and grouping them into orthologous gene clusters (orthogroups). ECOP also addresses the complex task of paralog detection by reconciling gene and species trees to pinpoint speciation and gene duplication events critical for understanding gene family expansions, which are prominent in echinoderms. As one of the main goals of Echinobase, ECOP output contributes to gene nomenclature standardization, ensuring that echinoderm gene names align with established gene naming conventions without inconsistencies or misidentifications. Because ECOP is reproducible, customizable, and automated, it can be seamlessly applied to integrate orthologous relationships as new echinoderm proteomes become available. Additionally, its flexible design allows it to be adapted for use in both model and non-model species with available proteomes, making it a valuable resource for broader comparative genomics studies.
ECOP is freely available for testing and application at https://gitlab.com/echinobase-pipelines/ECOP.

Differential gene expression within a multinucleated cell: dorsal-specific expression of genes in the skeletogenic syncytium of the sea urchin embryo

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Most eukaryotic cells possess a single nucleus, but certain specialized cell types, such as mammalian skeletal muscle cells and osteoclasts, contain multiple nuclei. Surprisingly, individual nuclei within multinucleated cells (syncytia) can express distinct localized genetic programs. The larval sea urchin is an excellent model organism to study the mechanisms underlying localized gene expression within a syncytium. Specifically, its morphologically complex skeleton develops within a syncytium containing several dozen nuclei and involves multiple spatially restricted gene expression patterns. Our current understanding is that local epithelial regions overlying the syncytium release signaling ligands, driving gene expression. VEGF3 signaling pathway contributes significantly to ventral skeletal development; but a different, unidentified pathway regulates dorsal syncytial gene expression. My research aims to identify this unknown signaling pathway. One approach has focused on dissecting the cis-regulatory elements that drive dorsally-restricted expression of the transcription factor encoding-gene, *gata3,* within the syncytium. Using reporter plasmids and transgenesis, I pinpointed a small (138bp) region upstream of the transcriptional start site that is sufficient for robust dorsal expression and contains a 16-bp element that is required for this expression. Second, I have found that a specific inhibitor of Type I BMP receptors (K02288) a) blocks SMAD phosphorylation in the dorsal region, b) inhibits the formation of dorsal skeleton, and c) reduces the expression of the *gata3* reporter construct. My current studies are focused on identifying specific inputs into the 138bp upstream element of *gata3* and further analyzing the importance of BMP signaling for dorsal development and gene expression using K02288.

Illuminating unpredicted cellular machineries in germ cell production, gonad development, and reproduction

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Differentiating germ cells must execute fascinating, dynamic, and precisely coordinated cellular and developmental programs for successful fertilization. Deficiencies in these early processes are the leading cause for infertility, miscarriages and gonadal tumors, but the mechanistic defects are unknown because we lack fundamental understanding of the natural processes. In my lab we employ a multidisciplinary holistic strategy to the developing zebrafish ovary, including by advanced quantitative and live microscopy of whole ovaries, as well as genetics, genomics, proteomics and biochemical approaches etc.,. By contributing to our fundemanteal understanding of the earliest stages of egg production, our studies generate knowledge that is directly relevant to human reproduction.

In my talk I will share unpublished data from three main stories from the lab: 1) The discovery of the oocyte (and spermatocyte) zygotene cilium, from basic research in zebrafish to conservation in humans, and identification of ciliary mutants that likely underly premature ovarian insufficiency in patients, 2) Resolving mechanisms of oocyte polarity from symmetry breaking to molecular condensation of a conserved oocyte membraneless organelle, and the roles of the centrosome and microtubule regulation, and 3) The discovery of the developmental microenvironment of germline stem cells and how it uncovers a previously unrecognized mode of oogenesis. I hope to argue that illuminating these unpredicted cellular machineries in germ cell production and gonad development is foundational to making important discoveries in developmental biology and biomedical research.
Learn more here: https://www.yanivelkoubylab.com/

Gene regulatory network dynamics: a developmental transition in the control of the sea urchin skeletogenic network.

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Developmental gene regulatory networks (dGRNs) are dynamic; their circuitry changes as a consequence of autonomous mechanisms and as a response to external signals. Sea urchin skeletogenesis provides a striking example of the dynamic nature of dGRNs. During gastrulation, the skeletogenic dGRN switches from a cell-autonomous to a signal-dependent mode of regulation. This shift is manifested by a change in spatial patterns of gene expression from an early pattern in which effector genes are expressed uniformly by PMCs to a late pattern in which expression is restricted to discrete sub-domains within the PMC syncytium. The cell-autonomous phase of regulation has been well-studied, due partly to its evolutionary novelty, but we know much less about the architecture of the network later in development, when overt skeletogenesis occurs. The analysis of developmental changes in dGRN structure has been facilitated by new tools for the spatiotemporal control of gene expression in sea urchins. This presentation will summarize the current state of knowledge regarding dynamic changes in the skeletogenic dGRN during sea urchin embryogenesis.

Wnt inhibitory factor-1 fine-tunes early Wnt-mediated endomesoderm and neuroectoderm patterning in sea urchin embryos

Fenner, JL, Gautam S, Ka C, Moore W, Grant M, Stocks C, and Range RC

Wnt signaling drives germ layer (endoderm, mesoderm, and ectoderm) specification and patterning along the anterior-posterior (AP) axis during early embryonic development of across metazoans from cnidarians to humans. Despite the importance of Wnt signaling in many developmental processes we still have a limited understanding of how diverse Wnt signals are modulated during embryogenesis in any model system. In sea urchin embryos AP axis formation is controlled by an integrated Wnt signaling network of canonical (Wnt/β-catenin) and non-canonical (Wnt/JNK and Wnt/PKC) pathways. We have previously shown that several extracellular Wnt modulators (e.g., DKKs and sFRPs) regulate the AP Wnt signaling network during positioning of each early germ layer gene regulatory network (GRNs) along the AP axis. Here, we examined the role of a poorly characterized extracellular Wnt signaling modulator, Wnt inhibitory factor-1 (Wif1), during early sea urchin embryogenesis. We show that *wif1* expression in the posterior endomesoderm is regulated by canonical and non-canonical Wnt signaling but that BMP2/4/7 signaling activates *wif1* expression in the dorsal ectoderm. Perturbations of Wif1 result in downregulation of key genes in the endomesoderm GRN leading to gastrulation defects. Our functional assays also indicate that during late blastula stages Wif1 in the dorsal ectoderm works synergistically with Wnt7 to position specific anterior neuroectoderm (ANE) GRN components around the anterior pole. Together, our findings show that Wif1 is a previously uncharacterized member of the posterior Wnt signaling landscape that establishes critical regulatory subcircuits in the sea urchin endomesoderm GRN. And that activation of Wif1 by dorsal BMP2/4/7 signaling and Wif1's subsequent refinement of patterning within the ANE territory establishes an important link between anterior-posterior and dorsal-ventral axial patterning processes.

Chromatin Remodelers are Required for Normal Development

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Chromatin remodelers regulate gene expression by dynamically altering the structure of chromatin. We have previously characterized their expression profiles in the early embryonic development of L. variegatus. Through these experiments, we identified a number of genes encoding chromatin remodelers that are active from fertilization through the pluteus stage. Now, we aim to determine the specific roles these genes play in development, and more specifically whether they impact skeletal patterning. We performed morpholino microinjections to knock down chromatin remodeler genes to test whether gene knockdown leads to morphological perturbations. In embryos in which chromatin remodelers expressed earliest in development have been knocked down, gastrulation occurs but development is stunted during or shortly after primary skeleton formation, suggesting that these genes are essential in the transition between primary and secondary skeletal patterning. Additionally, preliminary data suggests altered patterning and gene expression profiles in the primary mesenchyme cells, which are responsible for secretion of the larval skeleton. Further experiments will continue to characterize both the temporal and spatial effects at the morphological and molecular level that result from

blocking expression of chromatin remodeler genes.

The evolutionary conservation of inflammation

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Innate immune responses rely on the detection of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRR) that trigger intracellular signaling cascades to produce immune mediators including pro-inflammatory cytokines. One function of these cytokines is to induce their own expression in a feed-forward loop to amplify the nascent immune response leading to a physiological state commonly referred to as inflammation. In mammalian model systems for which the components are well-understood with interleukin-1β (IL-1β) being one of the essential cytokines. In contrast, our knowledge about the molecular basis of inflammation is much more limited and restricted to receptors and intracellular messengers that are readily identified based on sequence conservation. In contrast, only a single pro-inflammatory cytokine family has been described in echinoderms and other invertebrates. Here we describe the discovery and characterization of the first members of the IL-1 superfamily in invertebrates and related immune factors. Our observations support a model that a central signaling pathway, the inflammasome axis, that was originally described in mammals is in fact an ancient immune response module that emerged in an ancestor of cnidarians.

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Single-Cell Gene Expression Analysis for the PMC Response to Embryonic Patterning Cues

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The sea urchin larval skeleton provides an elegant, morphologically simple model to study developmental pattern formation. In this two-component system, mesodermal cells called PMCs produce a calcium carbonate skeleton in response to patterning cues that are expressed in discrete spatial regions by the adjacent ectodermal cells. To understand how the PMCs respond to patterning cues, we performed temporal single cell transcriptomics on PMCs from control embryos and from embryos with loss-of-function for one of four patterning cues via

pharmacologically inhibition. We identified and spatially mapped gene expression trajectories within the PMCs using ICAT, single molecule FISH, and automated detection of the cells and the FISH signals within them. Our findings demonstrate that PMC trajectories arise in spatially discrete domains and show that patterning cue responses in PMCs involve a combination of static and dynamic spatial expression changes. Finally, we found that each patterning cue loss-of-function resulted in discrete gain and loss patterns for PMC subpopulations. This extensive spatiotemporal analysis of the PMC response to normal and abnormal cue states reveals that patterning relies on multiple ectodermal cues that each direct local diversification-based patterning of spatially discrete PMC subpopulations to drive the skeletal pattern formation process.

The regulation of sea urchin spicule matrix proteins - from transcription to deposition

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Biomineralization is a vital biological process through which organisms produce mineralized structures such as shells, skeletons, and teeth. A common feature of all biomineralizing organisms is the expression of phylum specific matrix proteins that are transported into a dedicated biomineralization compartment where they regulate the properties of the mineral. Here we use the sea urchin larval skeletogenesis to investigate the regulation of the expression and trafficking of matrix proteins to the biomineralization compartment. The gene that encodes the spicule matrix protein, SM50, is expressed at the active growth zones at the tips of the sea urchin skeletal rods. We revealed a positive feedback circuitry where VEGF signaling activates ERK in the skeletogenic tip-cells, and ERK drives the expression of VEGF receptor (VEGFR) and SM50 in these cells. ERK is responsible for the clearance of SM50 from the cells at the back. The SM50 protein overlaps with microtubule filaments that elongate from the Golgi to the spicule cavity. The microtubule filaments elongate from around the skeletogenic nuclei to the biomineralization compartment where they overlap with active focal adhesion kinase (FAK). Apparently, VEGF and ERK pathways control *SM50* gene expression, then, the SM50 protein is packaged in vesicles that are trafficked on microtubules to the spicule cavity, where their exocytosis is assisted by focal adhesions. We propose that while matrix proteins and their transcriptional regulation are phylum specific, the microtubule-guided vesicle transport into the biomineralization compartment could be a common mechanism in Eukaryotes' biomineralization.

Neural expression of CPEB2: A shared regulatory mechanism in Ctenophores and Bilaterians

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As the sister group to the rest of animals, ctenophores (comb jellies) are key lineage for reconstructing the biology of the last common animal ancestor (LCA). Two primary views on ctenophore neuron evolution suggest either independent origins or presence in the last common animal ancestor. Classical studies on neurotransmitter systems, ion channels, and nerve cell development homology remain inconclusive on ctenophore-bilaterian neuron relationships. Cytoplasmic Polyadenylation Element-Binding Proteins (CPEBs) are post-transcriptional

regulators that interact with specific target mRNAs to drive timely and localized translation. These early animals established two CPEB families: CPEB1, which functions in eggs and early development, and CPEB2, which regulates sperm development and neuronal protein synthesis. Our results from single-cell RNA sequencing demonstrates that CPEB2 is present in both mature and developing neurons in neural cell types of the comb jelly Mnemiopsis. Moreover, our HCR in situ hybridization shows that, during early development, CPEB2 is specifically active in the tentacle bulbs and tentacles. Our current aim is to investigate the function of CPEB2 in Mnemiopsis neurons, compare it to its role in bilaterian animals, and reconstruct its potential function in the last common animal ancestor. We provide new insights into nervous system evolution and cell types of the last common animal ancestor.

Melatonin Synthesis in *S.purpuratus* larvae

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Melatonin, often referred to as the "hormone of darkness", is an evolutionary conserved molecule across the phylogenetic tree and plays a pivotal role in regulating sleep in vertebrates. However, the presence of melatonin and its potential link to the circadian clock and sleep-like states in deuterostome invertebrates, such as the Echinodermata phylum, which is sister group to chordates, remains largely unknown. While the genome of the sea urchin *Strongylocentrotus purpuratus* contains melatonin receptors, no clear orthologues of arylalkylamine N-acetyltransferase (AANAT), the canonical rate-limiting enzyme in melatonin synthesis, have been identified. We therefore used liquid chromatography-mass spectrometry (LC-MS) to test for melatonin synthesis in *S. purpuratus* larvae and to examine whether diel differences in melatonin concentration exist in this species. Our preliminary results from fed and unfed urchin larvae provide evidence of melatonin in *S.purpuratus*, challenging the previous assumption that endogenous melatonin synthesis is absent in this species. We are now using phylogenetic and protein-folding analyses to search for candidate alternative melatonin-synthesizing enzymes in the *S.purpuratus* genome. This work provides a foundation for understanding the evolution of melatonin synthesis and its potential relationship to the larval sea urchin circadian clock and behavioral patterns.

Unveiling the role of maternal factors in regulating egg polarity and Wnt/β-catenin signaling during early *Nematostella* embryogenesis

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Oocytes from most animals have a primary polarity referred to as the animal-vegetal (AV) axis that is established during oogenesis by the asymmetric distribution of maternal determinants. In many taxa the AV axis strongly influences the specification and patterning of the primary embryonic axis through the localized activation of the Wnt/β-catenin (cWnt) signaling pathway by maternal determinants that are asymmetrically distributed along the primary egg axis. In the cnidarian *Nematostella vectensis,* localized activation of cWnt signaling pathway in animal pole-derived blastomeres specifies endomesodermal cell fates at this pole and initiates patterning of the primary embryonic axis, the oral-aboral axis. In contrast, in bilaterians such as echinoderms localized activation of cWnt signaling at the vegetal pole regulates endomesoderm specification and initiates patterning of the anterior-posterior (AP) axis. This observation has led to the hypothesis that both endomesoderm specification and gastrulation evolved at the animal pole in pre-bilaterians by the asymmetric activation of Wnt signaling, whereas these processes have been shifted to the vegetal pole during bilaterian evolution. Identification of upstream maternal regulators of Wnt signaling will be crucial to design experiments to test this hypothesis. To this end I carried out mass-spectrometry proteomics of isolated animal and vegetal halves from *Nematostella* eggs. This analysis identified proteins that are potentially differentially enriched along the AV axis and are involved in specifying egg polarity and cWnt pathway activation during *Nematostella* embryogenesis. Preliminary functional tests of the roles of these factors in regulating egg polarity and cWnt activation will be presented.

The evolution of the Cadherin-Catenin-Complex in the ctenophore species *Mnemiopsis leidyi*

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The Cadherin-Catenin-Complex (CCC) is a crucial component of adherens junctions in epithelia for both maintaining homeostasis, normal embryonic development, and cell-cell adhesion. The CCC allows epithelial cells to adhere to their neighboring cells through a mechanical interaction between classical cadherins and the actin cytoskeleton through the interaction between β- and α-catenin. Despite this importance in all animals surveyed, the CCC has been poorly characterized in ctenophores, the sister lineage to the rest of animals. Genome sequencing in ctenophore species revealed that they possess a full set of CCC genes. Nevertheless, a previous bioinformatic study showed that *Mnemiopsis leidyi* cadherins don't possess a β-catenin binding site, casting doubt on the conservation of the β-catenin function in cell-to-cell adhesion in ctenophores. However, we previously showed that β-catenin was localized at the cell-cell junctions during *M. leidyi* embryonic development, suggesting that the role of β-catenin in cell-

to-cell adhesion might be conserved in *M. leidyi*. Additionally, single-cell data from over 23,000 cells revealed that the CCC components were co-expressing in many cells during *M. leidyi* embryonic development. To validate these data, we are currently developing hybridization chain reaction on the 3 CCC genes to see if they are coexpressing in vivo. Finally, to test if interactions within the CCC are conserved, we plan to carry out both co-immunoprecipitation coupled with mass spectrometry, and Yeast 2-Hybrid screening. Further experiments are necessary to fully characterize the CCC in the ctenophore *M. leidyi*.

Genetics, automation and the juvenile nervous system of Lytechinus pictus
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The defining biological feature of the sea urchin is its capacity to produce virtually unlimited numbers of embryos. This attribute was well-exploited during the biochemical era for ensemble measurements of protein and gene expression. However, as genetics and imaging have become primary analytical tools, leveraging this biological feature has become challenging due to the labor-intensive nature of transient genetic methods and low-throughput imaging techniques. In this presentation, I will summarize our recent advances in transgenesis and knock-in technologies for the sea urchin *Lytechinus pictus*, and their integration into automated analysis platforms. I will also introduce our fully automated hybridization chain reaction (HCR) pipeline, capable of large-scale gene expression pattern profiling. This pipeline, termed high-throughput HCR (HT-HCR), can process up to 192 gene probe sets on whole-mount embryos within 32 hours. Finally, I will discuss how integrating automation, knockout, knock-in, and transgenesis approaches is enabling novel studies in our laboratory on the impacts of anthropogenic changes on nervous system development and function.

A hemichordate single cell atlas reveals the extensive lineage-specific turnover and diversification in neuron type selector and effector programs
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Metazoans harbor a diversity of terminally differentiated cell types; in particular, some bilaterians possess a high diversity of morphologically and molecularly distinct neurons. While the shared evolutionary origin of the bilaterian neuron has been established, it remains poorly understood when and how this neuron diversified into the many types present in extant lineages. We applied single cell RNA sequencing to the worm *Saccoglossus kowalevskii* to catalog the neural populations present in a representative of the phylum Hemichordata and gain further insight into the evolutionary history of neural cell populations within Deuterostomia. We identify a relatively high molecular diversity of terminally differentiated neurons in the hemichordate despite its simple behavioral repertoire and the primarily decentralized nature of its nervous system. Leveraging this new dataset and gene expression atlases from members of Echinodermata, Chordata, and Arthropoda, we make comparisons of neuronal gene expression programs across phyla and find that a few gene modules, including neurotransmitter pathways, are deeply conserved in their co-expression. However, in terms of their overall selector and effector gene expression, few cross-phyla pairs of neuron populations share any strong

similarities. Hemichordate neurons, like those of model bilaterians, show highly type-specific expression of unique sets of transcription factor genes, primarily homeodomain genes, but the combinations of such genes are distinct from those coexpressed in other species. These results suggest that, following the divergence of the bilaterian phyla, there was large-scale turnover and elaboration in the transcriptional regulatory circuits that determine the molecular phenotype of each type of terminally differentiated neuron.

Thyroid hormones reversibly inhibit metamorphic development in ophiuroid larvae

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The timing of metamorphosis and settlement is essential for the survival and reproductive success of marine animals with biphasic life cycles. Thyroid hormones (THs) are known to regulate developmental timing in chordates, notably influencing metamorphosis in amphibians, teleosts, and other groups. Recent findings indicate that THs may also play a role in metamorphosis across non-chordate taxa, including echinoderms, annelids, and mollusks. In echinoderms, TH effects during early embryogenesis have been documented in echinoid larvae, yet information on TH signaling in the metamorphosis of other echinoderm groups groups, such as ophiuroids (brittle stars), remains limited. Our research reveals that THs, particularly 3,5,3′,5′-Tetraiodo-L-thyronine (T4), reversibly inhibit metamorphic development and settlement in the daisy brittle star (*Ophiopholis aculeata*). Furthermore, exposure to thiourea, a TH synthesis inhibitor, accelerates metamorphic development, indicating a developmental point-of-no-return in ophiuroid metamorphosis that is stage-specific. Additionally, starvation of *O. aculeata* promotes juvenile morphogenesis and settlement and mitigates the inhibitory effects of thiourea on TH function. These observations suggest that TH synthesis may delay metamorphosis when food is plentiful. Our findings highlight the role of TH signaling in ophiuroid metamorphic development and propose that exogenous thyroid hormone sources could influence metamorphic timing in *O. aculeata*. Collectively, this study underscores the significance of endocrine signaling in regulating metamorphosis across a spectrum of invertebrates.

Building to Regrow: The Sea Star Blastema and Larval Regeneration

Veronica Hinman, Whitney Marine Laboratory

Understanding how injury induces cellular plasticity and tissue regeneration remains a fundamental question in developmental and regenerative biology. Sea star larvae provide a tractable and evolutionarily informative model for dissecting the molecular and cellular transitions that underlie whole-body regeneration. In this study, we leverage larval bisection in Patiria miniata to investigate the early events that drive blastema formation and regenerative competence. We identify a previously uncharacterized cell population in normal larva that exhibits characteristics consistent with a wound-responsive, fate-plastic cell type. We further define a novel transcriptional state induced specifically by bisection, preceding visible regenerative outgrowth. Integrative analysis of chromatin accessibility and gene expression reveals a distinct set of non-coding regulatory elements that are either re-deployed from embryonic development or uniquely activated during regeneration. These regeneration-specific enhancers are associated with the transcriptional programs that establish regenerating blastema. Together, our findings offer new insights into how injury cues reconfigure gene regulatory landscapes and mobilize cellular responses to enable regeneration.

Host microbe interactions Between *Lytechinus pictus* Larvae and Black Spot Disease Associated Bacteria, *Vibrio cylcitrophicus,* and *Shewanella electrodiphila.*

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The immune response of larval sea urchins to bacteria, particularly *V. diazotrophicus,* has been well characterized. However, little is understood about how bacteria stimulate and respond to larvae. This is increasingly important as microbial populations change in response to global environmental shifts. Understanding which species of bacteria negatively impact larval health is essential for predicting the long-term health of sea urchin populations.
To understand larval responses to potentially pathogenic bacteria they encounter in the environment, we first characterized the species of bacteria associated with black spot disease in adult *S. purpuratus* and *L. pictus using* metagenomic and molecular methods. Two species of bacteria, *Vibrio cyclitrophicus* and *Shewanella electrodiphila* were chosen for further study. Bacteria were grown in culture and *L. pictus* larvae were exposed to each bacteria at a concentration of 10^6 cfu/mL. Compared to *V. diazotrophicus* exposed larvae, different phenotypes were observed. Larvae exposed to *S. electrodiphila* showed inflammation of the ectoderm, and intestinal epithelia, and increased pigment cell association with skeletal rods.
To gain insight into how bacteria are causing these phenotypes, we looked at bacterial gene expression. Exposed larvae were HCR-probed for pan-bacterial 16SrRNA signal (EUB338), species-specific 16S, and virulence gene probes to identify compartments of colonization or niche establishment. Previously characterized larval immune genes were used to pinpoint areas of host-pathogen interactions.
Collectively, our results show that disease associated bacteria found in adults may influence larval development and survival. Future work will clarify the mechanisms of pathogenesis in larvae and adult sea urchins.

Using HCR-RNA FISH to investigate spatial gene expression in the sea urchin tooth in developing life stages

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Biomineralization enables the production of vital protective and mechanical structures to many species. Sea urchin teeth are a unique model for understanding the mechanisms that underly biomineralization due to their extreme toughness and hardness. The adult tooth has been extensively studied so its material properties including a self-sharpening mechanism and chemical composition consisting of magnesium-rich calcium carbonate with a magnesium gradient are well understood. However, the structure and composition of the tooth at intermediate

growth stages and the molecular mechanisms that underpin the tooth development are less well-known. To improve knowledge of the genetic mechanisms involved in sea urchin tooth development, we used hybridisation chain reaction RNA fluorescent *in-situ* hybridisation to survey the spatial expression of skeletal genes in the juvenile tooth. Using a candidate approach, we designed probes for genes active in the genetic regulatory network (GRN) for embryonic skeletogensis to implicate their involvement in tooth development. This was followed by immunohistochemistry to localise skeletal proteins in the juvenile teeth. Transcription factors *Alx1* and *Ets1/2* and differentiation genes *Sm50* and *Caral7a* were expressed in the juvenile tooth. This suggests that mechanisms active in embryonic skeletogenesis are conserved in the juvenile tooth, suggesting the GRN for tooth formation may resemble the GRN for embryonic skeletogenesis. We will examine the expression of these genes on cross-sections of the adult tooth to identify developmental differences in gene expression. Finally, this will be supported by the generation of a transcriptome for the plumula of the tooth to allow insight into all genes expressed.

VitelloTag: a tool for high throughput cargo delivery into oocytes and other ovarian cell types

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Developmental and reproductive biology research broadly relies upon the delivery of molecular and genetic tools into oocytes to perform perturbations and study various biological processes. In diverse model organisms, including mouse, zebrafish, xenopus, and echinoderms, this is achieved through microinjection. Microinjection, while highly effective, has challenges in terms of cost of setup, the technical skill required, and the limited numbers of injected oocytes obtainable. It also proves challenging in organisms with delicate oocytes or restricted spawning seasons. To overcome these limitations, we have developed VitelloTag, a simple, cost effective, and high throughput method of delivery into oocytes, comparable in terms of technical difficulty to transfection. Vitellogenesis is a yolk-protein uptake pathway, highly conserved across many animal phyla. It involves the yolk protein precursor vitellogenin, which is recognized by vitellogenin receptors on the oocyte cell surface, triggering its endocytosis and ultimately resulting in the formation of yolk platelets in the developing oocyte. Here, we present a delivery system that employs conserved regions of vitellogenin, recombinantly fused with the protein of interest for delivery via receptor-mediated endocytosis and endosome escape. We demonstrate this tool's utility and cross-taxa applicability by delivering GFP and Cas9/sgRNA complexes, with successful gene knockout phenotypes, in two distantly related species. We also show VitelloTag can deliver cargo to multiple cell types within the ovary and that it can be used to study the process of vitellogenesis in diverse species.

Transcriptomic response of sea urchin coelomocytes to UV-B induced DNA damage

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Our innate immune system plays a role in recognizing and eliminating DNA-damaged cells to avoid genomic instability. However, the mechanisms by which the DNA-damage response (DDR) and innate immunity interact to eliminate DNA-damaged cells remain poorly understood. Sea urchins are a promising neoplastic-disease-resistant animal model that can be leveraged to identify and characterize the links between the innate immune system and the DNA-damage response, which will provide new insights into innate immunity and reveal novel pathways important for cancer prevention. While the response of the sea urchin immune system to pathogenic challenge is well-characterized, the response to DNA damage is largely unknown. To characterize this response, we employed UV-B radiation to induce DNA damage to *Strongylocentrotus purpuratus* immune cells (coelomocytes) and quantified differentially expressed genes at 0, 1, 3, 6, and 24 hours of recovery *in vitro* using single-cell transcriptomic and bulk transcriptomic sequencing. Our results revealed a striking transcriptional response to UV radiation, with upregulated DNA-damage response genes (including those involved in cell cycle arrest, DNA repair, and autophagy) and innate immune system genes, including Toll-like receptor (TLRs), NOD-like receptor, scavenger receptor cysteine-rich (SRCR), and tripartite motif (TRIM) family members. This *in vitro* work provides foundational data to understand the interactions between the DDR and the global immune-regulatory mechanisms that protect these animals from cancer, and sets the stage for follow-up work that will map the interactions of the DDR and sea urchin innate immune system *in vivo*, with the goal of determining how healthy immune cells respond to those with DNA damage.

Estrogen is awesome

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Sex steroids are essential in many reproductive systems, and are involved in development of secondary sexual characteristics, gonadal maturation, germ cell proliferation, and sexual behavior. Unlike humans who are born with a finite pool of oocytes that are depleted mid-life, echinoderms show no reproductive senescence, implying the existence of stem cells for eggs that are constantly replicating. We hypothesize that echinoderms use estrogenic signaling mechanisms to regulate long-term stem cell function for oocyte proliferation. First, we found that the genomes of sea urchins and sea stars have genes expressed in their ovaries required for estrogen biosynthesis. Second, we challenged ovaries from different ovarian stages of maturation with estrogen and measured expression of potential estrogen responsive genes by DEG-seq2. From that result, qPCR, single cell RNA-seq and in situ hybridization of estrogen responsive candidates were tested. Our qPCR results revealed that while several genes were sensitive to estrogen, (myp, wwox, nvd, stard5), variation between ovaries was significant. This indicates that gene expression is likely to be highly specific to the cell types and stages of the ovary. The localization of transcripts did not change with estrogen treatment, but in some cases, e.g. MYP, the abundance changed dramatically. We will next determine estrogen's role by investigating the estrogen responsive DNA elements responsible for this activity in ovaries as well as in embryogenesis.

Establishing *Mespilia globulus* as a new model species

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We have established *Mespilia globulus*, a species of tropical euechinoid urchin, as a model species for developmental and evolutionary biology. To achieve this goal, we have generated a chromosomal-scale genome assembly with full annotation for *M. globulus*. We have also captured the regulatory and transcriptomic landscape during embryonic, larval and postlarval development with ATAC and bulk RNA sequencing. With the aid of the comprehensive genomic resources generated, we investigate the evolution of novelties in echinoderms, such as the water vascular system, tubefeet and pentaradial symmetry. This will be achieved through transcriptomic analysis of gene expression modules coupled with a comprehensive phylostratigraphy of expressed genes. *M. globulus* has also been proven to be amenable to the hybridisation chain reaction (HCR), a high resolution molecular imaging technique. We have already applied this technique to view the hox cluster across different developmental stages. To aid in these results, we have successfully closed the life cycle of *M. globulus* under laboratory conditions, from fertilisation through to metamorphosis and adult growth. This achievement solidifies *M. globulus* as a powerful model species for molecular, developmental, and evolutionary research.

In Pursuit of Pharmacological Inhibitor for DEAD-box Helicase 6

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In this project, we use *Lytechinus variegatus* (Lv) as a model to investigate the functional role of DEAD-box helicase 6 (DDX6) in pattern formation and the regulation of developmental timing. DDX6, an RNA helicase, is a necessary component of P-bodies, which are liquid-liquid phase separated cytoplasmic organelles that sequester and inhibit RNAs and proteins. DDX6 is required for normal skeletal patterning and other aspects of development. To better understand the role of DDX6, we are working to identify a pharmacological inhibitor that specifically inhibits it. DEAD-box helicases are inhibited by a family of chemicals known as rocaglates. In collaboration with the Porco lab in the Chemistry Department at Boston University who are rocaglate experts, we performed a chemical screen of over 500 rocaglate compounds. We identified a small number of them that produced phenotypes, and one in particular that exhibits a phenotype which is very similar to that obtained with LvDDX6 knockdown. We will share experiments that address the specificity of this candidate DDX6 inhibitor. Successful identification of a specific DDX6 inhibitor will significantly enhance our ability to perform multi-omics analysis on DDX6 loss-of-function embryos.

Molecular and structural signatures of the oviduct in *Lytechinus variegatus*

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Organisms with female gonads usually have oviducts for transporting eggs to sites of internal fertilization or for releasing eggs outside of the body. The oviduct of the sea urchin is a duct connecting the aboral tip of each adult female gonad directly to the gonopore. Observed through careful dissection, the oviduct of *Lytechinus variegatus* does not terminate at the aboral tip of the ovary and instead extends down through the center of the ovary, connecting all the way to the oral tip of the gonad. It is hypothesized that the intra-gonadal extension of the oviduct serves as structural support to the ovary, a mechanism for egg transportation during spawning, and as a component in *L. variegatus* ovarian development. Preliminary structural analyses indicate presence of an epithelium and maintenance of the tubular structure within the gonadal extension of the oviduct. Transcriptomic analyses of the oral/aboral regions of the ovary denoted molecular gradients along the length of the gonad and, separately, distinct molecular signatures within the oviduct as compared to the surrounding lobes of the ovary. A member of the homeobox gene family, Six4, was particularly enriched within the oviduct. Such enrichment suggests a possible developmental role of the oviduct in ovarian oral-aboral axis formation. CRISPR-Cas9 experiments will test insight into the developmental implications of the *L. variegatus* oviduct, underscoring the potentially expanded role of this structure within the developmental biology of the gonad in related organisms.

Involvement of the Tyrosine Kinase RET and of the orphan receptor GDNF receptor alpha-like (GFRAL) upstream of nodal expression

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Axis specification is an essential process during embryogenesis in metazoa. In some species, this process is initiated by maternal factors that are localized in distinct regions of the unfertilized egg, while in others, it is entirely dependent on zygotic factors. In the highly regulative sea urchin embryo, establishment of the dorso-ventral (D/V) axis critically depends on the zygotic expression of the TGF-β ligand Nodal. nodal expression is induced ubiquitously in the 32-cell embryo and progressively gets restricted to the presumptive ventral ectoderm during the early stages of development, making nodal one of the first genes to be asymmetrically expressed along the D/V axis. This early spatial restriction of nodal expression depends on the maternally expressed TGF-β ligand Panda. panda mRNA is asymmetrically deposited in the unfertilized egg in a shallow dorsal to ventral gradient. In the absence of Panda, nodal expression is no longer restricted and expands dramatically throughout the ectoderm, despite the presence of Lefty. Thus, in the sea urchin embryo, Panda has the properties of a maternal determinant of the D/V axis. However, the mechanism by which Panda works remained enigmatic. We reported recently that Panda works as an antagonist of the type-II receptor ACVRII. We have traced the antagonistic activity of Panda to the presence of a single Proline residue in its ACVRII binding region, which likely confers a dominant negative activity to this factor .We will present our recent work on additional mechanisms by which Panda orients the D/V axis. In particular, we will describe a second mechanism that involves the receptor tyrosine kinase RET, the orphan

receptor GDNF receptor alpha-like (GFRAL) and the downstream MAPK pathway as potential regulators of nodal expression and of D/V axis specification.

A Gene Regulatory Network for the Response to Embryonic Patterning Cues

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Gene regulatory networks (GRN) are essential to understand complex, biological spatiotemporal processes such as sea urchin skeletal pattern formation. However, its regulatory mechanisms remain poorly understood. To understand the regulation of embryonic patterning cues, we combined our single-cell RNAseq dataset with orthologous cross-species transcription factor (TF) binding motifs to infer the underlying GRN using SCENIC. We identified nine major regulons within the mesodermal cells along with the hub TFs that drive them. Knockdown of these hub TFs results in skeletal patterning defects, implying that these TFs are specifically required for the response to ectodermally expressed patterning cues. We corroborated ~ 30% of the SCENIC GRN predictions via bulk RNA-seq analysis, specifically validating most of the hubs. Finally, we found that each patterning cue loss-of-function targets distinct regulons in the GRN. Altogether, our analysis provides insights into how spatiotemporal and network integration drives the skeletal pattern formation process.

The P-body component LvDDX6 is necessary for normal morphogenesis in sea urchin embryos

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Early developmental patterning and morphogenesis are fundamental processes that are still not well understood. Perturbing these processes results in a range of disorders including cancer, intellectual disability, and physical disabilities. The RNA helicase DEAD-box helicase 6 (DDX6) is required for the formation of processing bodies (P-bodies), which are cytoplasmic liquid-liquid phase separated compartments involved in mRNA repression and sequestration of RNAs and proteins. DDX6 and P-bodies are required for major phenotypic transitions including epithelial-to-mesenchymal transition, maternal-to-zygotic transition, and the entry and exit from pluripotency; DDX6 plays a role in numerous disorders including cancer, viral hepatitis, and intellectual disability. We found that LvDDX6 is necessary for normal morphogenesis of the sea urchin embryo and larva, with DDX6 perturbations impacting the gut, mesoderm, skeleton, and nervous system. These results indicate that DDX6 is required for normal development of all three germ layers and suggest that DDX6 is involved in the gene regulatory network that reflects the instructive signaling between the ectoderm and PMCs during skeletal patterning.

Modulations depending on life history: Wnt signaling in posterior patterning of hemichordate embryos

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In most classical model systems, the adult body plan is formed directly during embryogenesis, a life history strategy called direct development. However, the life history strategy of most metazoan phyla is indirect, meaning the embryo first becomes a larva and has a body plan radically different to that of the adult. Enteropneust hemichordate worms are a class of marine deuterostomes that contain representatives from both of these life history strategies. In the indirect developing hemichordate, *Schizocardium californicum*, the larval ectoderm corresponds to the anterior territories of the adult while the posterior, Hox-expressing trunk regions are only added later during metamorphosis. The mechanisms of trunk development have been studied in detail in the direct developing species *Saccoglossus kowalevskii* and are known to be under the control of Wnt signaling. We hypothesized that the delay in anteroposterior trunk patterning in an indirect developer is controlled by differences in the timing of activation of Wnt signaling. To investigate this possibility, we identified and characterized the expression of components of the Wnt signaling pathway in multiple stages of development in *S. californicum* and compared these expression patterns with corresponding stages in *S. kowalevskii.* We treated the *S. californicum* embryo with a small molecule Wnt signaling activator to identify gene expression changes and chromatin accessibility changes during early AP patterning. These data suggest that the function of Wnt signaling is conserved in inhibiting anterior patterning, but does not promote posterior ectodermal fates in the indirect developing hemichordate.

Exploring animal evolution through chromosome-level comparative genomics

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Advances in sequencing technologies have granted us unprecedented access to chromosome-level genomes, offering new opportunities to investigate animal evolution. But how can we utilize this data to answer key evolutionary questions? In this talk, we will share recent progress from our lab, where we use these genomic resources to explore the evolution of animal genome structure. We will begin with bryozoans, where chromosome fusion and fission processes have led to the partitioning of genes from their Hox clusters onto multiple chromosomes. This provides valuable insights into the evolutionary history of Hox genes and highlights their close relationship with brachiopods. Next, in annelids such as earthworms and leeches, we will examine how highly rearranged genomes offer fresh perspectives on genome evolution within bilaterians. We will also discuss the conserved role of key signaling pathways in brachiopods, shedding light on body patterning mechanisms across different animal groups. Finally, we will present findings from coral genomics that clarify the relationship between corallimorpharians and stony corals, deepening our understanding of coral calcification. This talk will demonstrate how chromosome-level genomics can provide critical insights into complex evolutionary processes and help resolve long-standing

questions in animal phylogeny. We hope to offer a thoughtful exploration of how these tools are advancing our understanding of the animal tree of life.

Plasticity of Invertebrate Cell Division in a Warming Ocean
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Sea surface temperatures are forecasted to rise 1-4°C across the next century. It has been shown that ocean warming has devastating impacts on adult marine organisms, which raises another ecological question: will marine embryos be able to adapt? We explore this using sea stars to define the thermal resilience of early cellular events. Live imaging reveals that *Patiria miniata* embryos from the Pacific are acutely sensitive to temperature; a 2°C increase from their normal temperature causes incomplete cleavage at the first cell division. These failed cytokinesis attempts display a loss of coordination between cell cycle progression and cytokinetic ring constriction as daughter nuclei progress into the next cell cycle before cleavage furrow ingression is completed. This may be due to altered biophysical properties of the cortex, or dysregulation of contractile machinery. However, some embryos recover in the subsequent cell cycle through a plastic, multipolar division. To explore how thermal tolerance is tuned to different environments, we test the resilience of cytokinesis in embryos from *Asterias forbesi,* a warmer water Atlantic sea star with significantly smaller oocytes. The embryos display similar incomplete cleavage and recovery phenotypes to *P. miniata,* but at 3°C higher temperatures. Surgically reducing the size of larger *P. miniata* oocytes towards that of *A. forbesi* rescues cytokinesis at elevated temperatures where normal-sized oocytes would experience incomplete cleavage. These findings suggest that adult adaptation to warmer waters correlates with embryonic thermal tolerance, and that multipolar compensation during embryonic cell division may be a conserved corrective response to thermal stress.

Transfection and transduction in urchin embryos and urchin derived cell lines
Carl Manner, David McClay, Greg Wray

Scalable delivery of genetic cargo remains a challenge in the sea urchin, where all genetic perturbation is accomplished by microinjection. Clearing this barrier is the last remaining obstacle to unlocking the power of forward genetic screening for this model. In this work, we have investigated the use of Lipid NanoParticle (LNP) based transfection in the urchin embryo and in urchin derived cell lines, alongside several serotypes of AAV. Additionally, we report advances in lentiviral targeting to urchin cells, where we employed AI-based peptide docking prediction and alphafold3 to generate novel envelope proteins targeting sea urchin cell surface receptors.

***Leptosynpata tenuis* in the lab: a clear, tiny, and portable sea cucumber from Cape Cod.**
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Sea cucumbers, members of the echinoderm class Holothuroidea, exhibit unique developmental traits such as whole-body and organ regeneration. Our research focuses on establishing the local sea cucumber species, *Leptosynpta tenuis* as a new model to study development and regeneration. While other sea cucumbers are big and heavily pigmented with thick body walls, *L.tenuis*, a member of Apodida, is small, transparent, and slender, and found along the US East Coast, including Cape Cod. Because husbandry and shipping for sea cucumbers is challenging, very little research has been done so far on this species that is still quite unknown. In our preliminary work, we established protocols to keep *L. tenuis* in the lab and the MBL Marine Resource Center. Taking advantage of the fact that this is the only adult echinoderm that is completely clear, we are exploring its anatomy and physiology through immunofluorescence and behavioral experiments. Other aspects that have not been explored yet are the reproductive behavior and life cycle of *L. tenuis*. We are tracking gonad maturation over time and find a correlation between seawater temperature, which in Cape Cod changes dramatically over the months, and oocyte size. Since *L. tenuis* requires little care in the lab, can be easily shipped, and it is not endangered, we envision this as a promising model to study regeneration, behavior, and neurobiology in an adult echinoderm.

Building a gut.

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Sea urchin gastrulation displays what is probably the prototype for deuterostomes. All but a few cells derived from the vegetal half of the embryo either enter the blastocoel via an epithelial-mesenchymal transition or invaginate as a sheet of endoderm cells that establishes the archenteron. Late in gastrulation the stomodaeum, invaginating from the ventral side, connects with the archenteron to form the through-gut. Specification of this process occurs in two phases. Early specification of mesoderm and endoderm are well documented in the Gene Regulatory Network (GRN) established through the work of many labs over the past 25 years. Further specification to establish parts of the mesoderm and gut occur during gastrulation, with substantial contributions from Wnt pathways. Here we will show the dynamics that occurs both in the movement of cells and the expression and function of Wnts1,5,7,8 and 16. Concentric rings of Wnt expression move outward during gastrulation as mesoderm and endoderm cells converge toward and through the blastopore. As this dynamic movement and expression occurs cells are programmed toward their several differentiated states.

Translation regulation of cell cycle genes at fertilization

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mRNA translation is stimulated upon fertilization, and is required for progression through the first cell cycles of the sea urchin embryo. The translational activation occurs in an mTOR (mechanistic Target of Rapamycin) dependent manner and involves the assembly of the canonical eIF4 initiation complex on the cap structure of the maternally stored mRNAs. By a polysome profiling approach, we have identified a pool of mRNAs specifically recruited into

polysomes in response to fertilization. The set of translated mRNAs encodes for proteins implicated in different regulatory circuits, such as those controlling the cell cycle. We will present data regarding the regulation of polysome recruitment of mRNAs encoding several cell cycle proteins, by signaling pathways activated at fertilization.

Oocyte-derived Microtubule Projections: a Novel Putative Signaling Organelle in Oocyte Development

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Sexual reproduction requires the production of egg cell precursors called oocytes. Oocytes develop in the ovary in follicles, where they are enveloped by somatic follicle cells which support their development. However, oocytes and follicle cells are separated by thick extracellular layers. How do these cells communicate across such a barrier? Communication is canonically considered to be mediated by actin-based structures called transzonal projections (TZPs) that extend from follicle cells toward oocytes. However, in prophase I-arrested oocytes in the sea star *Patiria miniata*, we observe thin, microtubule-based projections (which we call OMPs, or oocyte-derived microtubule projections) that emanate from the oocyte surface toward the follicle cell, in the opposite direction as TZPs. To our knowledge, such structures, which are structurally distinct from both cilia and microvilli, have not been seen before in any animal oocyte. We hypothesize that OMPs may be important for oocyte-follicle cell communication. Using time lapse imaging of live oocytes at high spatial and temporal resolution, we show that OMPs contain microtubules, mainly lack actin, are dynamic on the order of minutes, and can contact follicle cells. In contrast to cilia, OMPs do not contain acetylated tubulin, but some OMPs do contain components of the intraflagellar transport complex. OMPs are lost during the process of meiotic maturation, suggesting that they play a specific developmental role. We propose that OMPs are likely dynamic signaling organelles that are important for oocyte-follicle cell communication during oocyte development.

Single-Larva RNA Sequencing Reveals That Red Sea Urchin Larvae Are Vulnerable to Co-Occurring Ocean Acidification and Hypoxia

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Anthropogenic carbon dioxide emissions have been increasing rapidly in recent years, driving pH and oxygen levels to record low concentrations in the oceans. Eastern boundary upwelling systems such as the California Current System (CCS) experience exacerbated ocean acidification and hypoxia (OAH) due to the physical and chemical properties of the transported deeper waters. Research efforts have significantly increased in recent years to investigate the deleterious effects of climate change on marine species, but have not focused on the impacts of simultaneous OAH stressor exposure. Additionally, few studies have explored the physiological impacts of these

environmental stressors on the earliest life stages, which are more vulnerable and represent natural population bottlenecks in organismal life cycles. The physiological response of the ecologically and commercially important red sea urchin (Mesocentrotus franciscanus) was assessed by exposing larvae to a variety of OAH conditions, mimicking the range of ecologically relevant conditions encountered currently and in the near future along the CCS. Skeleton dissolution, larval development, and gene expression show a response with clearly delineated thresholds that were related to OAH severity. Skeletal dissolution and the induction of Acid-sensing Ion Channel 1A at pH 7.94/5.70 DO mg/L provide particularly sensitive markers of OAH, with dramatic shifts in larval morphology and gene expression detected at the pH/DO transition of 7.71/3.71 mg/L to 7.27/2.72 mg/L. Experimental simulations that describe physiological thresholds and establish molecular markers of OAH exposure will provide fishery management with the tools to predict patterns of larval recruitment and forecast population dynamics.

The ECM is really cool

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The extracellular matrix (ECM) is a complex network of multidomain-containing macromolecules including laminin and collagen. The ECM serves as a physical scaffolding and provides a dynamic microenvironment that influences development and differentiation. Cells interact with this matrix through integrins, receptors for specific ECM proteins. We sought to identify gene functions in the ECM in sea urchins (*Strongylocentrotus purpuratus and Lytechinus variegatus)* and in the sea star (*Patiria miniata*). We identified candidate ECM transcripts by blasting to known ECM sequences, and by proteomic analysis of ECM extracts from larvae and ovaries. Using RNA *in situ* hybridization, single cell RNA seq analysis, and newly generated monoclonal antibodies, we have identified the expression of over 40 ECM components and receptors. These are expressed in diverse profiles in the embryo including the primordial germ cells (PGCs). In sea urchins, the four PGCs reach the tip of the archenteron during gastrulation and enter the coelomic pouches. Our data suggest that multiple ECM components affect this accomplishment of the PGCs and qPCR analysis of germ-cell factors shows that gene expressions unique to the PGCs are in disarray when specific ECM gene expressions are altered. We also found that knockdown of various integrins, especially the integrin subunits α6 and βc, result in coelomic pouch/PGC defects These findings highlight the functional roles of specific ECM components in the regulation of cellular specification and morphogenesis of the somatic cells and the germline in echinoderms.

Single cell transcriptomics reveal the pre-chordate origins of conserved ovarian cell types and regulatory systems

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Sexual reproduction in animals requires the development of oocytes, or egg cells. This process, termed oogenesis, does not occur autonomously but rather through complex interactions amongst germline and somatic cell types in the ovary. How did these cell types and their signaling interactions evolve? Here we used the sea star *Patiria miniata* as a non-chordate deuterostome representative to define the ovarian cell type toolkit in echinoderms. Sea stars continuously produce millions of new oocytes throughout their lifespan, making them an excellent system to understand the mechanisms that drive oogenesis. We performed scRNA-seq on sea star ovaries, combined with high-resolution 3D-imaging, to reveal the ovarian cell types and their spatial organization. Our data reveal the presence of actively dividing oogonial stem cells and suggest the presence of granulosa-like and theca-like cells, which display similarities with their mammalian counterparts. Last, our data support the existence of an endocrine signaling system between oogonial stem cells and ovarian neurons with similarities to the vertebrate hypothalamic-pituitary-gonadal axis. Overall, this study provides molecular evidence supporting the possible pre-chordate origins of conserved ovarian cell types, and the presence of an intrinsic neuroendocrine system controlling oogenesis that predates the formation of the hypothalamic-pituitary-gonadal axis in vertebrates.

Leveraging quantitative physics-based approaches to investigate fluid flows in marine invertebrate larvae

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The ocean is filled with ciliated invertebrate planktonic larvae that thrive in a highly viscous fluid environment. Many of these larvae use ciliary beating to drive fluid flows essential for swimming and feeding. Their diverse body shapes, local morphologies, and ciliation patterns create intricate and dynamic three-dimensional flows that are challenging to investigate in laboratory conditions. Conventional microscopic imaging techniques typically involve gently confining the soft larvae between a glass slide and cover slip to observe their flows in a quasi-two-dimensional setting. However, a comprehensive framework for understanding the physics of larval scale fluid flows under such confinement has remained elusive given their complex body shapes and morphologies.
We show that vortices surrounding larvae increase in number with stronger confinement and deduce the underlying physical mechanism. By experimentally measuring confinement-induced flows in sea star and sea urchin larvae, we observed strikingly consistent patterns: under weak

confinement, all larvae generated two vortices, whereas strong confinement led to the emergence of multiple vortices. Combining experimental observations with theoretical physics analysis, we developed a comprehensive framework for understanding confinement-induced flows in a wide range of ciliated marine invertebrate larvae.
We leverage our physics-based experimental assays and computational data analysis techniques to quantify the effects of PFAS compounds on sea urchin larvae. We quantitatively demonstrate that PFAS compounds such PFOA and Gen X perturb ciliary behavior and swimming.

Alizarin Red mediates skeletal patterning defects in sea urchin embryos via elevation of reactive oxygen species

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Alizarin Red (AZ) is a calcium-binding fluorochrome used for labeling skeletal structures that has been shown to produce toxic effects in many species. AZ is also used as a textile dye and can pollute water systems and have adverse effects on the development of aquatic species. However, the extent of these effects and the mechanisms by which they arise are mostly unknown. Our study is one of the first to connect these mechanisms to developmental defects mediated by AZ in the sea urchin Lytechinus variegatus, an ideal model for studying developmental patterning. Embryos exposed to AZ have abnormal anterior patterning and skeletal element rotation. Immunostains reveal delayed migration and ectopic clusters of the skeleton-secreting primary mesenchyme cells, while polychrome labeling confirms delayed initiation of biomineralization and spurious elements. We also find mild dorsal-ventral (DV) defects, demonstrated by partial radialization and incomplete ciliary band restriction, along with patterning defects in the serotonergic neurons. These results suggest that, despite its calcium-binding function, AZ perturbs the ectoderm to mediate DV and skeletal patterning defects. Additionally, prior studies showed that AZ inhibits catalase. We also find that AZ-treated embryos have an elevated concentration of reactive oxygen species (ROS), consistent with the inhibition of catalase. Most AZ-mediated skeletal patterning defects are phenocopied both by catalase inhibition and by H2O2 treatment, providing further evidence that elevated ROS mediates the majority of AZ-induced abnormalities. These findings support a model in which AZ disrupts catalase function to promote ROS accumulation that, in turn, produces developmental defects.

Extracellular control of the anterior-posterior Wnt signaling network in sea urchin embryos

Ryan C Range, Che Ka, Boyuan Wang, Sujan Gautam and Jennifer Fenner

Embryogenesis of multicellular organisms depend on the cell's ability to interpret the extracellular signaling environment through precise ligand/receptor interactions at the cell surface. In sea urchin embryos, anterior-posterior (AP) axis formation is governedby a complex landscape of Wnt receptors, co-receptors and extracellular modulators that control three different, but interconnected, canonical and non-canonical Wnt pathways (Wnt/Beta-catenin, Wnt/JNK and Wnt/PKC). Here, we give an overview of our current understanding of this Wnt landscape at the cell membrane, focusing on two newly discovered Wnt signaling components in the sea urchin – a receptor tyrosine kinase, ROR1/2, and a secreted Wnt antagonist TIKI1/2. Using morpholino and dominant negative interference assays, we establish ROR1/2 as a co-receptor for the non-canonical Wnt1/Wnt8-Fzd5/8-JNK-ATF2 signaling pathway that positions the anterior neuroectoderm (ANE) gene regulatory network (GRN) around the anterior pole during blastula states. We also show that ROR1/2 is necessary in a separate developmental mechanism to maintain the expression of the entire endomesoderm GRN by the beginning of gastrulation. Our functional perturbations of TIKI1/2 expression show that it works in concert with other secreted Wnt antagonists (Dkk1/2/4, sFRP1/5, and sFRP-like) to prevent the elimination of the ANE GRN by precocious Wnt/Beta-catenin and Wnt/JNK signaling activity. We also establish that TIKI1/2 is expressed in the posterior endomesoderm where it modulates specific Wnt dependent endomesoderm gene expression. Together, these results highlight strengths of using pre-gastrula sea urchin embryos as a model for untangling the complex Wnt signaling environment that establishes the AP axis in many metazoan embryos.

Development of the sea lamprey adaptive immune system

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Lymphocytes maintain proliferative capacity and stem-like qualities to carry out clonal functions in the immune systems of vertebrates. Most of what is known about these cells comes from studies in mammals where they orchestrate adaptive immunity mediated by somatically diversified B cell and T cell receptors. Jawless vertebrates have similar cell types that carry out immunity using Variable Lymphocyte Receptors (VLRs), an analogous but structurally unrelated family of diversified cell surface receptors and soluble antibodies. In comparison to jawed vertebrates, investigations in the living Agnatha (hagfishes and lampreys) provide a window into the early evolution of vertebrate adaptive immunity and its underpinnings in invertebrate immunity. We have performed scRNAseq on several lymphopoietic tissues, VLRB repertoire analyses, and CRISPR/Cas9 perturbation studies to characterize the development and function of lamprey B cell-like lymphocytes. Both typhlosole (a gut associated immune tissue) and the anterior kidney are lymphopoietic and show signatures of VLRB assembly and maturation relative to peripheral blood lymphocytes. Experimental and genomic analyses identify markers for developing B-like cells in these tissues and elucidate the relationship of these cells to jawed vertebrate B cells. Although lamprey B-like cells exhibit many parallels to systems in the jawed vertebrates, they also display striking differences. Given the ancient separation of these two lineages and their divergent genomic trajectories, these differences are expected and promise insights into the early state of vertebrate adaptive immunity and its origins in the immune systems of more distant deuterostome common ancestors.

How do the eyeless larvae of the blood fluke Schistosoma mansoni detect light?

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Human blood flukes are parasitic trematode flatworms from the genus Schistosoma. They infect 237 million people worldwide and cause the disease schistosomiasis (Bilharizia or snail fever) which is responsible for 1.0–2.6 million Disability Adjusted Life Years and over 11,000 human deaths annually. The life cycle of schistosomes is complex, involving parasitic stages in two hosts (a freshwater snail and a mammal), with two free-swimming larval stages in between (the miracidium and cercaria). Both larval stages are short-lived and non-feeding, with limited energy reserves with which to find and infect a host to propagate the life cycle. Light triggers a positive phototactic swimming response in both larvae which brings them into the bright, shallow waters where the air-breathing snail host and mammalian hosts are found. However, the mechanisms that drive the photoresponse are puzzling as Schistosoma species lack eyes. In the case of flatworms, eyes are composed of two cell types: photoreceptor cells and a cup-shaped pigment cell. Pigment cells are clearly missing but we hypothesize that there must be photoreceptor cells. We have generated single cell atlases of both larval stages and custom-made antibodies against opsins (putative photopigments) to investigate the molecules and cells that drive the photoresponse. Our preliminary findings show that both larval stages have opsin+ cells adjacent to the brain, suggestive of vestigial eyes, and opsin+ cells in transient larval cell types and organs that indicate novel ‘extra-ocular’ photoreception.

A collagenous extracellular matrix regulates germline gene expression in the sea star embryo

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The extracellular matrix (ECM) is a network of proteins necessary for structural support, cell signaling, and tissue repair. Collagen is a fundamental component of the ECM network that coordinates binding with other proteins such as laminin and integrins. β-aminopropionitrile (BAPN) is a well-known inhibitor of lysyl oxidase (LOX), an enzyme important for the cross-linking and stabilization of mature collagen in the ECM. The function of ECM in the development and formation of germline cells was tested in the sea star *Patiria miniata.* Germline formation in this animal uses inductive mechanisms and is distinct from the sea urchin. Here, we use BAPN treatments to test the significance of ECM integrity in forming the sea star germline. DEG-RNA seq experiments suggest that disruption of ECM by BAPN and Col003, a more specific collagen processing inhibitor, dysregulates germline gene expression and normal gut development at specific times of development. We selected the top upregulated and downregulated genes and by RT-qPCR results learned that both inhibitors resulted in the same effects. We also identified an uncharacterized gene from our screenings as BAPN Associated Target with Malformed Archenteron (BATMAN) since it was consistently upregulated in the BAPN treatments and spatial expression is in the defective archenteron. Additionally, two germline genes, Piwi and Nanos3, were similarly affected by both treatments, with Piwi being downregulated and Nanos3 upregulated. We conclude that the ECM is essential for the inductive mechanism of germ line formation, and believe the sea star is an excellent model for identifying causative mechanisms.

Lysyl Oxidase is Essential for Germ Layer Formation

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The extracellular matrix (ECM) is a critical component of embryonic development, providing both structural support and a dynamic signaling environment for cell migration, adhesion, and tissue organization. Collagen, the most abundant protein in the ECM, plays a pivotal role in these processes by forming a supportive network through crosslinking, which is mediated by the enzyme lysyl oxidase (LOX). Dysregulated LOX activity disrupts the mechanical integrity of the ECM.
Sea urchins offer a robust model for studying LOX and ECM dynamics in embryonic development due to their rapid, transparent development and traceable cell lineages, including germ cell movements. Through an invagination during gastrulation which forms the three primary germ layers, somatic cells and germ cells migrate to their respective locations using the ECM as a scaffold. Previous studies using the pan-monoamine oxidase/LOX inhibitor, Beta-aminopropionitrile (BAPN), suggested an essential role of LOX in gastrulation and in maintaining ECM integrity. Here, we incorporate BAPN along with newly developed LOX inhibitors (PXS-4787 and PXS-5153) and morpholinos targeting LOX2 to inhibit LOX activity. Using LOX activity assays, qPCR, and microscopic imaging, we explored the effect LOX inhibition has on cell fate decisions with a particular focus on germ cells. We find that

gastrulation is particularly sensitive to LOX inhibition, and the mRNA levels of several candidate genes (FoxY, Nanos, Wnt8, and Nodal) increase several-fold, suggesting a compensatory transcriptional response in the absence of a high-fidelity ECM.
We conclude from this data, that germ cells monitor the status of the LOX-dependent ECM and respond transcriptionally.

Revisiting Davidson's Postulate: modulators of genomic regulatory code readout for development

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Eric H. Davidson was a strong proponent of explaining metazoan development in terms of causal gene regulatory network dynamics. During the last 30 years of his life, he coaxed a large community of scientists to collaborate creating the empirical foundation of a gene regulatory network model for early sea urchin development. This became an inspiration to those working in other developmental genetic systems. However, his concept of how the network should operate rested on a deeper postulate about the way that regulatory information should be encoded in metazoan genomes and, especially, what should be rate-limiting about the way that transcription factors read this information. Davidson saw combinatorial transcription factor binding as the primary determinant of gene expression in all contexts and transcription factor site occupancy in any real cell nucleus to be overwhelmingly determined by specific target motif recognition. Thus, expression specificity would be predictable based on the presence of clustered motifs for the right factors and absence of motifs for the wrong factors in genes' cis-regulatory regions. Self-evident?

In the decade since Davidson's death, abundant genomic research in multiple systems and results of numerous perturbation experiments in embryonic and non-embryonic systems have offered fresh perspectives from which to consider Davidson's postulate and its corollaries. This talk will focus on rich molecular genetic data from mammalian hematopoiesis, a stem-cell based developmental system. These results show unexpected constraints and modulators of Davidson's postulate and different conditions under which they apply, and point toward the new initiatives needed for deeper understanding.

Exploring the pancreatic-like cell composition of a sea urchin at a single-cell resolution

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The digestion of food sources relies on the production of digestive enzymes and hormones involved in metabolic homeostasis. In vertebrates, the organ responsible for controlling such processes is the pancreas. Even though forming a specific pancreatic organ is a vertebrate innovation, pancreatic-like cell types have been found in many invertebrates, together with a highly conserved expression of canonical pancreatic genes such as *Pdx-1* (pancreatic and duodenal homeobox-1). *Pdx-1* is a transcription factor that in humans guides pancreas formation, B-cell differentiation, and subsequently controls insulin production in the latter, resulting as a causal factor in some types of diabetes. Despite the evident conservation of *Pdx-1* expression in gut cells of most deuterostomes, most studies focus on the exocrine/digestive cells, leaving the rest of the "pancreatic landscape" and the evolutionary origin of the organ pancreas still unknown. Using single-cell transcriptomics, we here explore for the first time the pancreatic-like cell type composition of the Mediterranean sea urchin *Paracentrotus lividus* larva raised under a differential feeding regime and aim to put it in an evolutionary context by comparing it to other deuterostome species.

Immune regulation and metabolic exchange between the acoel *Symsagittifera roscoffensis* and its photosymbiont

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Acoels are marine flatworm-like invertebrates characterized by a simple body plan and belong to the phylum Xenacoelmorpha. The acoel *Symsagittifera roscoffensis* shows a photosymbiotic association with the green alga, *Tetraselmis convolutae*. *S. roscoffensis* establishes the symbiosis after hatching (i.e., horizontal transmission), and the symbiont is located in extracellular space in the worm's body. To elucidate the mechanism underlying the maintenance of photosymbiosis, we investigated the effects of various abiotic factors on *S. roscoffensis* through physiological, morphological, and RNA-seq analyses under different nutrient concentrations and photoperiod conditions over one week. The number of symbiotic algae increased under nutrient-enriched conditions and decreased in darkness. This result suggested that the algal density within worms was regulated by the host's immune system, similar to the symbiosis observed between cnidarians and dinoflagellates. To validate this hypothesis, we focused on the Toll-like receptor (TLR) signaling pathway which play a crucial role in the innate immune system. Interestingly, typical TLRs composed of TIR and LRR domains were absent in *S. roscoffensis*; however, several TLR-like genes were identified. Most of TLR-related genes were downregulated under nutrient-enriched conditions and upregulated in darkness, suggesting that the TLR signaling pathway may be involved in regulating symbiont abundance. Additionally, we identified candidate transporters potentially involved in metabolic exchanges in *S. roscoffensis*. Electron micrographs revealed that symbiotic algae attached to the peripheral parenchyma, suggesting that parenchymal cells play a key role in maintaining the symbiosis. Further studies are needed to determine the specific cell types that express these genes.

A novel role for host defense peptides in transducing bacterial cues for marine invertebrate metamorphosis via TLR signaling.

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Many marine invertebrate species utilize bacterial cues to trigger metamorphosis. However, the mechanisms microbial cue sensing and signal transduction remain under-studied. We identified an antimicrobial "host defense" peptide (AMP) that links bacterial presence with innate immunity pathways to induce metamorphosis. The echinoderm-specific AMP, *Strongylocin 2* was identified in the painted sea urchin, *Lytechinus pictus*. Renamed *pictocin*, this gene is developmentally upregulated only in late larval stages that approach metamorphotic competency. Using HCR RNA-FISH, we localized *pictocin* transcripts to lipid-rich cells that are present in the rudiment, the base of the pedicellaria, and within the ciliary band. Pictocin protein co-localized to endosomes in these cells and in complexes along larval cilia. Pictocin protein vesicles were also present within the neurons underlying the ciliary band. Pictocin^{+} vesicles expand across the nervous system of the larva at the onset of metamorphosis. Notably, mammalian AMPs similar in structure to the pictocin peptide will form nanocrystalline structures with microbial dsDNA to activate Toll-like receptor (TLR)-mediated inflammatory signaling. Perturbation studies with TLR pathway inhibitors in *L. pictus* caused a dose-dependent and significant reduction in metamorphosis. CRISPR/Cas9 knockdown of *pictocin* resulted in reduced metamorphosis rates and retention of decaying larval tissue at the aboral surface of larvae that do complete metamorphosis. Newly developed transgenic tools in the *L. pictus* system will allow us to trace pictocin-mediated signaling in live larvae and identify downstream targets. In summary, our work suggests that a deeply conserved TLR pathway is amplified by microbial cues to activate sea urchin metamorphosis. This provides a foundation for testing how the innate immune system orchestrates developmental events and extensive tissue remodeling in marine invertebrates. This may also provide novel application to studies on overactive AMP signaling in autoimmune disease with features of dysregulated tissue shedding (*e.g*. psoriasis).

Defining the cleavage plane stimulus-response system at the nanoscale level in sea urchin embryos

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Rappaport's micromanipulation experiments identified overlapping arrays of astral microtubules as the delivery mechanism for the stimulus that define the site of contractile ring assembly. We

now know that the stimulus is comprised of Centralspindlin, a complex of MKLP1 (mitotic kinesin-like protein) and a GTPase-activating protein (Cyk4) that in turn recruits the RhoGEF responsible for Rho activation and ring assembly. At its earliest stages, contractile ring (CR) components such as anillin, septin, and myosin II (MyoII) are organized as small clusters or nodes; and as the CR matures, these nodes coalesce into a highly organized contractile assemblage. However, it is unknown whether these nodes represent local contacts between Centralspindlin and the CR precursors. MKLP1 strongly colocalized with early MII-containing nodes, but as the ring matured, MKLP1/MyoII colocalization diminished. MKLP1 recruitment to nodes required neither actin nor myosin II contractility, suggesting that MKLP1 does not require local tension for association with anillin/septin/MyoII clusters. However, blocking either MII or ROCK activity blocked the ability of MKLP1 to bundle microtubules at the cell surface, providing support for the notion that there exists a feedback loop between the stimulus (MKLP1) and the response (Myosin II). Current efforts are focused on capturing MKLP1/node interactions in live cells as well as blocking Centralspindlin activation to examine its effects on node assembly at the cell equator.

Go Nads - Puberty in the sea urchin and development of the unique transcriptional profile of gonads

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Adult *Lytechinus variegatus* is a valuable model for researching important questions of reproduction, and gonad function. The study of gonad development is enhanced by knowing the timeline post-metamorphosis for formation, growth, and gamete proliferation in gonads. We tested F1 progeny post-metamorphosis for developmental milestones of the somatic and germline cells in the developing gonad. Under controlled environmental conditions with ad libitum feeding, we measured size and wet weight of the developing juveniles, and learned that the F1 animals were asynchronous in their growth kinetics but that gonad and germ cell development is correlated to size of the animal, not the time of development. At a test width of 20mm (wet weight avg. 0.83 g) the gonadal precursor appears, and continually developed in direct relation to the size of the juvenile, reaching maturity and gamete formation at test width 32mm (wet weight avg. 3.92 g) within 4.5 months. External features of the pubescent animals, such as open gonopores, were a non-invasive confirmation of reproductive maturity. To focus on subsequent gonadal development we first performed DEG-RNA-seq on the 16 major tissues in the adult and the resulting whole body tissue atlas revealed the diversity of organ and tissue functions. Gene candidates per organ were confirmed by qPCR and explored by in situ RNA hybridization. Merging the datasets from the puberty study with the adult whole body tissue atlas now enables gene prioritization for functional studies based on knockouts of genes uniquely expressed in the adult gonad.

Characterizing Immune Cell Development in an Ancient Cidaroid Sea Urchin

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Cidaroidea is a primitive monophyletic subclass of sea urchin, sister group to Euechinoidea, the modern urchin. Fossil records have placed the emergence of this group ~270 million years ago during the Guadalupian epoch of the mid-Permian. Preliminary research has demonstrated that the process of embryogenesis of cidaroids differs from euechinoids in key aspects, particularly in regard to the differentiation of skeletal and non-skeletal mesenchyme tissues. There still exists, however, a major knowledge gap concerning the GRNs that drive the processes of embryogenesis in cidaroid urchins. We propose to stage the development of the cidaroid urchin *Eucidaris tribuloides* from fertilization to metamorphosis. Additionally, we aim to gain novel insights into the GRN controlling hematopoiesis with a focus on the differentiation of an adjacent population of mesoderm cells that give rise to immune cells. We will use *in vivo* imaging to define developmental stages and anatomical features. To study the GRN of this system, we will focus on *gata1/2/3, gcm,* and *irf4*, which have been implicated in *S. purpuratus* immune cell development. Gene expression will be localized using in situ hybridization chain reaction and further quantified through qPCR and RNA sequencing. Finally, regulatory connections will be confirmed using reporter constructs in which predicted transcription factor binding sites can be mutated. Comparative methods will be used to assess the architecture of the E. tribuloides GRN in the context of echinoderm evolution. These methods will thoroughly examine the underlying processes of *E. tribuloides* development and immune cell differentiation.

Extending the gene regulatory network underlying immune cell development in *Strongylocentrotus purpuratus*

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The sea urchin larval immune system is mediated by give morphologically distinct cell types. These develop from two regions of non-skeletogenic mesoderm (NSM) specified during mid-blastula stage. Previous work demonstrated that orthologs of transcription factors that regulate vertebrate hematopoiesis also play important roles in maintaining pluripotency in sea urchin immune cell precursors (GataC and Scl). We hypothesize that these transcription factors form the beginning of a complex gene regulatory network (GRN) that specifics immune cell development. Embryos injected with GataC Morpholino Antisense Oligonucleotides (MASO) were used in RNA-Seq analysis to identify downstream targets of GataC. From this analysis, we find that the expression of another transcription factor, Irf4, is dependent on GataC activity. Hybridization Chain Reaction (HCR) analysis indicates that Irf4 and GataC are co-expressed in the oral mesoderm during mid-blastula stage. MASO perturbation of Irf4 results in embryos with a significantly high number of pigment cells, which is consistent with an expansion of the aboral mesodermal marker *gcm* during blastula stage. These data point to a clear role for Irf4 in sea urchin larval hematopoiesis. This function mirrors that in vertebrates, where Irf4 is required for lymphocyte differentiation. Additional downstream targets of GataC include *Cebpa* and *Sequestosome1*, which involved in the development and maintenance of vertebrate immune system. Together these findings suggest that the sea urchin larval immune system is built using an ancestral deuterostome toolkit.

Post-transcriptional regulation during early development

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All living organisms localize RNAs in regions of the cell to control local gene expression. We found select microRNAs and their targets localize to the mitotic spindle to regulate local translation during early development. One of the RNAs that we examined is the *Fascin* RNA, which encodes a protein that is involved in actin bundling and microtubule polymerization. *Fascin* RNA and its regulatory miR-1 and miR-31 are enriched on the mitotic spindle in dividing blastomeres and perinuclear in non-dividing blastomeres. Localization of these RNAs to the mitotic spindle is evolutionarily conserved, as we also observe this spindle localization in mammalian cells. We found miR-31 regulates local translation of these transcripts to ensure proper cell division. Forced ectopic translation of Fascin at the cell periphery in dividing embryonic cells resulted in chromosomal segregation defects, developmental delay, and even embryonic death, demonstrating the importance of local translation at the spindle. These exciting results prompted us to investigate the mechanism of subcellular transport of these RNAs. We use high-throughput biochemical approaches to identify RNA-binding proteins that mediate subcellular RNA transport. Additionally, comparing the proteomes of control and miR-31 inhibited embryos, we further discover functions of miR-31 during early development. Overall, this work contributes to fundamental understanding of post-transcriptional regulation, RNA localization, and cell division which are critical for proper development.

Comparative genomics and the evolution of deuterostomes

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Deuterostomes, including Ambulacraria (hemichordates and echinoderms) and Chordata (amphioxus, tunicates, and vertebrates), represent a major group of bilaterian animals. Due to the diverse body plans of deuterostomes, it has been challenging to reconstruct their ancestral condition and to decipher the genetic changes that drove the diversification of deuterostome lineages. We have generated chromosome-level genome assembly of *Ptychodera flava*, an indirect-developing hemichordate that produces planktonic larvae before metamorphosing into an adult body plan. We used comparative genomic approaches to infer the chromosomal architecture of the deuterostome common ancestor and delineate lineage-specific chromosomal modifications. We also analyzed the transcriptomes and chromatin accessibility of multiple developmental stages of *P. flava* to understand how the genome is activated during development. Based on the ATAC-seq dataset, we identified a *cis*-regulatory element (CRM) at the hemichordate *brachyury* gene locus containing a conserved syntax comprised of binding sites of four TFs with strict order and orientation. We further identified the syntax in CRMs of *brachyury* orthologs in various non-chordate animals and even in *Capsaspora*, a unicellular relative to animals. These non-chordate CRMs exhibited activity in the notochord, a defining feature of chordate embryos. Our results indicate the ancient association of the regulatory syntax with

brachyury, likely predating the origin of animals. We propose that during chordate evolution, the newly established links acting on the conserved regulatory syntax promote the acquisition of chordate novelties. Together, these results provide molecular bases for understanding deuterostome ancestral conditions and their evolution.

FoxY is essential for cellular transfating to germline in the sea urchin embryo

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Some animals have the ability to restore missing body parts, though loss of the germ line cells usually results in adult infertility. Sea urchin embryos, however, recover a new germline that supports adult fertility. The primordial germ cells (PGCs) in the sea urchin are determined in the small micromeres at the 32-cell stage. Previous reports showed that if the micromeres, precursor cells to the small micromeres, were removed from the embryo, new PGCs were formed and the adult regained fertility. Here we seek to understand how new germ cells appear in micromere-less (mic-less) embryos. Detailed observations of mic-less embryos of *Strongylocentrotus purpuratus* revealed that new PGCs were formed at the onset of coelomic pouch formation. FoxY, which is a crucial transcription factor for coelomic pouch formation in normal embryos, was expressed in the foregut along with the germline factor, Nanos2, beginning from the early gastrula stage in mic-less embryos. When FoxY was knocked down with morpholino-antisense oligonucleotides in mic-less embryos, new PGC formation was inhibited. Surprisingly, Nanos2 expression in mic-less embryos is not needed for PGC transfating. Animal half embryos, which normally do not make PGCs, do so when β-catenin signaling is activated with LiCl. These results imply that FoxY, but not one of its germ cell targets, Nanos2, is an essential component for cell transfating to germline and ß-catenin signaling is an important input for activating FoxY. Thus, germ cell transfating appears to use a genetic program distinct from the intact embryo for reproduction.

Deuterostome Ancestors and Chordate Origins

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The Deuterostomia are a monophyletic group, consisting of the Ambulacraria, with two phyla, Hemichordata and Echinodermata, and the phylum Chordata, containing the subphyla Cephalochordata (lancelets or Amphioxus), Tunicata (Urochordata) and Vertebrata. Hemichordates and echinoderms are sister groups and are critical for understanding the deuterostome ancestor and the origin and evolution of the chordates within the deuterostomes. Genomic comparisons show that cephalochordates share synteny and a vermiform body plan

similar to vertebrates, but phylogenomic analyses place tunicates as the sister group of vertebrates. Tunicates have a U-shaped gut and a very different adult body plan than the rest of the chordates, and all tunicates have small genomes and many gene losses, although the GRNs underlying specific tissues, such as notochord and muscle, are conserved. Echinoderms and vertebrates have extensive fossil records, with fewer specimens found for tunicates and enteropneusts, or worm-like hemichordates. The data is mounting that the deuterostome ancestor was a complex benthic worm, with gill slits, a cartilaginous skeleton, and a CNS. Two extant groups, echinoderms and tunicates, have evolved highly derived adult body plans, remarkably different than the deuterostome ancestor. We review the current genomic and GRN data on the different groups of deuterostomes' characters to re-evaluate different hypotheses of chordate origins. Notochord loss in echinoderms and hemichordates is as parsimonious as notochord gain in the chordates but has implications for the deuterostome ancestor. The chordate ancestor lost an ancestral nerve net, retained the central nervous system, and evolved neural crest cells.

Echinobase: a community resource for echinoderm research.

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Echinobase (www.echinobase.org), a third generation resource, supports the echinoderm research community by hosting genome assemblies and functional genomic data, literature and community links, and reagent information. Echinobase is a copy of Xenbase with shared web servers and databases, and is migrating to use the open source Ubuntu, Apache Tomcat web, and PostgreSQL systems. Search and BLAST tools are available directly from the landing page or through the gene pages. Gene pages also display gene model HGNC compliant names, multispecies orthology, GO terms, JBrowse genome browser, a gene expression plotting tool and published, manually curated antibodies, morpholinos and gRNAs and cis-regulatory elements (CREs) aligned to the genome. Orthology analysis is ongoing in order to increase the number of gene models with gene symbols. Tabs beyond the summary gene page provide gene specific literature, transcripts, expression data, protein sequences and interactants. Automated literature collection has retrieves publications for automated and manual curation. The Echinoderm Anatomical Ontology (ECAO) has been developed with standardized anatomy terms for developmental stages and parts that are organized into a hierarchy with a visualization tool to graph the relationships between anatomical structures as they develop. The ECAO has now been updated to be used to label functional genomic data for use with multiple species. To support the community, collections of data, protocols and other resources are shared using EchinoWiki and a Download site. To enable collaborative studies, descriptions and contact information of community members are available and searchable.
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A novel regulatory paradigm: microRNA induced translational-dependent mRNA decay

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Following fertilization, Metazoan embryos undergo a series of rapid and coordinated mitoses. In the sea urchin cleavage embryo, microRNA-31 (miR-31) regulates local translation of *Fascin* at the mitotic spindle. Fascin is a cytoskeletal modulator that bundles F-actin and regulates microtubule polymerization. Inhibition of miR-31 leads to an increase of newly translated *Fascin* on the mitotic spindle, chromosomal, and cytoskeletal defects. Curiously, both inhibition of miR-31 and removal of miR-31's binding to *Fascin* mRNA lead to a significant decrease of *Fascin* transcript and increased protein. Similarly, embryos injected with a *Renilla* luciferase construct fused with *Fascin* 3'UTR containing mutated miR-31 binding site was significantly decreased, indicating that miR-31's binding has a protective effect on the stability of its target transcript *Fascin.* Prevention of *de novo* translation significantly rescues the level of *Fascin* transcript in miR-31 inhibited embryos. These results lead to our hypothesis that miR-31 post-transcriptionally inhibits the translation of its target and concurrently protects the transcript from undergoing translation-dependent RNA decay. We will test this hypothesis with single molecule resolution live imaging to measure active translation and transcript stability by using the SunTag system, which contains GC4N repeats upstream of *Fascin* 3'UTR and PP7 elements (*Fascin-PP7*). Co-injection of an scFv antibody-GFP allows translational quantification of GC4N repeats (ie. Fascin translation) and a viral coat protein fused to 3x-mCherry that binds to the *Fascin-PP7* for RNA quantification. We will reveal the novel regulatory mechanism of miR-31 in dynamically modulating transcript stability and translation rates that impacts mitosis and early development.

p21 Activated Kinases Expression, Regulation, and their Roles in Sea Urchin Development and Skeletogenesis

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p21-activated kinases are key regulators of cell signalling, acting as major downstream effectors of the Rho GTPases Rac and CDC42. PAKs play essential roles in cytoskeletal remodelling regulation and MEK phosphorylation in the RAS/MEK/ERK pathway, both critical for morphogenesis. PAKs play a conserved role in axonal guidance from humans to fruit flies and nematodes. However, little is known on the regulation and role of PAKs in marine invertebrate development. Here we investigated the expression, regulation, and role of PAKs in the embryonic development of the sea urchin, *Paracentrotus lividus* (*P. lividus*). There are two PAK genes in the *P. lividus* genome, *Pl-pak1/3* and *Pl-pak4*. Both are dynamically and broadly expressed during sea urchin development. Inhibition of both PAKs activity using a broad PAK inhibitor interferes with gut development and skeletal growth. Although polymerized F-actin is enriched around skeletal rods, PAK inhibition does not visibly affect it. During sea urchin development, ERK is active in the skeletogenic cells near the tips of the skeletal rods, and in the ciliary band neurons. PAK inhibition reduced pERK signal in the skeletogenic cells and in ciliary band region, implying it plays a role in skeletogenesis and neurogenesis. *Pl-pak1/3* expression is enriched in the skeletogenic cells and its genetic perturbation by Morpholino antisense

oligonucleotides results in reduction in skeletal length. Our findings portray a role of PAK proteins in sea urchin skeletal and gut development, and an evolutionary conserved role in neurogenesis, providing insight into the role of PAKs in the development of marine invertebrates.

Investigating teratogenic effects of ethanol on neural development of sea urchin embryo

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Ethanol is a well-established teratogen, capable of inducing a wide range of structural, functional, and behavioral abnormalities in developing embryos and fetuses. While previous studies have shown that increasing concentrations of ethanol lead to various developmental abnormalities in sea urchin embryos, including skeletal patterning defects and altered gut development. Ethanol exposure increases levels of the neurotransmitter serotonin in sea urchin larvae at 48 hours post-fertilization without reducing neural numbers. We therefore hypothesize that neurons in developing sea urchins are refractory to the teratogenic effects of ethanol. Our current study investigates the effect of ethanol on specification of different neural cell types at later time-points in larval development to determine whether sea urchin neurons are refractory to ethanol treatment. We first examined the temporal and spatial expression of several enzymes essential for the synthesis of neurotransmitters that are commonly used as markers for fully differentiated neurons in other species. We will next examine the effects of ethanol exposure on their temporal expression as the larvae progress toward metamorphosis using RT-qPCR and HCR-FISH. Our results will define whether sea urchin neural development exhibit differences from vertebrates and mammals in their response to ethanol, and this thus is potentially biomedically relevant for preventing fetal alcohol syndrome.

Elucidating the role of Notch signaling in radial nerve cord and intestinal regeneration in the sea cucumber *Holothuria glaberrima*

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Understanding the genetic regulation of tissue regeneration, particularly the involvement of signaling pathways, remains a crucial area of investigation. Notch signaling, a highly conserved pathway in multicellular organisms, plays a pivotal role in various cellular processes, including proliferation, dedifferentiation, and fate specification. However, its specific function in post-traumatic regeneration remains poorly characterized. In this study, we employ a small-molecule inhibitor of the Notch pathway, DAPT, to investigate its role in CNS and intestinal regeneration in a novel model system, the sea cucumber *Holothuria glaberrima*. We induce evisceration of the intestines, induce injury to the radial nerve cord (RNC), and study the subsequent regeneration. Our observations reveal that DAPT inhibition of the Notch pathways causes a significant delay in cell dedifferentiation in both tissues. Additionally, DAPT treatment decreases cell proliferation in both tissues at 8 days post-injury, typically the peak period of proliferation. The observed delays in cell dedifferentiation, reduced proliferation, and altered tissue morphology suggest that Notch signaling regulates these aspects of regeneration. The fact that DAPT treatment similarly affects both CNS and intestinal regeneration suggests that Notch signaling plays a significant

role in the amazing regenerative abilities of echinoderms and points to a process that could provide insights into the regeneration processes in other organisms. Further studies are needed to fully elucidate the mechanisms by which Notch signaling influences regeneration in sea cucumbers and to explore its potential as a target for therapeutic interventions in regenerative medicine.

Characterization of Immune Cell Type Populations in the Sea Cucumber *Holothuria glaberrima*

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In holothurians, it has been inferred that coelomocytes, a heterogeneous population of free-circulating immune-associated cells, likely play an important role in the regeneration of organs. Experiments addressing this role are limited by the current characterization of coelomocytes mainly based on morphological features. To overcome this limitation, we have initiated a comprehensive characterization of the molecular profiles of coelomocyte populations. Thirteen clusters, portraying distinct expression profiles, were identified utilizing a single-cell RNA Sequencing of a 9-day regenerating intestine of the sea cucumber *Holothuria glaberrima*. Four clusters showed a high expression of genes related to the immune system suggesting that these cells are coelomocytes. Representative genes for each cluster were selected based on their expression profile, defining populations that expressed Fibrinogen-like protein A (FIBA), Insulin-like growth factor-binding protein complex acid labile subunit (ALS), SCO-spondin (SSPO), or Balbiani ring protein 3 (Brp-3). An In Situ (HCR-FISH) was performed on coelomic fluid and tissue samples collected at various regeneration stages to identify these clusters and verify their influence on the regeneration process. Current results have identified the morphology of two known immune cell types: Cells expressing FIBA portray a morphology comparable to lymphocytes while those expressing Brp-3 demonstrate a morphology similar to phagocytes. Ongoing experiments are focused on describing additional populations and further characterizing the genes expressed by specific groups. This research will serve as a framework for defining coelomocyte populations based on their gene expression profiles which will be key for determining cellular roles and identifying cell orthologues across different species.

β-catenin localization in the ctenophore *Mnemiopsis leidyi* suggests an ancestral role in cell adhesion and nuclear function

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The emergence of animal multicellularity marks a pivotal evolutionary event which was enabled by molecular innovations in the way cells adhere and communicate. β-catenin is significant to this transition due to its dual role as both a structural component in the cadherin-catenin complex and as a transcriptional coactivator involved during Wnt/β-catenin signaling. However, our

knowledge of how this protein functions in ctenophores- the earliest diverging extant metazoan taxon- is limited. To study β-catenin function in the ctenophore *Mnemiopsis leidyi*, we generated affinity-purified polyclonal antibodies targeting *Ml*β-catenin. This reagent was used to observe β-catenin protein localization in *Mnemiopsis* embryos. Evidence of consistent β-catenin protein enrichment at cell-cell interfaces in *Mnemiopsis* embryos was discovered, suggesting that *Ml*β-catenin has a role in cell adhesion. Additionally, β-catenin was found enriched in some nuclei, particularly restricted to the oral pole around the time of gastrulation, suggesting *Ml*β-catenin may have nuclear function in *Mnemiopsis* embryos.

We now aim to use these antibodies to identify proteins that interact with *Ml*β-catenin through co-immunoprecipitation and mass spectrometry. Additionally, by combining *in silico* AI-based protein interaction prediction tool like AlphaFold with *in vitro* transcription-translation assays, we aim to better understand how these proteins are interacting. Our ultimate goal is to conduct functional studies in *Mnemiopsis leidyi to unravel the regulation of embryonic* β-catenin in this lineage, providing insights into the evolution of animal multicellularity.

The Spikes of Spikey Skinned Animals.

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The root words of echinoderms are *spikey* and *skin*. In a sea urchin, these projections serve as protection from predators, contribute to movement, and in many cases, are heavily pigmented. Calestani et al., 2003 first identified genes important for pigment in cells of the larva including polyketide synthase (PKS), and flavin – containing monooxygenases (FMO 1-5). We now know these genes are also essential in adult pigmentation. PKS makes the core pigment molecule, a naphthoquinone, and KO of PKS causes albinism. FMO gene products modify the core metabolite and the FMO profile in spines correlates with color morphs e.g. *L. variegatus.* The resulting echinochrome pigment appears to be selectively antimicrobial, depending on the colormorph, suggesting that the pigments influence, positive or negative, microbial colonization. DEG RNA-seq of spine colormorphs reveal distinct transcriptomes and LCMS/MS showed unique metabolomes depending on pigment presence. We also tested the composition of secondary spines; in addition to their difference in pigmentation and morphology, secondary spines have dramatically distinct transcriptomes, even though their mineral composition appears the same as primary spines. Finally, we tested the presence of PKS and FMO on banded spines from the Tuxedo urchin. These spines alternate colors along the proximal/distal spine axis from white to red. Surprisingly, PKS and FMO3 are more enriched in the white (non-pigmented) segments of the spines. We believe that sea urchin spines provide a multimodal system for studying structural dynamics, patterning, immunity, and gene regulation in an easily accessible tissue.

Functional analysis of the *Nematostella* Wnt/β-catenin destruction complex provides insight into the evolution of a critical regulatory module in a major metazoan signal transduction pathway

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The genesis of signaling pathways likely drove metazoan evolution and diversification, but how these pathways evolved and acquired signaling activity is poorly understood. To this end we studied the Wnt/β-catenin (cWnt) pathway destruction complex (DC) - which regulates cytoplasmic β-catenin levels - in the cnidarian *Nematostella*. Cnidarians are the closest outgroup to bilaterians and in bilaterians β-catenin binding to Axin and APC proteins, and Axin-APC heterodimerization are required for DC function. Experimental analysis showed that both NvAxin and NvAPC-like regulate cWnt signaling in *Nematostella* embryos, but bioinformatic predictions showed that all non- bilaterian Axin and APC-like homologs lack domains required for bilaterian DC activity questioning if non-bilaterians have a functional cWnt DC. We found that *Nematostella* Axin (NvAxin) interacts with NvGSK-3β and NvAPC-like interacts with β-catenin, and NvAxin and NvAPC-like can heterodimerize to form a functional DC.

Unexpectedly, we noted that NvAxin binds β-catenin weakly despite lacking the bilaterian β-catenin-binding motif (βcatBM). Using AlphaFold, we identified two βcatBM-like sequences in NvAxin, one within the Axin-RGS domain and another near the C-terminus, and a single βcatBM-like sequence in Axin-RGS of placozoans, poriferans, and ctenophores. Our results suggest that the bilaterian βcatBM evolved from a low-affinity βcatBM-like sequence within the Axin-RGS in early metazoans and this sequence was duplicated in the cnidarian-bilaterian ancestor. The Axin-RGS copy was lost in the bilaterian lineage and the other acquired higher β-catenin affinity. We suggest that scaffolding proteins in ancestral metazoan signaling pathways had promiscuous activity that increased pathway evolvability during their broad deployment in bilaterians.

Turbo-devo: reaching metamorphosis ASAP

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Many marine invertebrates produce small eggs that develop into feeding larvae that spend weeks grazing on phytoplankton before acquiring sufficient mass to complete metamorphosis. Yet species that complete metamorphosis in just a few days have evolved dozens of times in disparate metazoan clades. What changes in developmental mechanisms are able to slash the time of pre-metamorphic development to a fraction of its ancestral duration? Our work with the sea urchin genus *Heliocidaris* provides insights into how this remarkable life history transformation evolved. We show that the maternal-to-zygotic transition and embryonic patterning is *delayed* in the species with faster pre-metamorphic development. While seemingly paradoxical, this result makes sense from a cell biological perspective. Despite this general delay, we find that establishment of the DV and LR axes occurs earlier in development, but with apparently conserved consequences. This timing change contributes to earlier establishment of the imaginal adult rudiment, which in turn contributes to quicker pre-metamorphic development. Taken together, our findings provide a holistic understanding of how and why deeply conserved developmental mechanisms evolve.

Skeletogenic potential in non-skeletal lineages : Single-cell insights into VEGF3-mediated transdifferentiation in sea urchins

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Embryonic cells have the capacity to alter programs of differential gene expression (transdifferentiate) in response to experimental perturbations. In euechinoid sea urchins, the skeleton-forming primary mesenchyme cells (PMCs) are ordinarily derived from the micromeres of 16-cell stage embryos, but can also arise from other cells via transdifferentiation. Previous studies have shown that VEGF3 overexpression induces the conversion of blastocoelar cells (BCs) to PMCs, yet the plasticity of other cell populations in response to VEGF signaling and the underlying regulatory mechanisms remain poorly understood. We employed single-cell RNA sequencing (scRNA-seq) to characterize the cellular and molecular dynamics of VEGF3-driven transdifferentiation. Our analysis identified cell types responsive to VEGF3 signaling, marked by co-expression of alx1, a conserved transcriptional regulator of skeletogenic specification, with gene markers for other cell types. Our findings indicate that several endomesoderm-derived cell

populations are involved in the transdifferentiation process. Using gene perurbations and chemical inhibitors, we investigated regulatory pathways downstream of VEGF3 signaling, and identified pathways that impact transdifferentiation. While some pathways did not directly affect alx1 expression, their disruption impaired the ability of cells to fully complete the transdifferentiation program, suggesting that other, unidentified factors are required. This study provides novel insights into the cellular plasticity and regulatory mechanisms underlying VEGF3-mediated transdifferentiation in sea urchin embryos. Future work will focus on dissecting the crosstalk between VEGF3 and pathways governing endomesodermal lineage specification. A deeper understanding of this extended network will illuminate how cell populations retain the capacity for reprogramming while navigating normal developmental trajectories.

Developmental single-cell transcriptomics illuminates molecular pathways involved in sea urchin neurogenesis

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Single-cell RNA sequencing (scRNA-seq) enables the detailed analysis of gene expression at the individual cell level and has broad applications across various organisms, including non-model species. In this study, we applied scRNA-seq to the Western Pacific sea urchin *Hemicentrotus pulcherrimus*, constructing a comprehensive atlas of stage-specific gene expression profiles from 24 to 96 hours post-fertilization. Our analysis focused on understanding neurogenesis by identifying and characterizing gene networks involved in neuronal specification and differentiation. This revealed distinct patterns of gene expression that define neural cell populations and their developmental trajectories. Notably, we observed the emergence of serotonergic neurons, a small yet critical population for larval behavior, and identified the regulatory gene networks underlying their differentiation. These findings highlight the intricate processes shaping neural development and provide evidence for conserved neurogenic mechanisms across deuterostomes. By integrating scRNA-seq data with developmental biology frameworks, this study emphasizes the utility of sea urchins as a model for understanding neural system evolution in non-model organisms. The data generated from this study will be publicly available on HpBase (https://cell-innovation.nig.ac.jp/Hpul/), offering an invaluable resource for exploring gene expression dynamics and advancing research in developmental and evolutionary biology.

Nodal is expressed and regulates axis formation much earlier during embryogenesis in *Heliocidaris erythrogramma*

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The sea urchin *Heliocidaris erythrogramma* (*H.e.*) exhibits significantly accelerated embryonic development compared to planktotrophic sea urchins. Previous embryo dissection experiments revealed that the dorsal-ventral (D-V) axis in *H.e.* is established much earlier than in planktotrophic species. While the transforming growth factor-beta (TGF-β) family members Nodal and Lefty are known to regulate D-V specification in planktotrophic sea urchins, it remains unclear whether the same mechanism operates in *H.e.*, albeit at an earlier developmental stage. To address this question, we employed single-cell RNA sequencing (scRNA-seq) and in situ hybridization chain reaction (HCR) to examine the expression patterns of Nodal and Lefty during early embryonic development. Our findings demonstrate that both genes are expressed at significantly earlier stages in *H.e.*. Perturbation assays using dicer-substrate siRNAs (dsi-RNAs) and chemical inhibitors to knock down Nodal and Lefty resulted in radialized embryos, indicating that the Nodal-Lefty gene network still governs D-V specification in *H.e.*. Furthermore, time-specific drug perturbation experiments revealed that the sensitive period for Nodal-Lefty activity occurs much earlier in *H.e.* compared to planktotrophic sea urchins. These results suggest that while the core regulatory mechanism of D-V specification is conserved, its temporal dynamics have shifted significantly in *H.e.* due to accelerated development.

Decoding neuronal communication: a multi-modal approach to understanding information flow in sea urchin larvae

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Sea urchin larvae, tiny swimming pyramids of the marine world, exhibit remarkable phenotypic plasticity, altering their morphology and behaviors in response to environmental cues such as food and light. Despite their simple nervous system, the chemical cocktail of neuronal signaling is diverse and complex, making them an ideal model to explore the interplay between genetics, environmental factors, and neural circuit plasticity.

Our research focuses on understanding how sensory inputs are processed and integrated by neuronal circuits and signaling networks to regulate behavior and induce morphological changes via developmental gene regulatory networks. Using the Mediterranean sea urchin *Paracentrotus lividus*, we aim to construct a two-modality, whole-larva neural network atlas by integrating high-resolution microscopy with single-cell transcriptomics. This approach will map neuronal morphologies to their molecular signatures and identify ligand-receptor pairs critical for intercellular communication.

Through transgenic and functional experiments, we seek to uncover how simple neuronal systems process environmental information, providing key insights into the evolution of nervous systems and the regulatory mechanisms driving phenotypic plasticity.