Developmental Biology of Sea Urchins and other Marine Invertebrates, 2023

Tuesday, October 17

Pre-meeting Workshop

HCR and high-resolution imaging techniques 14:30 – 15:30
Carrie Albertin, Marine Biological Laboratory
Laurent Formery, Stanford University and University of California Berkeley

Coffee Break 15:30 – 16:00

Single cell/nucleus transcriptomics as a tool to understand animal evolution and development 16:00 – 17:00
Anne Meyer, Carnegie Mellon University
Periklis Paganos, Marine Biological Laboratory

Dinner Break **17:00 – 19:00**

Plenary Session 1: Introduction and Welcome to the Meeting **19:00 – 19:15**
Chair: Maria Ina Arnone, Stazione Zoologica Anton Dohrn

EMBO Keynote Lecture: Kristin Tessmar-Raible, Max Perutz Labs **19:15 – 20:15**
Environmentally-informed molecular lab experiments: The relevance of time and light
Early Investigator Lecture: Zak Swartz, Marine Biological Laboratory **20:15 – 21:15**
Cell division in development, aging, and a changing world

Social Mixer **21:30 – 23:00**

Wednesday, October 18

Plenary Session 2: Evolutionary Novelty and Diversification of Cell Types
Chair: Sebastian Fugmann, Chang Gung University

Veronica Hinman, Carnegie Mellon University 08:30 – 09:00
The evolution of gene regulatory networks for novel traits in echinoderms
Arun Chavan, Yale University 09:00 – 09:30
Origin and diversification of gut-derived organs in chordates
Maria Ina Arnone, Stazione Zoologica Anton Dohrn 09:30 – 10:00
Cell type dynamics across sea urchin development

Coffee Break 10:00 – 10:30

Plenary Session 3: Regeneration in Marine Invertebrates
Chair: José García Arrarás

Mansi Srivastava, Harvard University 10:30 – 11:00
Considering stem cells, regeneration, and development from an acoel perspective

Elaine Seaver, Whitney Laboratory for Marine Bioscience 11:00 – 11:30
Regeneration in annelids

Paola Oliveri, University College London 11:30 – 12:00
Genomic control of arm regeneration

Lunch Break **12:00 – 13:20**

Plenary Session 4: Evolution from an Organismal Perspective
Chair: Chris Lowe, Stanford University

Stephan Schneider, Academia Sinica 13:20 – 13:40
Of cilia and worms: Approaches to dissect spiralian ciliary cell type diversity and function

Greg Wray, Duke University 13:40 – 14:00
The development and evolution of cell fate specification

Yi-Hsien Su, Academia Sinica 14:00 – 14:20
Insights into deuterostome evolution from the biphasic gene regulatory program of hemichordates

Billie Swalla, University of Washington 14:20 – 14:40
The tale of degenerate ascidian tails

Smadar Ben-Tabou de-Leon, University of Haifa 14:40 – 15:00
Interplay between mechanical and genetic information processing controls skeletal growth in the sea urchin embryo

Coffee Break **15:00 – 15:30**

Concurrent Session 5a: How to build an animal
Chair: Nat Clarke,

César Arenas-Mena, CUNY Graduate Center and College of Staten Island 15:30 – 15:45
Widespread priming of transcriptional regulatory elements by incipient accessibility or Pol II pause in early embryos of the sea urchin Strongylocentrotus purpuratus*.*

Amber Rock, Harvard University 15:45 – 16:00
Reprogramming of cell fate in an embryo with stereotyped cleavage

Alejandro Berrio, Duke University 16:00 – 16:15
Single-cell transcriptomics reveal developmental trajectories of micromereless sea urchin embryos

Jane Swart, Duke University, 16:15 – 16:30
Where wnt went: Changes to wnt signaling following a life-history change in Heliocidaris erythrogramma

Margherita Perillo, Marine Biological Laboratory 16:30 – 16:45
A new model for understanding tube morphogenesis and the origins of birth defects

Brennan McDonald, Duke University 16:45 – 17:00
Reconstruction of the sea urchin larval body plan in preparation for metamorphosis

Concurrent Session 5b: Novel approaches in marine invertebrate developmental biology
Chair: Brad Shuster

Rossella Annunziata, Stazione Zoologica Anton Dohrn (virtual) 15:30 – 15:45
Holothuria tubulosa *as a new experimental system for evo-devo studies*

Elliot Jackson, Scripps Institution of Oceanography, UC San Diego 15:45 – 16:00
Foundational transgenic building blocks in the sea urchin Lytechinus pictus

Akshay Kane, Marine Biological Laboratory 16:00 – 16:15
VitelloTag: a tool for high throughput cargo delivery into oocytes

Carl Manner, Duke University 16:15 – 16:30
Tools for genetic perturbation of the urchin embryo

John Henson, Dickinson College 16:30 – 16:45
Constructing the cytokinetic contractile ring in the early sea urchin embryo: Insights from new imaging, pharmacological, and computer modeling approaches

Yoon Lee, Scripps Institution of Oceanography, UC San Diego 16:45 – 17:00
A cost-effective pipeline for fully automated high-throughput spatial transcriptomics of whole-mount marine embryo samples

Dinner Break **17:00 – 19:00**

Plenary Session 6: Eric H. Davidson Memorial Lecture **19:00 – 20:00**
Chair: Kate Buckley, Auburn University

Marianne Bronner, California Institute of Technology
Differential neural crest gene regulatory subcircuits along the body axis

Poster Session 1 **20:00 – 21:30**

Mixer **21:30 – 23:00**

Thursday, October 19

Plenary Session 7: Environmental Influences on Marine Invertebrate Evolution
Chair: Jia Song

Donal Manahan, University of Southern California — 08:30 – 09:00
The metabolic pace of development: environmental challenges from the beginning

Andrea Bodnar, Gloucester Marine Genomics Institute — 09:00 – 09:30
Genomic signatures of exceptional longevity and negligible aging in the red sea urchin

Gary Wessel, Brown University — 09:30 – 10:00
"Get a spine!" shouts the invertebrate: A mechanistic explanation of echinoid colormorphs

Coffee Break — **10:00 – 10:30**

Plenary Session 8: Broadening Participation in Developmental Biology
Chair: Michelle Roux-Osovitz, University of Tampa

Michael Barresi, Smith College — 10:30 – 10:50
Reimagining the laboratory experience: Mentoring diverse research students from a teacher's perspective

Panel Discussion — 10:50 – 11:30
Catherine Schrankel, San Diego State University
Smadar Ben Tabou de Leon, University of Haifa
Becoming ambassadors for the next generation of developmental biologists

Open Forum: Echinobase Townhall — **11:30 – 12:00**

Memorial for R. Andrew Cameron — **12:00 – 12:20**
Pedro Martinez, L. Courtney Smith and Donal Manahan

Lunch Break — **12:20 – 13:20**
Echinobase Scientific Advisory Board Meeting

Plenary Session 9: Embryonic and Post-Embryonic Axial Patterning
Chair: Jennifer Croce

Cyndi Bradham, Boston College — 13:20 – 13:40
Voltage-gated sodium channel activity mediates sea urchin larval skeletal patterning through spatial regulation of Wnt5 expression

Dave McClay, Duke University 13:40 – 14:00
Transfating replacement of micromere/skeletogenic cells after removal at the 16-cell stage

Jennifer Fenner, Auburn University 14:00 – 14:20
TGF-β and non-canonical Wnt signaling interactions coordinate anterior-posterior and dorsal-ventral axis formation in sea urchin embryos

Laurent Formery 14:20 – 14:40
Stanford University and University of California Berkeley
Axial patterning in asterozoan and the diversification of echinoderm pentaradial body plans

Shunsuke Yaguchi 14:40 – 15:00
Shimoda Marine Research Center, University of Tsukuba
Sea urchin larvae utilize light for regulating the activity of digestive tract

Coffee Break 15:00 – 15:30

Concurrent Session 10a: Development of specialized structures: cilia, neurons, biomineralization and skeletogenesis
Chair: Smadar Ben-Tabou-de Leon

Nat Clarke, Massachusetts Institute of Technology 15:15 – 15:30
Seeing clearly: visualization of whole, intact organ systems in adult echinoderms

Macie Chess, Carnegie Mellon University 15:30 – 15:45
Genome-wide identification and spatiotemporal expression analysis of cadherin superfamily members in echinoderms

Majed Layous, University of Haifa 15:45 – 16:00
The co-option of the mechanosensing and mechanotransduction during the evolution of biomineralization in metazoans

Alexandra Lion, Boston University 16:15 – 16:30
Perturbation of the P-body component DDX6 reveals a potential connection between developmental timing and skeletal patterning

William Chang, Christian-Albrechts-Universität zu Kiel 16:30 – 16:45
Regulation of intracellular pH homeostasis in calcifying cells of sea urchin larva

Vivek Prakash, University of Miami 16:45 – 17:00
Squeeze confinement-induced changes in fluid flows generated by ciliated marine larvae

Concurrent Session 10b: Immune systems & regeneration
Chair: Periklis Paganos, Marine Biological Laboratory

Jon Lee Andrade, Carnegie Mellon University 15:30 – 15:45
Single-cell transcriptome analysis of regenerating sea stars

José García Arrarás, University of Puerto Rico 16:00 – 16:15
Pluripotency of the regenerating intestinal rudiment epithelia shown by single cell analysis

Sebastian Fugmann, Chang Gung University 15:45 – 16:00
Evolutionary conservation of immune signaling pathways in echinoderms

Catherine Schrankel, San Diego State University 16:45 – 17:00
A new animal model for testing the roles of drug transporters in gut-specific immune response and disease

L. Courtney Smith, The George Washington University 16:30 – 16:45
SpTransformer proteins drive phagocytosis, bind to phagocytes, and change gene expression in the innate immune system of the purple sea urchin

Jonathan Rast, Emory University 16:15 – 16:30
The genomic organization of sea lamprey variable lymphocyte receptor loci and the development and function of the agnathan adaptive immune system

Dinner **17:00 – 19:00**

Plenary Session 11: Keynote Lecture **19:00 – 20:00**
Chair: Athula Wikramanayake, Miami University

Christopher Lowe, Stanford University
TBD

Poster Session 2 **20:00 – 21:30**

Mixer **21:30 – 23:00**

Friday, October 20

Plenary Session 12: Early axis patterning
Chair: Cyndi Bradham

Jenifer Croce, CNRS LBDV 08:30 – 09:00
Stage-specific functions of nuclear β-catenin in cell fate specification, maintenance, and restriction during early sea urchin embryogenesis

Ryan Range, Auburn University 09:00 – 09:30

The conserved role of integrated canonical and non-canonical Wnt signaling during anterior-posterior axis formation in ambulacrarian deuterostome embryos

Jr-Kai Yu, Institute of Cellular and Organsimic Biology, Academia Sinica 09:30 – 10:00
Asymmetric β-catenin nuclearization regulates mesendoderm formation in amphioxus early embryos

Coffee Break **10:00 – 10:30**

Plenary Session 13: Cell biology of embryos
Chair: Zak Swartz

Alex McDougall, Sorbonne Université/CNRS 10:30 – 11:00
Ascidian invariant cleavage

Charles Shuster, New Mexico State University 11:00 – 11:30
Cellular mechanics of oocyte maturation

Jia Song, University of Delaware 11:30 – 12:00
miR-31-mediated local translation at the mitotic spindle is important for early development

Lunch Break **12:00 – 13:20**

Plenary Session 14: Emerging Systems for Advancing Developmental Biology in Marine Invertebrates
Chair: Catherine Schrankel, San Diego State University

Nipam Patel, Marine Biological Laboratory 13:20 – 13:40
TBA

Carrie Albertin, Marine Biological Laboratory 13:40 – 14:00
The molecular embryology of cephalopod molluscs: conservation, convergence, and innovation

Amro Hamdoun, UC San Diego 14:00 – 14:20
Building a better sea urchin

Charles Ettensohn, Carnegie Mellon University 14:20 – 14:40
Analysis of gene regulatory network dynamics using a Tet-On system for conditional control of gene expression

Coffee Break **14:40 – 15:00**

Concurrent Session 15a: Neurogenesis, signaling and skeletogenesis
Chair: Jennifer Fenner

Tsvia Gildor, University of Haifa (virtual) 15:00 – 15:15
Actomyosin remodeling regulates biomineral formation, growth and morphology during eukaryote skeletogenesis

Ali Elagoz, Ku Leuven 15:15 – 15:30
A cephalopod molecular approach of evolving the largest invertebrate nervous system

Jessica Stock, Marine Biological Laboratory 15:30 – 15:45
The role of BMP signaling during neural development in the squid Doryteuthis pealeii

Tal Gordon, Stanford University 15:45 – 16:00
Insights into the cellular and molecular mechanisms of the highly regenerative tunicate Polycarpa mytiligera

Cheikhouna Ka, Auburn University 16:00 – 16:15
TIKI is required for anterior neuroectoderm and skeletal patterning in sea urchin embryos.

Malcolm Arnott, University of Delaware 16:15 – 16:30
Transcription of miRNAs is regulated by developmental signaling pathways

Concurrent Session 15b: Early development and reproduction
Chair: TBA

Jérémy Sallé, Jacques Monod Institute - CNRS 15:00 – 15:15
Contribution of ER network to microtubule aster centration in the early Paracentrotus lividus *embryo*

Ruben X. G. Silva 15:15 – 15:30
University of Aveiro, CESAM Centre for Environmental & Marine Studies
Marine heatwave driven bleaching and its impacts on the reproduction of the stenophagous nudibranch Berghia stephanieae

Sonia Dufour, Station Biologique Roscoff, CNRS, Sorbonne Université 15:30 – 15:45
The mTOR pathway regulates a translational network stimulating lysosome biogenesis in the sea urchin early embryo

Julia Morales, Station Biologique Roscoff, CNRS, Sorbonne Université 15:45 – 16:00
A translational network regulates the dynamics of cell division in sea urchin early embryo

Cosmo Pieplow, Brown University 16:00 – 16:15
Foundations for understanding the biology of the echinoderm ovary

Periklis Paganos, Marine Biological Laboratory 16:15 – 16:30
Molecular and cellular mechanisms controlling sea star reproductive longevity

Business Meeting **16:30 – 17:30**

Closing Banquet & Awards Ceremony **18:00 – 23:00**

Posters

Deema Abayawardena, University of Miami
An RNAseq analysis of isolated animal and vegetal halves from Patiria miniata oocytes

Tanya Alessandro, Stazione Zoologica Anton Dohrn (virtual)
The molecular basis of circadian rhythms in echinoderm larvae

Sophie Bodine, Boston University
PFAS compounds PFOA and GenX are teratogenic to sea urchin embryos

Maria Cocurullo, Stazione Zoologica Anton Dohrn (virtual)
Single-Cell Transcriptomic Analysis Reveals the Molecular Profile of Go-Opsin Photoreceptor Cells in Sea Urchin Larvae

Tarrin Dewberry, Brown University
TBA

William Douglas, Carnegie Mellon University
Repressive interactions between GRNs: Alx1-mediated repression of NSM GRNs ensures skeletogenic specification

Benjamin Fang, Boston University
The Actin Cytoskeleton is Required for Normal Skeletal Patterning and PMC migration

Rachel Ferrigno, Boston University
Chromatin Remodeling Enzymes are Required for Normal Skeletal Patterning

Lydia Francis, University of Tampa
Sea Urchins as Indicators of Seasonal Environmental Stress

Aidan Furze, Brown University
Sea urchin spines as a model specimen for innate immune cell chemotaxis

Nathan Harry, North Carolina State University
The role of heterochronic gene expression and regulatory architecture in early developmental divergence

John Henson, Dickinson College
α-Actinin Localization and Myosin II Inhibition Indicate that the Early Assembly of the Cytokinetic Contractile Ring is Independent of Actomyosin Contraction

Eva Jimenez Guri, Stazione Zoologica Anton Dohrn (virtual)
Plastic leachate-induced toxicity during sea urchin embryonic development

Riss Kell, Gloucester Marine Genomics Institute
Profiling the DNA damage response at the single-cell level in sea urchin coelomocytes

Ellie Kim, Brown University
Estrogen is awesome

Nick Kraieski, Auburn University
Dissecting the role of copper in sea urchin embryogenesis

Che-Yi Lin, Academia Sinica
Improvement of scRNA-seq analyses by extending gene annotation to 3' UTR using the Iso-Seq technology

Lauren Lubeck, Stanford University
Cell type comparisons between hemichordates and vertebrates

Jenna Luc, San Diego State University
Exploring sea urchin larval immunity in response to microbial isolates behind black spot disease in Lytechinus pictus

Jamie MacKinnon, Marine Biological Laboratory
Cell division during animal reproduction in a changing ocean

Megan Maloney, Auburn University
Pigment cell development in late larval stages of the painted sea urchin, *Lytechinus pictus*

Stanley Marjenberg, Boston University
Emx is required for normal skeletal patterning and gut development in *Lytechinus variegatus* embryos

Valeria Montenegro, New Mexico State University
Investigating tropomyosin isoform localization and function during sea star development.

Nathalie Oulhen, Brown University
Echinoderm ovaries are awesome

Sydney Popsuj, Georgia Institute of Technology
Getting meta*more*phosis for your money: studying tailed and tailless tunicate metamorphosis to identify neurodegenerative gene *dkk3* form and function

Zachary Pracher, Duke University
Inducible CRISPRi technology to investigate rapid diversification of embryonic immune cell lineages in the green sea urchin, *Lytechinus variegatus*

Marko Radulovic, Boston University
Using Polychrome Labeling to Study Skeletal Patterning

Gabriela Reyes, New Mexico State University
Physical association of the microtubule-based cytokinetic signaling protein MKLP1 with the nascent contractile ring in sea urchin embryos

Gerardo Reyes, Brown University
Which came first? Red, Green, or White

Maria Lorenza Rusciano, Stazione Zoologica Anton Dohrn (virtual)
Characterization of mesenchymal photoreceptors in *Paracentrotus lividus* larvae

Tyler Smith, Auburn University
Delineating the gene regulatory network that controls blastocoelar cell differentiation in *Strongylocentrotus purpuratus*

Lauren Stoeltje, San Diego State University
Drug transporter knockout and gut microbiome analysis in *Lytechinus pictus*: Building a model for commensal interactions and disease mechanisms

Yu Sun, Auburn University
Dynamic evolution of the Nod-like receptor (NLR) gene family within echinoderms

Haruka Suzuki, University of Tsukuba, Brown University
Formation, and reformation, of the anterior - posterior axis in halved sea urchins embryos

Amy Tan, Texas A&M University
Epigenetic changes shaped by early environmental conditions in S. purpuratus

Jake Tatum, Auburn University
Motility is required for the pathogenicity of Vibrio diazotrophicus in purple sea urchin larvae

Cheryl Telmer, Carnegie Mellon University
Echinobase: a community resource for echinoderm research.

Michael Testa, University of Delaware
Elucidating the function of fascin isoforms in early embryogenesis

Gayatri Thorat, Boston University
Ethanol exposure perturbs sea urchin development and disrupts developmental timing

Emily Wilkins, Auburn University
Temperature influences immune larval cell development in Strongylocentrotus purpuratus

Amelia Williams, Auburn University
Analysis of the sea urchin larval response to bacterial challenge using single cell nuclei RNA-sequencing

TALK ABSTRACTS

Environmentally- informed molecular lab experiments: The relevance of time and light

Kristin Tessmar-Raible

Max Perutz Labs, University of Vienna, Vienna Bio Center, Dr. Bohr-Gasse, Vienna, Austria

Since its beginnings, life has been exposed to the regular environmental cycles caused by sun and moon, causing adjustments of the molecular mechanisms underlying physiology and behavior. Yet, lab experiments -no matter the research topic- are typically performed under conditions that differ significantly from the natural environment. Of course, the level of molecular details and causal relationships could not be reached by (theoretically) performing lab experiments “in the wild”. However, knowledge about the natural environment, and the controlled implementation of some of its cues is critical to not just understand how physiology and behavior can be controlled, but how the underlying molecular mechanisms can actually be modulated and adapted to the various predictable and unpredictable environmental changes.

My lab attempts to address this challenge by studying how (especially moon-controlled) timing systems and different light conditions impact on the development, physiology and behavior of the marine bristle worm *Platynereis dumerillii*. Using the power of molecular biology and functional genetics, combined with information from the worm’s natural marine environment, we address question such as: How can different worms across a population reliably synchronize their physiology and behavior to the same moon phase? Does lunar timing influence daily timing?

How do different light conditions impact on growth and life span? And can the study of molecular timing mechanisms of marine bristle worms help to understand some of the scientifically reported, but “just weird” correlations between human physiological/behavioral rhythms and the lunar cycle?

Cell division in development, aging, and a changing world

S. Zachary Swartz

Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA 02543, USA

Animal reproduction relies upon a fragile developmental window: the transition of oocytes (egg cells) through meiosis and fertilization to embryonic cleavage divisions. During this window, a complex set of cellular events must be intricately coordinated in time and space. The success of these transitions requires robust control from the transcriptional to post-translational levels, and is influenced by cellular age, disease, and environmental factors. In our lab, we are asking several fundamental questions: 1) how do egg cells develop and age, 2) how is cell division modulated for reproductive and developmental processes, and 3) how adaptable is animal reproduction to anthropogenic changes in our environment? I will discuss our lab's recent progress in addressing these questions, and the development of new experimental tools, primarily using the sea star *Patiria miniata* as a research organism.

The evolution of gene regulatory networks for novel traits in Echinoderms

Annie Meyer, Will Hatleberg, Carolyn Ku, Greg Cary, Veronica Hinman

Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213, USA

Cell types are the building blocks of metazoan biodiversity and offer a powerful perspective for inferring evolutionary phenomena. The development of single-cell transcriptomic techniques allows for new evaluations of cell types, which in turn allows a conceptual reassessment of traditional definitions of novel cell types and their evolution. Echinoderms are especially well suited to studies of the evolution of cell types and novelty. There are multiple cell types that are considered to be specific to some clades and absent in others across the five classes of echinoderms. Additionally, the developmental GRNs for the specification of these cell types are especially well known. We developed a gene expression atlas using single nuclear RNA sequencing (snRNA-seq) across various embryonic stages of the bat star, *Patiria miniata*. This atlas facilitates comparison with the sea urchin, Strongylocentrotus purpuratus, revealing significant insights into shared and divergent gene expression clusters. We then juxtapose these findings with the established GRNs of these cell types to gain insights into how developmental pathways have facilitated the evolution of novelty. This work suggests a new interpretation of the evolution of these well-studied cell types and a reflection on the definition of novel cell types.

Origin and diversification of gut-derived organs in chordates

Arun R Chavan[1], Jacob M Musser[2], Naomi Philip[1], Meena Ambati[1], and Ruslan Medzhitov[1]

[1] Department of Immunobiology, Yale School of Medicine, New Haven, CT 06510, USA
[2] Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT 06520, USA

Evolutionary diversification of organs likely occurs through subfunctionalization of an ancestral organ; a process that can be thought of as a lineage-splitting event in the phylogeny of organs. Organ diversification implies the diversification of its constituent cell types, giving rise to "paramorph" cell types, i.e. serial homologs that have diverged to become individuated and specialized. How do the diversification processes at these two levels of organization intersect and influence each other? We attempt to address these questions using the gut-derived organs of chordates as a model system. The phylogenetic distribution of gut-derived organs suggests that the ancestral chordate gut was a multifunctional organ that carried out all digestive functions, while these functions were subsequently allocated to organs specialized for secretory (liver and pancreas) and absorptive (intestine) functions in the vertebrate lineage. Here, we present single-cell transcriptomic data from gut tissues of a cephalochordate, amphioxus (*Branchiostoma floridae*) and a urochordate, *Ciona robusta*. In the gut of both of these species, we identify cell types homologous to those from the vertebrate digestive system, including the pancreatic exocrine cells, enteroendocrine cells, absorptive enterocytes, hepatocytes, as well as putative tissue-resident macrophages. We will present our ongoing efforts to trace the evolutionary origin of the liver and the pancreas in light of these findings, and discuss their implications for the origin and diversification of animal tissues.

Cell type dynamics across sea urchin development

Periklis Paganos[1], Jack Ullrich-Lüter[2], Alba Almazan[3], Giovanna Benvenuto[1], Filomena Caccavale[1], Maria Cocurullo[1], Lorenza Rusciano[1], Danila Voronov[1], Jenifer Croce[4], Jil Carl[2], Berit Zemann[2], Paolo Ronchi[5], Maria I. Arnone[1]

[1] Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Naples, Italy
[2] Museum für Naturkunde, Leibniz-Institut für Evolutions und Biodiversitätsforschung, Berlin, Germany
[3] Institut de Génomique Fonctionnelle de Lyon, Lyon, France
[4] Laboratoire de Biologie du Développement de Villefranche-sur-Mer, Villefranche-sur-Mer, France
[5] European Molecular Biology Laboratory, Heidelberg, Germany

The great variation in body plans occurring during the life cycle of indirectly developing echinoderms, from their bilaterally symmetric larvae to their pentameral adults, has led to many inquiries about how such a change in developmental programs can be carried out by the same genome. Testing the degree to which developmental Gene Regulatory Networks and cell types are shared between the sea urchin pluteus larva and the juvenile that emerges from it during metamorphosis is one method of resolving this conundrum. In this study, we use single cell and single nucleus transcriptomics to provide a general overview of the cell type families present at the mature rudiment and post-metamorphic juvenile stages of the sea urchin *Paracentrotus lividus* and compare them with those found in the 3-day *Strongylocentrotus purpuratus* larva. Overall, our findings demonstrate the presence of common cell types throughout development, with substantial regulatory module conservation within related cell types, while also demonstrating the enormous neuronal variety in the juvenile. In particular, we demonstrate that all of the opsin genes encoded in the sea urchin genome are expressed at the juvenile stage, highlighting the complexity of the photoreceptor cell (PRC) repertoire of these animals. Moreover, performing single nucleus RNA sequencing on isolated tube feet—organs thought to be mediating echinoderms' extraocular vision—we show that tube feet are in fact an opsin hub supporting their function as photosensitive organs.

Considering stem cells, regeneration, and development from an acoel perspective

Mansi Srivastava

Department of Organismic & Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA

All animals undergo embryonic development, the process by which a single cell produces a multicellular organism, to establish their body plans. Many, but not all, animals can recapitulate parts of this process after the completion of embryogenesis, when they undergo regeneration as adult animals. My research group focuses on the mechanisms of whole-body regeneration, the striking phenomenon where animals can regrow entire bodies from small fragments, often via the use of large populations of adult stem cells. The widespread but variable occurrence of regeneration across the tree of animal life raises the question of how regeneration evolved, and we seek to determine whether any fundamental, unifying molecular and cellular principles operate during regeneration and stem cell regulation in distantly-related animals. In particular, we seek to find mechanisms that are unique to regeneration, and are not mere redeployments of developmental processes. Over the last several years, we have focused on our newly established model system, the three-banded panther worm *Hofstenia miamia*, to develop frameworks for identifying these mechanisms. *Hofstenia* enables us to study both regeneration and development, and my talk will draw comparisons between the two processes using functional genomics and classical embryological approaches.

Identification of a localized stem cell cluster in the annelid *Capitella teleta*

Elaine C. Seaver

Whitney Laboratory for Marine Bioscience, University of Florida, FL 32611, USA

Annelids display a wide range of regeneration abilities, reproductive strategies and postembryonic development. Somatic tissue regeneration and regeneration of reproductive tissues require birth and patterning of new cells from resident adult stem cells, which reside either in specific locations within the body or can be more broadly distributed. *Capitella teleta* is an annelid that exhibits posterior regeneration, regenerates both somatic and reproductive tissues, and experiences environmentally induced sex change. Furthermore, *Capitella* has several favorable characteristics for *in vivo* studies of regeneration and stem cell biology, including availability of functional genomic tools, a stereotypic cleavage program and associated fate map, along with a sequenced genome. In this study, we identified a cluster of undifferentiated cells suspended by mesenteries in the ventral coelomic cavity of thoracic segments 4 - 6 in *C. teleta*. Cells in this cluster express genes of the multipotency cell program such as *vasa*, *nanos*, *piwi*, *PL10* and *PCNA*. Characterization of cell division profiles and gene expression patterns by in situ hybridization reveals heterogeneity among cells within this cell cluster. We characterized the maturation of this cluster during the life cycle and identified its progenitors in early-stage larvae. Transverse amputations performed at various axial positions in adults demonstrate that reformation of somatic and gonadal tissue regeneration success maps to a single segment whose identity changes over ontogeny. We suggest that this cluster is a stem cell niche and hypothesize that cells within the cluster can generate both somatic and germline descendants.

Genomic control of arm regeneration

Paola Oliveri

Department of Genetics, Evolution, & Environment, University College London, London UK

Amphiura filiformis, a prevalent species of brittle star found in the northern East Atlantic, possesses a remarkable ability to regenerate its arms after injury. In the wild, over 90% of adult individuals display evident signs of regeneration. This provides an excellent system for investigating the regeneration of complex structure. Through a combination of genome sequencing and analysis of transcriptome data, we aim to elucidate the genomic control governing arm regeneration in *A. filiformis.* The high-quality chromosome-scale genome assembly enabled the comparison with other echinoderms, which revealed several genomic rearrangements and a complex pattern of gene loss and gain within the *A. filiformis* genome. The analysis of dynamics of gene expression during different stages of regeneration identified three distinct phases of gene activity. These phases encompassed genes associated with immune responses, cell proliferation, and tissue differentiation, collectively orchestrating the regenerative process. Drawing parallels between our datasets and analogous ones from other species undergoing appendage regeneration, we pinpointed sets of genes that are commonly expressed during the regeneration processes. Moreover, our analysis of differential gene expression during explant regeneration identified potential regulators responsible for maintaining positional identity, shedding light on the intricate regulatory control underlying *A. filiformis* arm regeneration.

Of cilia and worms: Approaches to dissect spiralian ciliary cell type diversity and function

Stephan Q. Schneider

Academia Sinica, Taipei, Taiwan

Larval motility of most marine invertebrates depends on the activity of large numbers of cilia that provide the means to move within the water column, towards food sources, and towards appropriate substrates for settlement. These cilia are generated by multi-ciliated cell types (MCCs) that are organized in ciliary bands. The diversity of cilia in marine larvae are high displaying cilia in different positions and arrangements, and of different lengths and strengths. These differences must occur due to tweaking of developmental and cell biological mechanisms which are mostly unknown. To harness this diversity of cilia we follow two complementary approaches: We utilize phylogenomics in Spiralia, a clade of over 14 mostly marine invertebrate phyla, to unravel spiralian ciliary gene evolution. Secondly, we study the formation of MCCs in the marine annelid *Platynereis dumerilii* to decipher the cellular and molecular make-up of MCCs. To do so we have constructed a library of spiralian gene models, named SpiraliaDB, comprising of over 200 species, to identify molecular synapomorphies and ciliome specializations. For *Platynereis* larvae we have conducted a census of all MCCs. We leveraged two ultrastructural whole-body cellular atlases for a three-day old free-swimming larva, and for a six-day old settled juvenile worm to map each cilium including basal bodies for each individual MCC enabling the first complete count of cilia in an organism, and their precise arrangement within each cell. Bulk and single cell RNA-seq approaches are being used to identify the *Platynereis* 'ciliome', and to link profiles to distinct multi-ciliated cell types.

The development and evolution of cell fate specification

Gregory A. Wray

Department of Biology, Duke University, Durham, NC 27708, USA

The astonishing ability of development to compensate for perturbations was discovered and initially characterized in sea urchins by Hans Driesch and Sven Hörstadius between 1891 and 1935. A century later, a powerful set of tools is providing an increasingly clear understanding of the heart of this process, namely the molecular mechanisms that reprogram individual cell fates. More surprisingly, perhaps, this same information is also providing insights into the evolution of developmental mechanisms and their underlying gene regulatory networks (dGRN). We are investigating the development and evolution of the earliest patterning events in sea urchin development, which simultaneously establish the primary signaling center and specify the germ and skeletogenic cell lineages. Drawing on experimental perturbation, single cell sequencing, and multiplexed in situ hybridization, we are uncovering parallels and differences between the regulative capacity of embryonic patterning and the evolution of these same patterning mechanisms. Our results suggest that specific dGRN features and regulative properties may facilitate evolutionary changes in early development and contribute to adaptive traits.

Insights into deuterostome evolution from the biphasic gene regulatory program of hemichordates

Alberto Perez-Posada[1,2], Che-Yi Lin[3], Tzu-Pei Fan[3], Ching-Yi Lin[3], Yi-Chih Chen[3], José Luis Gómez Skarmeta[†,1], Jr-Kai Yu[3,4], Yi-Hsien Su[3], Juan J. Tena[1]

[1] Centro Andaluz de Biología del Desarrollo, Consejo Superior de Investigaciones Científicas-Universidad Pablo de Olavide-Junta de Andalucía, 41013 Seville, Spain
[2] Department of Biological and Medical Sciences, Oxford Brookes University, Headington Campus, Gipsy Lane, OX3 0BP Oxford, United Kingdom
[3] Institute of Cellular and Organismic Biology, Academia Sinica, 11529 Taipei, Taiwan
[4] Marine Research Station, Institute of Cellular and Organismic Biology, Academia Sinica, 26242 Yilan, Taiwan
[†] Deceased September 16, 2020

Among deuterostomes, hemichordates and echinoderms (collectively called Ambulacraria) are sister groups of chordates. Comparative studies involving these three groups provide valuable insights into deuterostome evolution. Indirect developing hemichordates produce planktonic larvae that bear resemblance to echinoderm larvae before undergoing metamorphosis into an adult body plan with anteroposterior polarity homologous to that of chordates. Therefore, understanding the developmental processes of indirect-developing hemichordates is key to unravelling the evolution of deuterostomes and the origins of chordates. In this study, we analyzed the transcriptomes and chromatin accessibility of multiple developmental stages of the indirect-developing hemichordate *Ptychodera flava* and discovered that it exhibits a biphasic developmental program controlled by distinct sets of transcription factors. Comparative analyses of transcriptomes and network analyses revealed that the gastrula transcriptome is relatively ancient, and the TFs orchestrating its gene expression are highly interconnected. By comparing the developmental transcriptomes of hemichordates, echinoderms, and amphioxus, we identified a high conservation of gene expression during gastrulation that extends to the neurula stages of amphioxus, along with remarkable similarity in larval transcriptomes across the three species. Additionally, we show that *P. flava* possesses conserved interactions of transcription factors necessary for the development of echinoderm endomesoderm and chordate axial mesoderm, including conserved cis-regulatory elements of the FoxA that is central to the two networks. These findings suggest the existence of a deuterostome phylotypic stage during gastrulation governed by gene regulatory networks with conserved cis-regulatory interactions. Overall, our results support the hypothesis that the indirect developing strategy is an ancestral trait in deuterostomes.

The Tale of Degenerate Ascidian Tails

Billie J. Swalla[1,2,3], Sydney Popsuj[3,5], Anna Di Gregorio[4], Alberto Stolfi[3,5]

[1] Biology Department, University of Washington, Seattle, WA 98195, USA
[2] Friday Harbor Laboratories, University of Washington, Friday Harbor, WA 98250, USA
[3] Station Biologique de Roscoff, 29680 Roscoff, France
[4] Department of Molecular Pathobiology, NYU College of Dentistry, New York, NY 10010, USA
[5] School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA 30332, USA

Ascidians are invertebrate chordates and develop into chordate tadpole larvae, with a head containing sensory organs and a tail with a notochord, dorsal neural tube, and muscle cells. However, in one clade of ascidians, the Molgulidae, both the larval tail and pigmented otolith have been lost multiple times independently during evolution. We have sequenced and analyzed the transcriptome of *Molgula occulta* embryos at different stages and compared it to those of a closely related tailed species, *Molgula 24culate* and of hybrid embryos obtained from tailless eggs fertilized with tailless species sperm. These two sister species live sympatrically near Roscoff, France and can be hybridized to produce a half-tailed larva with an otolith. Larval tails are formed through convergent extension and then swelling of 40 notochord cells. This process fails to occur in tailless molgulid species, leaving a “notoball”, a 20-cell aggregate. In hybrid larvae, notochords undergo normal convergent extension, forming a smaller, 20-cell notochord that lacks muscle. We are using this system to study changes in the gene regulatory networks that are responsible for the breakdown of the larval regulatory gene networks and lead to the lack of functional tissues in tailless ascidians. We have discovered that a maternal SHARK tyrosine kinase, the larval muscle actins and the larval tyrosinase genes have become pseudogenes and produce nonfunctional RNAs and proteins in the tailless embryos. Therefore, the intact tailed paternal genes can rescue the function of the pseudogenes in the hybrid embryos. We are continuing to examine the neural and notochord gene networks to understand how much of the normal larval regulatory gene networks are still intact in the tailless species, *M. occulta*, and how they are restored in the hybrids.

Interplay between mechanical and genetic information processing controls skeletal growth in the sea urchin embryo

Majed Layous[1], Tsvia Gildor[1], Eman Hijaze[1], Areen Qassam[1], Haguy Wolfenson[2], Smadar Ben-Tabou de-Leon[1]

[1] Department of Marine Biology, Charney School of Marine Sciences, University of Haifa, Israel
[2] Department of Genetics and Developmental Biology, Rappaport Faculty of Medicine, Technion, Haifa, Israel

Embryonic development is regulated by genetic information processing conducted by gene regulatory networks (GRNs) and mechanical information processing conducted by adhesion and cytoskeleton remodeling proteins. The information exchange between these two regulatory systems underlies polarized organ growth; yet, little is known on the genetic and mechanical information exchange in the context of the whole embryo. The sea urchin larval skeletogenesis offers a highly tractable system to address this gap in knowledge. The sea urchin skeleton is made of two frameworks of calcite rods, termed spicules, formed within a syncytial tubular cable generated by the skeletogenic cells. The spicules grow by localized deposition of mineral and matrix proteins at their tips. The GRN that controls sea urchin skeletogenesis is known in great details and shows remarkable similarity to the GRN that controls vertebrates' vascularization, while it is quite distinct from the GRN that controls bone formation. Here we unravel a network of adhesion and cytoskeleton proteins that controls the polarized growth of the spicules and strongly affects the sea urchin skeletogenic GRN. At the initial stages of spicule formation, Focal Adhesion Kinase (FAK) is recruited and activated at the membrane engulfing the initial biomineral grain. During spicule elongation, FAK is activated at the growing zone downstream of RhoA associated coiled-coiled kinase (ROCK). At this time, microtubule filaments extend from the Golgi apparatus in the skeletogenic cells toward the spicules. These microtubule filaments are possibly anchored to the focal adhesions and enable the transport of vesicles carrying matrix proteins and minerals to the growth zone. FAK activity promotes skeletal growth and inhibits ectopic skeletal branching. FAK and ROCK activate Erk signaling in the skeletogenic cells, which in turn, regulates the expression of key skeletogenic regulatory genes, at the tips of the rods. Thus, the interactions between focal adhesions, actomyosin remodeling and microtubule filaments and the skeletogenic GRN drive polarized skeletal elongation and prevent ectopic growth. This is a remarkable example of the interplay between the mechanosensing machinery to the GRN that controls organogenesis during embryo development.

Widespread priming of transcriptional regulatory elements by incipient accessibility or Pol II pause in early embryos of the sea urchin *Strongylocentrotus purpuratus*

César Arenas-Mena

CUNY Graduate Center and College of Staten Island

Transcriptional regulatory elements (TREs) are the primary nodes of GRNs, and their accessibility controls developmental potency. In embryo stages, larvae and adult differentiated red spherules, proximal and distal TREs are detected by Precision Run-On Sequencing (PRO-seq), which maps genome-wide the location of paused or elongating RNA polymerase Pol II at base pair resolution, thus enabling the genome-wide location of TRE transcription start sites along with their proximal pause and pause-release rates. In parallel, TRE accessibility is estimated by ATAC-seq. Our analysis identifies surprisingly early and widespread TRE accessibility in 4-cell cleavage embryos that is not necessarily associated with concurrent or subsequent transcription. TRE transcriptional differences identified by PRO-seq provide more contrast among embryonic stages than ATAC-seq accessibility differences, in agreement with the excess of accessible but inactive TREs during embryogenesis. Global TRE accessibility reaches a maximum around the late blastula stage, which coincides with the consolidation of major embryo regionalizations and peak histone variant H2A.Z expression. A transcriptional potency model based on labile nucleosome TRE occupancy driven by DNA sequences and the prevalence of histone variants is proposed in order to explain the basal accessibility of transcriptionally inactive TREs during embryogenesis. Our analysis also reveals that a large number of distal TREs become transcriptionally disengaged during developmental progression, in support of an early Pol II paused model for developmental gene regulation that eventually resolves in prompt transcriptional activation or permanent silencing. Thus, developmental potency in early embryos may be facilitated by widespread incipient accessibility and transcriptional pause at developmental TREs.

Reprogramming of cell fate in an embryo with stereotyped cleavage

Amber Rock and Mansi Srivastava

Department of Organismic & Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA

Animals begin development as a totipotent zygote and cell fate becomes increasingly restricted through progressive cleavages. This results in irreversible fate decisions being made as early as the first cleavage in some animals. The three-banded panther worm, *Hofstenia miamia*, is a regenerative acoel worm that undergoes a stereotyped cleavage program during early embryogenesis. After the first symmetric division, cells undergo a series of asymmetric cleavages; resulting in smaller micromeres and larger macromeres, with specific cellular fates assigned upon each division. At the four-cell stage, the micromeres are specified to become the only source of neuronal and pharyngeal tissue in the adult animal, while the macromeres give rise to muscle, gut, and neoblasts, the pluripotent stem cell population that allows the adult to regenerate. However, when a single four-cell staged macromere is isolated, it can develop into an adult worm, suggesting that the cell is totipotent despite having undergone a fate-specifying cleavage. Lineage tracing has shown that this maintenance of totipotency is not driven by the neoblasts. By leveraging RNA-sequencing and classical cut and paste embryology, we are searching for the mechanisms that drive reprogramming in this unique blastomere. Although no individual cell is totipotent past the four-cell stage, the blastomeres remain plastic in response to positional perturbation, although not in isolation, suggesting that cell-cell signaling can drive fate specification in the early embryo. This allows us to probe the developmental potency of blastomeres, both as an individual and in the whole of the embryo, throughout developmental time.

Single-Cell Transcriptomics Reveal Developmental Trajectories of Micromereless Sea Urchin Embryos

Alejandro Berrio, Duke University

Department of Biology, Duke University, Durham, NC 27708, USA

Sea urchins serve as a valuable model organism for investigating embryonic development and cell fate determination. Micromeres play a crucial role in generating the skeletogenic lineages. However, even in the absence of micromeres, skeletogenic cells can be rescued and embryos can still form an endoskeleton through a process known as transfating. Despite the importance of this process, the molecular mechanisms underlying transfating in sea urchin embryos remain elusive. In this study, we employed single-cell RNA sequencing and Waddington-OT to analyze the transcriptomes of micromereless sea urchin embryos. By comparing the developmental trajectories of micromereless and normal embryos, we identified genes and pathways that exhibit differential expression or regulation in the absence of micromeres. Our findings shed light on the role of micromeres in directing the development of other cell lineages and offer new insights into the gene regulatory network governing sea urchin embryogenesis.

Where wnt went: Changes to *wnt* signaling following a life-history change in *Heliocidaris erythrogramma*

Jane Swart

Department of Biology, Duke University, Durham, NC 27708, USA

Early signaling events in sea urchin embryos are necessarily highly coordinated and conserved across echinoids. The signaling center of the embryo is established prior to the blastula stage and is crucial in specifying cell lineages. However, direct developing sea urchin *Heliocidaris erythrogramma* has key components of the signaling center disrupted or delayed without delays to gastrulation. In both the typical indirect developing urchin and in *H. erythrogramma*, *wnt* signaling has been shown to be active beginning in the blastula, through gastrulation, and important in specifying both the endoderm and ectoderm. Disruptions of *wnt* signaling in *H. erythrogramma* indicate that it is playing a role in gastrulation and specification of the endoderm, though the phenotypes produced by these manipulations differ between direct and indirect developing embryos. Furthermore, indirect developing *Lytechinus variegatus* shows ligands *wnt1* and *wnt8* expressed throughout gastrulation, beginning at the vegetal pole of the embryo and moving into ectodermal cells. In *H. erythrogramma*, these ligands are still expressed but remain vegetal through gastrulation. Taken together, these results indicate that *wnt* signaling remains important in specifying the endoderm in a direct developing urchin, though the mechanism has changed.

A new model for understanding tube morphogenesis and the origins of birth defects

Margherita Perillo

Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, University of Chicago, Woods Hole, MA 02543, USA

Building a tube, or tubulogenesis, is the first step in organ development. Most of our organs start as a tube primordium, an epithelium surrounding a lumen that grows and elongates to form the final organ architecture. Malfunctions in tube morphogenesis cause severe birth defects in humans, like pulmonary hypoplasia, hypoplastic kidney, or situs inversus. However, as vertebrates have many organs all tightly packed together, the basic mechanisms of tube primordium formation that are common among organs and organisms are still poorly understood. Indeed, so far we lacked a system that is related to vertebrates but with a simpler anatomy to image this process *in vivo*. To address this deficit, we have developed a suite of powerful tools for *in vivo* long-term live imaging and set up CRISPR Cas9 genetic perturbations for the sea star *Patiria miniata,* a close relative to vertebrates that has a clear larva with only two organs. We discovered that cell mechanics, cell migration behaviors and proliferation patterns that drive elongation of the sea star hydro-vascular organ -a looped organ that we established as a new model for tubulogenesis- is highly conserved with vertebrates. We discovered that the Wnt signaling acts through a Frizelled1/2/7 receptor to drive tube orientation -an important aspect of tube morphogenesis that if impaired results in organ malfunctions. Moreover, to be fully functional many organs are made of different cell-types that during embryogenesis migrate from other tissues. We found that in the sea star larva cells from the growing gut (the other tubular organ of this system) move out of the epithelium to migrate as a cluster towards the growing tubes of the hydro-vascular organ, a critical step for the proper architecture of the final organ. Altogether, using the sea star larva as a new system for tubulogenesis will shed a light on the common players responsible for birth defects caused by improper organ morphogenesis.

Reconstruction of the sea urchin larval body plan in preparation for metamorphosis

Brennan D. McDonald, Abdull Jesus Massri, and Gregory A. Wray

Department of Biology, Duke University, Durham, NC 27708, USA

Over the past few decades, developmental biologists have gained a relatively strong understanding of early development in echinoderm embryos. However, echinoderm development does not end until after larvae undergo metamorphosis, which generally occurs several days to weeks after fertilization. This extended timeframe has previously made molecular studies challenging, but new technologies have begun to make the process more accessible for research. In this study, we analyzed a single cell RNA sequencing dataset of development in *Heliocidaris erythrogramma*, a direct-developing sea urchin species that resides in Australia. The dataset consisted of a time course from six through 60 hours post fertilization, when the pentaradial adult body plan is well established, including the water vascular and central nervous systems. We observed rapid cellular differentiation up through gastrula phase as tissues diversified into larval structures. Of all the timepoints examined, cell clusters at 30 hours post fertilization appear to be the most transcriptionally distinct from one another. Notably, most cell clusters after 30 hours began to gradually lose their distinctive identities as the larvae remodeled themselves in preparation for metamorphosis. We found that many cell type marker genes from the canonical sea urchin developmental gene regulatory network decreased in expression and localization over time, a pattern confirmed by hybridization chain reaction *in situ*s. Overall, these findings suggest that cells in *H. erythrogramma* larvae undergo a novel pattern of de-differentiation ahead of metamorphosis, indicating a widespread regulatory shift in the transition from a larval to adult body plan.

Holothuria tubulosa as a new experimental system for Evo-Devo studies

Margherita Perillo[1,2], Alfonso Toscano[2] and Rossella Annunziata[2]

[1] Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, 7 MBL St., Woods Hole, MA 02543, USA
[2] Stazione Zoologica Anton Dohrn, Villa Comunale 80121, Naples, Italy

Holothuroids, also known as sea cucumbers, are a highly diverse, ecologically and economically important group of animals belonging to echinoderms. Although most studies using sea cucumbers are performed on the adults (ecotoxicological assessments, isolation of bioactive compounds, exploration of their regeneration capacities, etc.), there is significant growing interest in dissecting the factors regulating and/or influencing their embryonic and larval development, for both aquaculture and basic research purposes. However, the few available functional data on sea cucumber development derive only from studies performed on two Pacific species (*A. japonicus* and *P. parvimensis*).

To expand the array of holothuroid species available for evo-devo studies and to uncover the ancestral traits of this group of animals, we established the sea cucumber *Holothuria tubulosa* as new experimental system for cell and developmental biology. This species is highly abundant in the Mediterranean Sea, the genome has been recently published and embryos develop through a planktotrophic larval stage. We setup a reproducible protocol to obtain gametes, mature oocytes, fertilize eggs and culture *H. tubulosa* embryos up to the auricularia stage. We performed a detailed morphological analysis combining immunohistochemistry and high resolution microscopy and highlighted critical cellular and developmental features that are unique of this animal. Transcriptomic analyses of different developmental stages are ongoing and will shed light on the molecular toolkit employed during embryogenesis and larval development.

This work represents a step forward in our understanding of holothuroids development and establishes *H. tubulosa* as an emerging and easily accessible experimental system for Evo-Devo studies and beyond.

Foundational transgenic building blocks in the sea urchin *Lytechinus pictus*

Elliot Jackson

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, UC San Diego, La Jolla, CA 92093, USA

Transgenesis is arguably the most transformative step towards unlocking the full potential of model organisms. While the sea urchin is one of the oldest animal models, it lags in comparison to others regarding the production of transgenic lines. This is largely because the long generation times of sea urchin species typically used (i.e. *Strongylocentrotus purpuratus*) does not incentivize the use of transgenics. For this reason, a more genetically tractable species - *Lytechinus pictus* - was chosen to develop a transposon-mediated approach for transgenesis in sea urchins. Here I will report on progress towards this approach to create a transgenic line that can serve as both a valuable imaging line as well as a landing pad line for large cassette exchanges. To achieve this, several transposable landing pad approaches are being pursued. One of them being a plasmid with cre/lox sites flanking a polyubiquitin promoter downstream of a H2B-mCerulean nuclear marker. Injection of this plasmid with the transposase resulted in stable, mosaic expression through metamorphosis. In contrast, injection of the plasmid without the transposase resulted in progressively fewer individuals with fluorescence, with precipitous decline in fluorescence upon metamorphosis. Fewer than 4% of juveniles injected with the plasmid alone showed mosaic, nuclear fluorescence, compared to 44% of those injected with the plasmid and transposase. Transgenic F0 animals are currently being raised to adulthood to determine germline integration. This modular transgenic line will serve as a foundational resource that will enable future combinatorial genetic tools (e.g. active genetics).

VitelloTag: a tool for high throughput cargo delivery into oocytes

Akshay U. Kane[1†], D. Nathaniel Clarke[2†], Margherita Perillo[1], S. Zachary Swartz[1]

[1]Eugene Bell Center, Marine Biological Laboratory, Woods Hole, MA
[2]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA
[†]These authors contributed equally to this work.

Developmental and reproductive biology research broadly relies upon the delivery of molecular and genetic tools into oocytes. In diverse model organisms, including mouse, zebrafish, xenopus, and echinoderms, this is achieved through microinjection into oocytes and egg cells. Microinjection, while highly effective, has disadvantages in terms of cost of setup, the high skill level required, and the limited numbers of injected oocytes obtainable. To overcome these limitations, we have developed a simple, cost effective, and high throughput method of delivery into oocytes, comparable in terms of technical difficulty to transfection. This delivery system employs conserved regions of vitellogenin, a yolk protein precursor, recombinantly fused with the protein of interest for cargo delivery via receptor-mediated endocytosis and endosome escape. We have optimized two minimal vitellogenin peptide sequences sufficient for uptake. We further establish that these approaches are compatible with an array of different model organisms, including sea stars, other echinoderms, and the acorn worm. These studies establish a tool for delivery of a variety of experimentally important cargos used in experimental biology.

New Tools for Genetic Perturbation of the Urchin Embryo

Carl Manner, Keen Wilson, Zach Pracher, David McClay, and Greg Wray

Department of Biology, Duke University, Durham, NC 27708, USA

Limited tools exist for genetic perturbation in the sea urchin embryo, and most knockdown is still accomplished using antisense morpholinos, which are expensive, difficult to solubilize, and are hindered by severe design constraints. New Cas9-based methods are emerging, but validated protocols in sea urchin embryo often rely on expensive synthetic sgRNAs and/or recombinant protein effectors. We have developed several novel technologies for perturbation of the sea urchin embryo and urchin-derived cell cultures to facilitate work in this system, with the eventual goal of enabling high-throughput forward genetic screening. For targeted gene knockdown, we employ an RNAi-based method offering substantial advantages in terms of cost, perdurance, ease of design, and ease of injection. We additionally developed a Cas9-based method for drug-inducible knockdown and transcriptional activation, and methods for lentiviral delivery of genetic payloads to cell lines derived from sea urchin embryos. In future work we hope to combine these tools to generate scalable temporal control of gene expression to enable massively parallel forward genetic screening in the developing embryo.

Constructing the Cytokinetic Contractile Ring in the Early Sea Urchin Embryo: Insights from New Imaging, Pharmacological, and Computer Modeling Approaches

John H. Henson[1,3], Gabriela Reyes[2,3], Jonathan B. Alberts[2,3], and C. Bradley Shuster[2,3]

[1] Department of Biology, Dickinson College, Carlisle, PA 17013, USA
[2] Department of Biology, New Mexico State University, Las Cruces, NM 88003, USA
[3] Friday Harbor Laboratories, University of Washington, Friday Harbor, WA 98250, USA

The precise mechanisms underlying the assembly and structural progression of the cytokinetic contractile ring (CR) in animal cells are not fully understood. We have studied the CR in early sea urchin whole embryos and isolated cortices using super resolution light microscopy (3D-SIM and STED) and platinum replica TEM. To date our studies have indicated that the CR initiates as a band of clusters containing myosin II, actin, septin and anillin, which then congress over time into a narrower, more linearized and contractile array within the cleavage furrow. Our recent imaging work indicates that MKLP1, the kinesin-like microtubule motor component of the Rho-activating Centralspindlin complex, localizes to the early CR, whereas the critical actin filament crosslinking protein alpha-actinin only associates with the late stage CR. iPALM imaging of cortices reinforces our previous SIM/STED evidence of the presence of a CRassociated septin filament network Our recent drug studies using Latrunculin A to disrupt actin filaments and the new myosin II ATPase inhibitor para-aminoblebbistatin (PAB) - a more soluble and photostable derivative of blebbistatin - suggest that the initiating myosin II clusters can assemble in the absence of actin filaments and/or myosin II motor activity. Agent-based computer modeling of myosin II clusters demonstrates that they can undergo congression and linearization in association with actin filaments *in silico*. These results highlight the importance of the myosin II clusters in setting up the organization of the sea urchin embryo's CR and we are currently investigating how deep into development this cluster-based CR assembly mechanism is maintained.

A cost-effective pipeline for fully automated high-throughput spatial transcriptomics of whole-mount marine embryo samples

Yoon Lee

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, La Jolla CA USA

Over the past several decades, fluorescent *in situ* hybridization (FISH) has powered spatial transcriptomics in sea urchin developmental biology. This has allowed for seminal work on the developmental gene regulatory network and identification of genetic endpoints of marine toxicant exposures. However, FISH assays have historically been laborious, error prone, low-throughput, and thus, costly. Sea urchin research does not have the issues which force projects to be low-throughput since they produce large sample sizes on demand, develop quickly and synchronously, can be imaged easily due to their optical transparency, and can be handled easily due to their small size. Taking full advantage of these features, we developed a novel FISH pipeline and techniques based on hybridization chain reaction (HCR) which enable completely automated miniaturized high-throughput FISH, from fixation to image production. Using automated confocal microscopy, robotic liquid handlers, and new FISH reagents which simplify the HCR assay, and thus, enable automation, we are able to perform automated FISH in a 384-well plate format. As a proof-of-concept, we show that using a single column of a 384-well plate, our pipeline can produce localization images of 80 genes across multiple developmental stages of *Lytechinus pictus* (painted sea urchin). Our pipeline can be extended to produce localization images of 1920 genes utilizing the full plate. We discuss the applications of large-scale spatial transcriptomic image datasets. In particular, we explore the utilization of our pipeline as a drug screening platform using optimized panels of genes which are diagnostically important and constitute conserved gene regulatory networks.

Differential neural crest gene regulatory subcircuits along the body axis

Marianne Bronner

California Institute of Technology, Pasadena, USA

Neural crest cells are multipotent, migratory stem cells that form at the border of neural plate border in vertebrate embryos. They then migrate from the neural tube along defined pathways, populate numerous sites, and differentiate into diverse cell types. However, neural crest populations differ along the neural axis; e.g., only cranial neural crest cells give rise to cartilage and bone of the face, and only cardiac/vagal neural crest cells contribute to the heart and enteric nervous system. We have performed transcriptome analysis of these different neural crest populations to identify gene regulatory networks (GRNs) that confer axial level specific identification to different neural crest cell populations. By characterizing the function of transcriptional and signaling components in these neural crest GRNs in diverse vertebrates from lamprey to amniotes, we aim to understand the steps that define the neural crest from the time of induction at the neural plate border to their differentiation into diverse cell types along the body axis and across evolutionary time.

The Metabolic Pace of Development: Environmental Challenges from the Beginning

Donal Manahan

Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089, USA

Early stages of animals have high mass-specific rates of metabolism (Metabolic Scaling Law) that must be sustained to maintain the pace of development. A major challenge is to predict how these processes will be impacted—in any species—by future scenarios of environmental change. Observed variance in biological response is not simply experimental error. There are many complex Nature–Nurture interactions involved that are difficult to dissect experimentally. Using select species of marine invertebrates, my research group studies the "Cost-of-Living" as an integrating framework to assess the capacity of developmental stages to respond to environmental change. Specifically, the analysis is based on determining: (1) genetic bases of adaptive variance, (2) limits of organismal-level tolerance, (3) physiological capacity for cellular energy trade-offs, and (4) molecular biological biomarkers of resilience. Regarding development and the environment, specific examples will be presented to illustrate how evolutionary "Winners" could be identified in a rapidly changing ocean.

Genomic signatures of exceptional longevity and negligible aging in the red sea urchin

Jennifer M. Polinski[1], Kate R. Castellano[1], Katherine M. Buckley[2] and Andrea G. Bodnar[1]

[1] Gloucester Marine Genomics Institute, Gloucester, MA 01930, USA
[2] Department of Biological Sciences, Auburn University, Auburn, AL 36849, USA

The red sea urchin (*Mesocentrotus franciscanus*) is one of the earth's longest living animals, reported to live more than 100 years with indeterminate growth, life-long reproduction, and no increase in mortality rate with age. To understand the genetic underpinnings of longevity and negligible aging, we constructed a chromosome level assembly of the red sea urchin genome and compared it to that of short-lived sea urchin species. Genome wide syntenic alignments identified chromosome rearrangements that distinguish short- and long-lived species. Expanded gene families in long-lived species play a role in innate immunity, sensory nervous system, and genome stability. Targeted analysis of the *M. franciscanus* immune gene repertoire revealed expansion of pattern recognition receptors and regulators of anti-viral immunity. The neural gene complement contained an extensive repertoire of GPCRs and transient receptor protein channels, and an expanded cholinergic signaling system. Genes associated with genome stability revealed the expansion of key tumor suppressor and DNA repair genes which may play a role in cancer resistance. Genes under positive selection in *M. franciscanus* are involved in nutrient sensing, protein homeostasis, genome maintenance, mitochondria and peroxisome function. Many of the positively selected genes form an integrated network and showed increased expression with age in the central nervous system suggesting a regulatory program for maintenance of neuronal function. Our results indicated that pathways known to promote longevity in model animals are also implicated in sea urchin longevity, but also suggested novel pathways to promote long-term maintenance of tissue homeostasis, disease resistance, and negligible aging.

"Get a spine!" shouts the invertebrate: A mechanistic explanation of echinoid colormorphs.

Gary Wessel[1], Madison Francoeur[2], Gerardo Reyes[1], Thomas Onarato[1], Cosmo Pieplow[1], Rushane Dunn[1], Aidan Furze[1], El Hebert[1], Nathalie Oulhen[1], Masato Kiyomoto[3]

[1]Providence Institute of Molecular Oogenesis (PRIMO), Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI 02906, USA
[2]Department of Earth and Environmental Sciences, Rutgers University-Newark, Newark, NJ 07102, USA
[3]Ochanomizu University, Bunko-ku, Tokyo, Japan

Echinoderms have marked morphological diversity and one prominent feature of the adults is diversity of color. The pigments responsible for much of this variation in sea urchins appear to be naphthoquinones, a polyketide made by an ancient and well conserved polyketide synthase (PKS). Here we focus on testing how the PKS product is modified in spines of the sea urchin to yield diverse pigmentation. We first examine the natural colormorphs of *Lytechinus variegatus* and learn that the spine color comes from cells distinct from the pigmented immune cells seen migrating throughout the spine. Instead, the pigment is present throughout the sub-epidermal regions. To identify genes responsible for these colormorphs, we compared the transcriptomes of spines from white, green, or red individuals and learned that the spine transcriptomes as well as the cognate proteomes from each colormorph are markedly different from each other, much more than could be ascribed to pigmentation. Examining the spines from different color groups by scanning electron microscopy and microCT analysis did not reveal any distinct structural differences that might explain such distinct gene activity. We then tested the presence of candidate genes from the transcriptomes responsible for pigmentation, including PKS, multiple flavin-containing monooxygenases (FMOs), and sulfotransferases. Using multicolor fluorescence hybridizations we conclude that the epidermal cells are responsible for expression of pigment genes as well as the structural genes involved in biomineralization. The accumulation of these candidate transcripts quantified by qPCR revealed distinct profiles consistent with the model that each spine color relies on a distinct combination of gene expressions, particularly of FMOs. We then tested the spine transcriptomes resulting from Cas9-mediated gene inactivation of pigment genes and learned that even in siblings, the presence or absence of genes whose activity yields pigment results in vastly different gene expression profiles. We surmise from these results that the enzymes necessary for pigment in adult echinoids also have important activities in other biochemical pathways that result in a distinct metabolome. Although not detrimental to the animal, these metabolic differences may alter broad transcriptional activities in the pigment-bearing structures.

Voltage-gated sodium channel activity mediates sea urchin larval skeletal patterning through spatial regulation of Wnt5 expression

Christopher F. Thomas, Dakota Y. Hawkins, Viktoriya Skidanova, Simone R. Marrujo, Janay Gibson, Ziqing Ye, Cynthia A. Bradham

Department of Biology, Boston University, Boston, MA 02215, USA

Defining pattern formation mechanisms during embryonic development is important for understanding the etiology of birth defects and to inform tissue engineering approaches. In this study, we used tricaine, a voltage-gated sodium channel (VGSC) inhibitor, to show that VGSC activity is required for normal skeletal patterning in *Lytechinus variegatus* sea urchin larvae. We demonstrate that tricaine-mediated patterning defects are rescued by an anesthetic-insensitive version of the VGSC LvScn5a. Expression of this channel is enriched in the ventrolateral ectoderm where it spatially overlaps with posterolaterally expressed Wnt5. We show that VGSC activity is required to spatially restrict Wnt5 expression to this ectodermal region that is adjacent and instructive to clusters of primary mesenchymal cells that initiate secretion of the larval skeleton as triradiates. Tricaine-mediated Wnt5 spatial expansion correlates with the formation of ectopic PMC clusters and triradiates. These defects are rescued by Wnt5 knock down, indicating that the spatial expansion Wnt5 is responsible for the patterning defects induced by VGSC inhibition. These results demonstrate a novel connection between bioelectrical status and the spatial control of patterning cue expression during embryonic pattern formation.

Transfating replacement of micromere/skeletogenic cells after removal at the 16-cell stage

Alejandro Berrio Escobar, Esther Miranda, Greg Wray, and Dave McClay

Department of Biology, Duke University, Durham, NC 27708, USA

Skeletogenic cells are replaced if removed from the sea urchin embryo at the 16-cell stage or later at the mesenchyme blastula stage. Absence of micromeres, the progenitors of the skeletogenic cells, removes early signaling of Wnt1, Wnt8, and Delta from the posterior end of the embryo. Despite the absence of those important signals the embryo recognizes the absence and replaces both the signals, and eventually, the skeletogenic cells. To understand the molecular sequence of replacement a scRNA-seq experiment was conducted with 7 timepoints covering the replacement time period compared to the same timepoints in control embryos. Several hypotheses were suggested by the scRNA-seq outcome. There is a 10-fold increase in neurons produced in the micromereless embryos over time reflecting the absence of Wnts which normally inhibit neurogenesis. This outcome indicates that neurogenesis in the anterior embryo is, in part, influenced by Wnt signaling from the most posterior end of the embryo. The micromereless embryos replace both skeletogenic cells and blastocoelar cells but not pigment cells, an expected result since previous work showed that Delta, produced by micromeres, is necessary for pigment cell specification. A long branch of the UMAP suggested a sequence of transfating beginning with endoderm, then endomesoderm, then mesoderm, and finally blastocoelar cells and skeletogenic cells. Tests involving perturbation of GRN components support that hypothesis.

TGF-Beta and non-canonical Wnt signaling interactions coordinate anterior-posterior and dorsal-ventral axis formation in sea urchin embryos

Jennifer L. Fenner, Sujan Gautam, Martina Martinez-Bartolome, Cheikounha Ka, M Grant, C Stocks and Ryan C. Range

Department of Biological Sciences, Auburn University, Auburn, AL 36849, USA

In deuterostomes, the specification and patterning of the anterior-posterior (AP) and dorsal-ventral (DV) axes spatiotemporally overlap. However, we lack a clear understanding of the explicit molecular mechanisms that control these axes in any developmental model organism. In sea urchin embryos an integrated network of canonical (Wnt/β-catenin) and non-canonical (Wnt/JNK and Wnt/PKC) signaling pathways specify and pattern the early germ layers along the AP axis. During this progressive AP patterning process, ventrally localized Nodal signaling initiates DV axis specification, establishing opposing Nodal and BMP2/4 signaling gradients activating DV GRNs in all three germ layers. Previously, we have shown that non-canonical Wnt16-Fzd1/2/7 signaling plays a critical role in the sea urchin AP Wnt signaling network. Here, we use functional knockdown experiments to show that Fzd1/2/7 signaling and a different Wnt ligand, Wnt6, are necessary for early Nodal signaling activity, as well as the transcription of *bmp2/4* and the dorsal ectodermal GRN. Unexpectedly, our data indicate that *nodal* transcription is normal in Wnt6 and Fzd1/2/7 knockdown embryos, but phosphorylation of its downstream target Smad2/3 is perturbed. Furthermore, we show that dorsal BMP2/4 signaling is necessary for the activation of a secreted Wnt ligand antagonist, Wnt inhibitory factor1 (Wif-1), in dorsal-anterior blastomeres. Wif-1 functional knockdown assays indicate that Wif-1 is essential for the positioning of the anterior neuroectodermal GRN around the anterior pole during AP patterning. Together, our results illustrate that in sea urchin embryos there are direct interactions among components of the early AP Wnt signaling network and the DV Nodal-BMP2/4 signaling pathways.

Axial patterning in asterozoan and the diversification of echinoderm pentaradial body plans

Laurent Formery[1,2], Daniel S Rokhsar[2], Christopher J Lowe[1]

[1]Department of Biology, Hopkins Marine Station of Stanford University, Pacific Grove, CA 93950, USA
[2]Department of Molecular and Cell Biology, University of California Berkeley, Berkeley, CA 94720, USA

Echinoderms are remarkable among animals because they exhibit a unique fivefold symmetry that evolved from a bilateral ancestor. Despite being united by their pentaradial body plan and the presence of a good fossil record, the evolutionary relationship between echinoderm classes have proven to be challenging to reconstruct based on morphological features alone. Recently, we have shown using a combination of spatial transcriptomics and HCR *in situs* in the asteroid *Patiria miniata* that positional information carried by conserved bilaterian axial patterning systems can be used to uncover hidden molecular anatomy and relate anatomical regions of the echinoderm pentaradial body plan to that of their bilateral relatives. In particular, we found that conserved modules patterning anterior structure in other bilaterians are deployed in the ambulacral ectoderm, with the midline of the arm corresponding to the most anterior territory. We postulated that this new axial paradigm would provide a key to reinvestigate regional homologies across extant echinoderm body plans. To test this idea, we surveyed anterior patterning network genes in ophiuroids, the sister group of asteroids. We found in *Amphipholis squamata* that the general principles of axial patterning are similar to that of *P. miniata*, although we also found important differences. Using the fossil record to polarize evolutionary changes, we show how modification of axial patterning in ophiuroids enabled the evolution of their ganglionated nervous system and flexible arm morphology. Our study exemplifies how coupling patterning data with the exquisite fossil record of echinoderms can help to reinvestigate key morphological transformations in light of regulatory changes.

Sea urchin larvae utilize light for regulating the activity of digestive tract

Junko Yaguchi[1] and Shunsuke Yaguchi[1,2]*

[1]Shimoda Marine Research Center, University of Tsukuba
[2]PRESTO, JST

Light plays a crucial role in sustaining life on Earth. For instance, visual systems heavily rely on light reflection, and the circadian rhythms of most organisms are regulated by the cycles of sunlight. However, the acquisition and diversification of light-dependent systems during deuterostome evolution remain poorly understood, mainly due to the limited knowledge of the light response signaling pathway in Ambulacraria, a major group of deuterostomes and a sister group of chordates. In this study, we demonstrate that sea urchin larvae utilize light to regulate digestive tract activity. Remarkably, our experiments reveal that exposure to light induces pyloric and anal opening even in the absence of food stimuli. Through our investigations, we have identified a light>Go-Opsin>serotonin>nitric oxide pathway responsible for regulating pyloric opening, while different opsins and neurotransmitters are involved in anal opening. Our findings shed light on the evolution of light-dependent systems related to digestive tract activities and neurotransmitter function, providing valuable insights into their establishment during the animal evolution. Drawing from previous research on brain-gut interactions in vertebrates, we speculate that one primitive function of anterior neuroectodermal neurons (brain neurons) might have been to regulate digestive tract function in the common ancestor of deuterostomes. Considering the fundamental importance of food consumption and nutrient absorption for animals, the acquisition and development of a sophisticated gut regulatory system driven by the brain could have played a significant role in deuterostome evolution.

Seeing clearly: visualization of whole, intact organ systems in adult echinoderms

Nat Clarke, Laurent Formery, Chris Lowe

Department of Biology, Hopkins Marine Station of Stanford University, Pacific Grove, CA 93950, USA

Studies of morphology and pattern in adult stages of many invertebrates are hindered by opaque structures, such as shells, skeletal elements, and pigment granules that block or refract light and necessitate dissection for observation of internal features. An inherent challenge in anatomical studies relying on surgical approaches is that cutting tissue is semi-destructive, and delicate structures, such as axonal processes within neural networks, are difficult to reconstruct once they are disrupted. To address this problem, we developed a hydrogel-based tissue clearing approach to render the bodies of opaque and calcified invertebrates optically transparent while preserving their anatomy in an unperturbed state, allowing for observation of intact organ systems. The resulting protocol can clear large (>1cm^3) specimens to enable deep-tissue imaging via confocal microscopy, and is compatible with molecular techniques, such as antibody staining and *in situ* hybridization to visualize protein and mRNA localization. To test the utility of this method, we performed a comparative microscopy study of intact nervous systems in multiple phyla of marine invertebrates; here, we will present observations on the organization of radial nerve cords and peripheral nervous system between multiple classes of echinoderms. We believe this technique is of broad interest and can allow comparative studies in the evo-devo field to extend further into development by enabling interrogation of structures in juvenile and adult life history stages.

Genome-wide identification and spatiotemporal expression analysis of cadherin superfamily members in echinoderms

Macie Chess, Josiah Saunders, Charles A. Ettensohn

Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213, USA

Cadherins are calcium-dependent transmembrane cell-cell adhesion proteins that are essential for metazoan development. Their functions include forming adherens junctions, establishing planar cell polarity (PCP), and regulating cell shape, proliferation, and migration. Because they are basal deuterostomes, echinoderms provide important insights into bilaterian evolution, but their only well-characterized cadherin is G-cadherin (McClay and Miller, 1997, Dev. Biol., 199:323-39). To better characterize echinoderm cadherins, we conducted phylogenetic analyses that included two echinoid, three asteroid, and one crinoid species and examined the spatiotemporal expression patterns of four cadherin-encoding genes in *Strongylocentrotus purpuratus*. These analyses identified ten echinoderm cadherins, including one deuterostome-specific ortholog, cadherin-23, and an echinoderm-specific atypical cadherin that possibly arose in an echinoid-asteroid ancestor. Catenin-binding domains in dachsous-2 orthologs were found to be a deuterostome-specific innovation that was selectively lost in mouse. The echinoderm cadherin toolkit is more similar to that of an ancient bilaterian predating protostomes and deuterostomes than it is to the suite of cadherins found in extant vertebrates, as it lacks vertebrate-specific innovations but contains two proteins that are present in protostomes and absent from mouse. The expression patterns of four embryonically expressed cadherins (fat atypical cadherins 1 and 4, dachsous-2, and protocadherin-9) were examined by in situ hybridization. All four genes' expression patterns mirrored those of a non-canonical Wnt PCP pathway receptor essential for archenteron morphogenesis (Croce et al., 2006, Development 133:547-57), suggesting that these cadherins facilitate gastrulation and organogenesis. Future experiments will examine cadherin expression in non-echinoid echinoderms and explore the functions of cadherins during echinoderm development.

The co-option of the mechanosensing and mechanotranduction during the evolution of biomineralization in metazoans

Majed Layous, Eman Hijaze, Tsvia Gildor, Haguy Wolfenson, Smadar Ben-Tabou de-Leon

Department of Marine Biology, Charney School of Marine Sciences, University of Haifa.

Embryonic development is driven by the interplay between genomically encoded gene regulatory networks (GRNs) and mechanosensing and mechanotransduction networks (MMNs) that sense and respond to the mechanical properties of the embryonic environment. The evolution of new organs necessitates the coordinated changes in GRNs and MMNs, especially during evolutionary innovations where the stiffness of the extracellular matrix changes dramatically, *e.g.* in the evolution of biomineralization. Biomineralization is believed to have evolved independently in different phyla, using distinct minerals, organic scaffolds and GRNs. Yet, all biomineralizing cells experience a distinct increase in the stiffness of their environment due to the formation of the biomineral, which is expected to activate the MMNs in these cells. Here we report that the same MMN regulates gene expression and biomineralization in the vertebrates' bone cells and in sea urchin skeletogenic cells, despite the distinct GRNs that control the specification of these cells. In vertebrates' osteoblasts, ECM stiffness activates Focal Adhesion Kinase (FAK), that activate the RhoGTPAse, RhoA, that activates RhoA associated coiled-coiled kinase (ROCK). ROCK activity enhances the strength and activity of the actomyosin network and leads to Erk signaling and to activation of osteoblasts transcription factors. We discovered that sea urchin FAK, ROCK and Erk are active in the skeletogenic cells and their activity is necessary for biomineral growth and skeletogenic gene expression. Perturbations of FAK, ROCK and a reduction of substrate stiffness result with a reduction of skeletal growth and an increase of ectopic branching. We propose that distinct GRNs across metazoans, had employed independently the mechanosensing and mechanostrasduction machinery, in response to the increase of ECM stiffness during the evolution of biomineralization.

Perturbation of the P-body component DDX6 reveals a potential connection between developmental timing and skeletal patterning

Alexandra T Lion[1,2], Benjamin Fang[3] and Cynthia A. Bradham[1,2,4,5].

[1] Molecular Biology, Cell Biology & Biochemistry Program, Boston University, Boston MA
[2] Department of Biology, Boston University, Boston MA
[3] College of Engineering, Boston University, Boston MA
[4] Bioinformatics Program, Boston University, Boston MA
[5] Biological Design Center, Boston University, Boston MA

Embryonic patterning and morphogenesis are complex processes that remain challenging research topics. Temporal transcriptome analysis in our lab showed that developmental gene expression profiles in *L. variegatus* are divided into four phases with low internal variation that are separated by three dramatic shifts in variation, two of which correspond to transcriptional bursts (Hogan et al., 2020). We have termed these "gene expression transitions" or GETs. DDX6 is a member of the DEAD-box helicase family of proteins that is essential for the assembly of processing bodies (P-bodies), which in turn regulate significant phenotypic transitions, including the exit from pluripotency and the onset of EMT. Intriguingly, LvDDX6 transcription dynamics exhibit reduced levels specifically at time points that correspond with each of the GETs, leading to the hypothesis that DDX6 negatively regulates the abrupt temporal transitions in gene expression that occur during development. Our preliminary data indicate that DDX6 is required for the normal timing of the first GET at hatching. I also find that DDX6 is necessary and sufficient for normal skeletal patterning and gut development, suggesting that these aspects of patterning are under DDX6-dependent control.

Regulation of intracellular pH homeostasis in calcifying cells of sea urchin larva

William Chung

Institute of Physiology, Christian-Albrechts University of Kiel, 24118 Kiel, Germany

Biomineralization which is essential for sea urchin larvae is processed by concentrating dissolved inorganic carbon (DIC) and removing protons from the site of mineral precipitation. However, the molecular regulatory mechanisms that orchestrate pH homeostasis and biomineralization of calcifying cells are poorly understood. We identified a proton-selective ion channel, Otop2L, expressing in the calcifying primary mesenchyme cells (PMCs) of sea urchin larvae and demonstrated its function on H^+ excretion and spicule formation through knock-down experiments. The following Otop2L current and PMC membrane potential measurements further support that Otop2L, which is driven by negative membrane potential, conducts outwardly H^+ flux in order to maintain the intracellular pH (pH_i) homeostasis in calcifying PMCs. In addition, we identified the acid-base sensing enzyme soluble adenylyl cyclase (sAC) expression in the PMCs during skeletogenesis. Live pH_i imaging of PMCs and pharmacological experiments revealed that sAC activity is required for pH_i regulation and biomineralization of PMCs. Finally, we demonstrated that sea urchin sAC, similar to its mammalian homologs, carries the splicing variants that express only the catalytic domains, and expression analysis revealed that the re-mineralization process alters the abundance of sAC splicing variants potentially associated with the regulation of sAC activity. Here we introduce the sensor and effector to the acid-base regulatory machinery of PMCs. This system maintains the pH_i homeostasis of calcifying cells in order to support the biomineralization process under environmental pH challenges.

Squeeze confinement-induced changes in fluid flows generated by ciliated marine larvae

Vivek N. Prakash[1,2,3], Bikram D. Shrestha[1], Santhan Chandragiri[1], and Melissa Ruszczyk[1]

[1] Department of Physics, University of Miami, Coral Gables, FL, USA
[2] Department of Biology, University of Miami, Coral Gables, FL, USA
[3] Department of Marine Biology & Ecology, University of Miami, Miami, FL, USA

Many ciliated marine invertebrate larvae swim and feed in a viscous low Reynolds number environment in the ocean. The larvae swim in three-dimensions (3D) using ciliary beating and their flow fields are often complex and challenging to quantify in experimental studies. The conventional microscopic imaging configuration of trapping larvae in between a glass slide and cover slip induces a quasi-two-dimensional (2D) confinement. We systematically quantify the fluid dynamical effects of this 2D squeeze-confinement on the flows generated by ciliated larvae at low Reynolds numbers (< 0.5) with both spherical and non-spherical morphologies. We vary the confinement parameter, i.e. the gap between the glass slide and cover slip, and observe changes in the number of vortices, vortex size and intensity. In non-spherical larvae with complex morphology of sea stars and sea urchin, we find that increasing confinement leads to larger number of vortices that come closer to the body surface. In spherical larvae of corals and the non-spherical larvae, we find that decreasing confinement gives rise to a pair of counter rotating vortices. Our results are broadly applicable for quantification of the fluid dynamical effects of squeeze confinement for ciliated larvae with a variety of morphologies.

Single-nucleus transcriptome analysis of regenerating sea star larvae"

Jon Lee Andrade, Anne Meyer, Adel Fergatova, Veronica Hinman

[1]Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213, USA

Larval sea stars possess the remarkable capability to fully regenerate after bisection through the anteroposterior axis. It is, however, not fully understood how specific populations of cells coordinate to produce regenerative outcomes. To shed light on this question, I have generated single-nucleus RNA-seq (snRNA-seq) data sets comprising uninjured and early regenerating larvae of the bat star, *Patiria miniata*. Using these data, I have characterized the expression patterns of the major transcription factor families across cell state and experimental condition, and I have identified previously unknown domains of gene expression within both characterized and as-of-yet uncharacterized cell states. I have also discovered and begun to characterize cell states detected specifically under uninjured or regenerating conditions, indicating measurable shifts in cellular identity in response to bisection. Further inspection reveals that these populations have elevated expression levels of genes associated with morphogenesis, differentiation, regeneration, stress and immune response, and signaling cascades including MAPK, WNT, and BMP. Future work will determine the contribution of these cells to regeneration and the mechanisms through which this is achieved.

Pluripotency of the regenerating intestinal rudiment epithelia shown by single cell analysis

Joshua Medina-Feliciano, Griselle Valentín-Tirado, Kiara Luna-Martínez, Saely Rosado-Ortiz, Yamil Miranda-Negrón, José E. García-Arrarás

Department of Biology, University of Puerto Rico at Río Piedras, San Juan, Puerto Rico 00925

In holothurians, the regenerative process following evisceration involves the development of a rudiment or “anlage” at the wounded end of the mesentery. This rudiment plays a pivotal role in the formation of a new intestine. Despite its significance, our understanding of the molecular characteristics inherent to the constituent cells of this rudiment has remained limited. To address this gap, we employed state-of-the-art single-cell RNA sequencing (scRNAseq) analysis, allowing us to discern the distinct cellular populations associated with the regenerating rudiments. Through this approach, we successfully identified a total of fifteen distinct cell populations. Among these, two populations exhibit characteristics consistent with putative mesenchymal cells, while four populations show features akin to immune or coelomocyte cell types. Notably, the remaining nine populations collectively form a substantial cluster encompassing the coelomic epithelia enveloping the rudiment. Within this cluster, we recognized previously documented cell populations such as muscle precursors and actively proliferating cells. Strikingly, our analysis also unveiled novel cell populations that had not been identified before. Consequently, our findings further strengthens the role of the rudiment's coelomic epithelia as a pluripotent tissue that gives rise to diverse cell types of the regenerating intestinal organ.

Evolutionary conservation of immune signaling pathways in echinoderms

Tse-Mao Lin[2], Chia-Yen (Erica) Lee[1,2], Shu-Ting (Robin) Chou[1], Ke Fan (Kevin) Liu[2], Sebastian D. Fugmann[1,2,3,4]

[1] Department of Biomedical Sciences, Chang Gung University, Taoyuan, Taiwan
[2] Graduate Institute of Biomedical Sciences, Chang Gung University, Taoyuan, Taiwan,
[3] Center of Molecular and Clinical Immunology, Chang Gung University, Taiwan,
[4] Department of Nephrology, Chang Gung Memorial Hospital, Linkou, Taiwan

Immune responses in multicellular organisms rely on the precisely coordinated action of numerous cell types, including highly specialized immune cells and non-immune tissue. The central signals that orchestrate such action are the initial recognition of pathogens by pattern recognition receptors followed by cellular crosstalk via secreted cytokines. Although respective signaling networks in vertebrates have been studied in depth, surprisingly little is known about their nature in invertebrates. Here we use *Strongylocentrotus purpuratus* to gain such insight at the cellular and molecular level and. Three novel findings of our study are a surprising evolution of protein-protein interaction surfaces critical for TLR signaling, the proteolytic processing of SpIL-17s (homologs of the mammalian proinflammatory IL-17 cytokines important for T cell responses against bacterial infections at mucosal surfaces) that appears to be evolutionarily conserved, and the first identification of an inflammatory caspase in invertebrates.

This work was supported by grants of the Chang Gung Memorial Hospital CMRPD1J0253] and the NSTC Taiwan [108-2320-B-182 -018 -MY3].

A new animal model for testing the roles of drug transporters in gut-specific immune response and disease

Catherine S Schrankel, Jenna Luc, Lauren Stoeltje, E Darin, Katherine Nesbit, and Amro Hamdoun

San Diego State University

Cellular defense systems dictate organismal survival in an increasingly changing world. This is particularly relevant for animals that develop in urbanized marine ecosystems. In marine invertebrates, two systems offer protection: 1) the immune system, and 2) the xenobiotic transporter system (the “chemical immune system”). We are exploring these cellular systems in the genetically enabled sea urchin *Lytechinus pictus*. We have identified larval immune cell types that are conserved at the molecular and morphological level with *Strongylocentrotus purpurpatus* immunocytes. Early *L. pictus* larvae respond robustly to infection with *Vibrio diazotrophicus, Flavobacterium*, and *Pseudoalteromonas* species. To better understand natural commensals and potential opportunistic pathogens present in larval *L. pictus*, we are also characterizing the gut microbiome up to metamorphosis. In parallel, we will identify the commensal community structure of larvae homozygous mutant for the drug transporter ABCB1/P-glycoprotein. Notably, ABCB1-/- larvae experience high baseline levels of larval gut inflammation compared to wild-types. This phenotype is conserved with established ABCB1-/- mice models for IBD and Chron’s disease. We hypothesize that the lack of ABCB1 activity in mutants causes xenobiotic molecules from commensals to accumulate in host gut epithelia, and that this accelerates the pathogenesis of gut inflammation and disease. Future work will identify the bacterial-derived compounds that are enriched in transporter-null larvae, and how their immune system responds to inflammation.

SpTransformer proteins drive phagocytosis, bind to phagocytes, and change gene expression in the innate immune system of the purple sea urchin

Ryley S. Crow, Chloe G. Shaw, L. Courtney Smith

Department of Biological Sciences, George Washington University, Washington, DC 20052, USA

The purple sea urchin, *Strongylocentrotus purpuratus*, relies exclusively on an innate immune system to combat the many pathogens that are prevalent in marine systems. The *SpTransformer* (*SpTrf*) gene family functions within this innate immune system as deduced from their expression in response to immune challenge, and based on their sequence diversity within and among animals. When the recombinant (r)SpTrf proteins are cross-linked to inert beads, they enhance phagocytosis compared to beads cross-linked to BSA. To elucidate whether unbound SpTrf proteins bind to the surface of phagocytes, fixed and impermeable cells were incubated with seven different rSpTrf proteins. All proteins display binding to the cell surface at varying levels. The specificity of surface binding was verified using the same and different pairs of proteins in binding competition, which suggests the presence of a cell surface receptor(s) on phagocytes. To evaluate the downstream effects of cell surface binding, the expression levels of key immune response genes (*SpTrf*, *SpIL17-9*, *SpEchinoidin*) were quantified in coelomocytes incubated with different rSpTrf proteins. All rSpTrf proteins influence the expression of the *SpTrf* gene family, and a subset of the proteins influence the expression of *SpIL17-9*, while none alter *SpEchinoidin* expression. These findings suggest that the receptor(s) on phagocytes may recognize secreted SpTrf proteins, signaling that an immune response is underway, and may trigger negative feedback to modulate the expression of immune genes and the correlated protein production and secretion. The diverse recognition and responses exhibited by different rSpTrf proteins underscore the complexity of the innate immune system in the purple sea urchin.

The genomic organization of sea lamprey variable lymphocyte receptor loci and the development and function of the agnathan adaptive immune system.

Jonathan P. Rast

Department of Pathology and Laboratory Medicine and the Emory Vaccine Center,
Emory University School of Medicine, Atlanta, GA 30322, USA

The evolutionary origins of the jawed vertebrate adaptive immune system have been a mystery despite intense investigations carried out over many decades. The molecular hallmarks of this system, including somatically diversified immunoglobulin (Ig) and T cell receptors (TCRs), and antigen presenting major histocompatibility proteins, are absent in jawless vertebrates and invertebrates. Intriguingly, the living jawless vertebrates, including lampreys and hagfishes, have an alternative system of diversifying receptors based on leucine-rich repeat proteins (the Variable Lymphocyte Receptors or VLRs). The lymphocyte-like cells that carry VLRs on their surface show striking parallels to those that bear Ig and TCR in jawed vertebrates. The lamprey VLR system thus provides a long-sought pathway to elucidate the early origins of vertebrate adaptive immunity. Five VLR subtypes have been identified in the sea lamprey: VLRB, which, like jawed vertebrate Ig, is expressed as both a cell surface receptor and as a secreted antibody; and VLRA, VLRC, VLRD, and VLRE which are cell surface transmembrane receptors. We have mapped the complex loci of all five VLR germline genes along with hundreds of associated donor cassettes used in the assembly of mature VLR. These donor cassettes are distributed in at least nine widespread clusters on five different chromosomes in the sea lamprey genome assembly. This genomic organization is conserved across several lamprey species with available chromosome-scale genome sequence assemblies. Notably, VLRA/C/D/E share extensive cassette usage in the construction of mature VLR genes. Understanding how VLR genomic organization relates to the generation of antigen recognition diversity, the development of VLR bearing cells, and to the chromosomal histories of the loci that encode different VLR subtypes will lend insight into the function and origins of this form of vertebrate adaptive immunity.

Christopher Lowe, Stanford University

TBD

Stage-specific functions of nuclear β-catenin in cell fate specification, maintenance, and restriction during early sea urchin embryogenesis

Guy Lhomond, Michael Schubert, Jenifer Croce

Evolution of Intercellular Signaling in Development (EvoInSiDe) team, Laboratoire de Biologie du Développement de Villefranche-sur-Mer (LBDV), Villefranche-sur-Mer, France.

In animals, embryonic development commonly relies on signaling pathways to efficiently control germ layer specification, maintenance, and restriction. One of the most important signaling pathways in these processes is the canonical Wnt/b-catenin pathway, which, in some animals, further exhibits duration-, stage-, context-, and/or dose-specific developmental functions. In sea urchins, perturbation experiments targeting the canonical Wnt/b-catenin pathway have already revealed its critical requirement for the development of the vegetal tissues (skeletogenic mesoderm, non-skeletogenic mesoderm, and endoderm) and for the spatial restriction, within the animal hemisphere, of the ectoderm and the anterior neuroectoderm lineages. The experiments documenting these effects have, however, all been initiated before or at fertilization, leaving open the questions of whether this pathway may have stage-specific requirements as well as particular functions at selective later stages during embryogenesis. Using an inducible, conditional knockdown approach, we have determined that, during sea urchin embryonic development, each of the three vegetal tissues requires different, specific temporal regimes of b-catenin activity to be correctly specified. We also determined that two of the vegetal tissues (non-skeletogenic mesoderm and endoderm) necessitate even further sustained nuclear b-catenin activity for their maintenance through time and that distinct durations of b-catenin nuclearization gradually control the respective restriction of the ectoderm and the anterior neuroectoderm territories along the animal-vegetal body axis. Together, our work is the first to functionally define the sequential requirements of b-catenin nuclearization during sea urchin embryogenesis, demonstrating in a new animal model the pleiotropic functions played by this key nuclear effector during animal early development.

The conserved role of integrated canonical and non-canonical Wnt signaling during anterior-posterior axis formation in ambulacrarian deuterostome embryos

Jennifer L. Fenner, Sujan Gautam, Christopher J. Lowe, Ryan C. Range

Department of Biological Sciences, Auburn University, Auburn, AL 36849, USA

One of the first events to occur in animal development is the formation of the Anterior-Posterior (AP) axis. This fundamental step in embryogenesis is largely controlled by a deep evolutionarily conserved posterior-to-anterior gradient of Wnt/β-catenin signaling that patterns the early germ layer (endoderm, mesoderm, ectoderm) gene regulatory networks (GRNs) along this axis. In the sea urchin, AP specification and patterning is controlled by an integrated Wnt signaling network of canonical (Wnt/β-catenin) and non-canonical (Wnt/JNK and Wnt/PKC) signaling pathways. Here we use mRNA overexpression and siRNA knockdowns to show that many components of the sea urchin AP Wnt signaling network are also critical for AP axis pattering in the in the hemichordate, *Saccoglossus kowalevskii*. In sea urchins Wnt8-Fzd5/8-JNK signaling is critical for the restriction of the anterior neuroectoderm (ANE) GRN to a territory around the anterior pole. Our functional perturbations show that Wnt8, Fzd5/8 and JNK are necessary for ANE restriction in *Saccoglossus.* We also show that knockdowns of the Wnt antagonist Dkk1 leads to the elimination of the ANE GRN. Finally, our data indicate that Fzd1/2/7 in *Saccoglossus* does not antagonize the Wnt/JNK restriction mechanism, as it does in sea urchins, but rather functions synergistically with Fzd5/8 to restrict the ANE GRN around the anterior pole. Echinoderms and Hemichordates are members of the Ambulacrarian phyla that forms the immediate sister group with chordates. Thus, collectively these data indicate that interactions among canonical and non-canonical Wnt signaling pathways during AP axis formation were present in the last common chordate/ambulacrarian ancestor.

Asymmetric β-catenin nuclearization regulates mesendoderm formation in amphioxus early embryos

Cheng-Yi Chen, Hui-Ju Wu, Che-Yi Lin, Shu-Shuo Yeh, Tsai-Ming Lu, Jr-Kai Sky Yu*

Institute of Cellular and Organsimic Biology, Academia Sinica, Taipei, Taiwan

Signaling pathways comprise an essential part of developmental gene regulatory networks. The activities of signaling pathways lead to changes of the gene regulatory states, and consequently determine different cell fates in particular areas within a developing embryo. The canonical Wnt/β-catenin pathway has been implicated in playing important functions for early embryogenesis. In a wide range of metazoan animals, vegetal nuclear accumulation of β-catenin defines an initial axis of the embryo and is essential for the germ layer specification. However, in Cephalochordates (amphioxus), whether β-catenin accumulates in the vegetal nuclei had been controversial, and the function of β-catenin in early germ layer specification remains unclear. Here we show that Wnt/ β-catenin signaling is active in the vegetal hemisphere in early amphioxus embryos, and ectopic activation of Wnt pathway before blastula stage greatly expands mesoderm/endoderm formation at the expense of ectodermal tissues. Transcriptome profiling on isolated blastomeres show differential enrichment of maternal transcripts associated with mRNA metabolism between the animal-tier and vegetal-tier blastomeres, suggesting diverged molecular regulatory functions among blastomeres in the early cleavage-stage amphioxus embryo. In sum, our results provide critical information to fill the gap in our knowledge regarding the conservation of early patterning mechanism in chordates.

Ascidian invariant cleavage

Alex McDougall, Janet Chenevert, Remi Dumollard, Herve Turier

Ascidian BioCell Group, Sorbonne Université/CNRS, Laboratoire de Biologie du Développement de Villefranche-sur-mer (LBDV), 06230 Villefranche sur-mer, France

Although different classes of embryos display predictable patterns of early cleavage divisions up to the blastula stage, we still do not understand what generic rules lead to one or another embryonic shapes. Difficulty in answering this question is due to the lack of mechanistic understanding of how cell shape, cell tension, cell adhesion and cleavage patterning and timing are coordinated. Even though significant progress has been made in identifying the genes involved in driving cell fate decisions, this reductionist one gene-one function approach has led to comparatively less progress in understanding how cleavage patterns and overall embryonic shape emerges.
We exploit the invariant cleavage pattern displayed by ascidian embryos to tease apart the cell biological and biomechanical forces involved in underpinning the invariant cleavage pattern. Invariant cell positioning in the ascidian embryo is important for cell-cell signaling starting at the 32-cell stage when 4 animal blastomeres are induced by vegetal blastomere derived FGF to become neural precursors. We have so far found a number of mechanisms that work together to create the invariant cleavage pattern: cell cycle asynchrony, unequal cell division, long apical length dependent spindle positioning and mitotic apical relaxation. I will present what we have done and are doing and try to give an overview of how the ascidian invariant cleavage pattern emerges.

Cellular mechanics of oocyte maturation

Debadrita Pal[1], Maria F. Visconti[1], Francina Montenegro-Vazquez[1], Elena Pearson[1],Clara Ross[1], Zak Swartz[2], John Henson[3] and Charles Bradley Shuster[1]

[1] Department of Biology, New Mexico State University; Las Cruces, NM 88003, USA
[2] Whitehead Institute for Biomedical Research, Cambridge, MA 02142, USA
[3] Dickinson College, Carlisle, PA 17013, USA

Stimulation of G2-arrested oocytes by maturation hormone triggers a series of events that leads to nuclear and cytoplasmic maturation in preparation for fertilization and development. Accompanying these changes is a dramatic change in the mechanical properties of the oocyte. Prior to hormone stimulation, cortical tension levels are amongst the highest ever measured for any animal cell type and is dependent on Rho and Myosin II activity. Following hormone stimulation, cortical Rho activity and cortical tension levels decline leading up to CDK1 activation and germinal vesicle breakdown. While the changes in cortical mechanics was independent of CDK1, the drop in tension was dependent on PI3K activity and Rac, thus linking the changes in cellular mechanics to the same signaling pathway as meiotic re-entry. Similar phenomena has been observed in mice, but the functional role of this remodeling is unknown. To explore the physiological significance of Rho downregulation on oocyte maturation, activated and dominant negative mutants of Rho were expressed, and their effects on known meiotic processes were analyzed. Constitutively active Rho had no effect on polar body formation, nor did it affect cortical granule translocation to the cortex. However, Rho downregulation appeared to be necessary for Dishevelled localization to the vegetal cortex. Thus, while there are likely multiple aspects of oocyte maturation affected by Rho and its downstream effectors, we have identified one element of meiotic maturation that requires Rho downregulation: the recruitment of developmental determinants to the vegetal pole.

miR-31-mediated local translation at the mitotic spindle is important for early development

Carolyn Remsburg, Kalin Konrad, Michael Testa, and Jia L. Song

Department of Biological Sciences, University of Delaware, Newark, DE 19716, USA

miR-31 is a conserved microRNA that plays critical roles in cell proliferation, migration, and differentiation. Using the sea urchin embryo as a model, we discovered that miR-31 and its validated targets have a cell cycle-dependent dynamic distribution: they are enriched on the mitotic spindles in dividing cells and in the perinuclear region of non-dividing cells. Localization of miR-31 and its targets at the mitotic spindles is evolutionarily conserved, as we also observe this in mammalian cells. To test its function, we inhibited miR-31 and observed that miR-31 inhibition led to developmental delay that correlated with cytoskeletal and chromosomal segregation defects. To understand the mechanism of these defects, we identified miR-31 to directly target several actin remodeling transcripts, such as *β-actin, Gelsolin*, *Rab35*, and *Fascin*. We hypothesize that miR-31 regulates local translation of transcripts encoding cytoskeletal remodeling proteins to ensure proper cell division. In support of this hypothesis, miR-31 inhibition results in subcellular increase of Fascin and Rab35 proteins. Further, forced ectopic localization and translation of *Fascin* transcripts at the cell cortex leads to delayed development and significantly increased lethality rate at the blastula stage, indicating local translation of *Fascin* at the mitotic spindle is essential for development. This study revealed the novel and evolutionarily conserved role of miR-31 in mediating local translation of cytoskeletal modifying transcripts that impact mitosis and early development. Overall this work contributes to the fundamental understanding of early cell division, birth defects, and predisposition to cancer.

Developmental Biology of Sea Urchins and other Marine Invertebrates, 2023

Nipam Patel

The molecular embryology of cephalopod molluscs: conservation, convergence, and innovation

Caroline Albertin

Marine Biological Laboratory, Woods Hole, MA

Cephalopods (octopus, squid, and cuttlefish) have a highly derived body plan and a suite of morphological innovations with no obvious correlates in other animals. They also demonstrate a loss of spiral cleavage, which is characteristic of non-cephalopod molluscs and other spiralians. As cephalopod embryogenesis itself is a novelty, comparisons with the otherwise highly conserved spiralian developmental program could provide important insights into the evolution of developmental processes. Our recent work, including sequenced genomes and the first CRISPR-mediated knockouts in cephalopods, has opened up the possibility of accessing new questions in this group at a molecular and cellular level. Leveraging these new resources, we are exploring the evolution of cephalopod development and their extraordinary body plan.

Building a better sea urchin.

Amro Hamdoun, Evan Tjeerdema, Elliot Jackson, Svenja Kling, and Yoon Lee

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92037, USA

Animal models are essential for understanding the mechanisms of development, and for uncovering the early life origins of disease. The utility of these models depends on the effective application of stable genetic manipulation methods to establish causal relationships between genes and phenotypes. While sea urchins have long been premier model organisms for the study of early development, they have been bottlenecked by the lack of stable genetics. Here I will report on how we are leveraging advances in research scale mariculture, molecular genetics and high content imaging to address this bottleneck and build a fully genetically enabled urchin, *Lytechinus pictus*. I will describe the systematic process towards the generation of inbred, knockout, knockin and landing pad lines, as well as the complementary efforts to leverage the resulting animals in miniaturized, semi-automated high content imaging pipelines. I will conclude with a perspective on how these approaches may be applied to diverse problems across the spectrum of sea urchin research.

Analysis of gene regulatory network dynamics using a Tet-On system for conditional control of gene expression.

Jian Ming Khor, Jennifer Guerrero-Santoro, and Charles A. Ettensohn

Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213, USA

Gene regulatory networks (GRNs) are inherently dynamic. Because molecular approaches that are typically used to explore network architecture result in constitutive, ubiquitous perturbation of gene function, and because many genes have essential roles during early embryogenesis or function in multiple tissues, it has been difficult to analyze GRN architecture in specific cell types at late developmental stages. We optimized a Tet-On system to conditionally induce gene expression in sea urchin embryos and used this approach to investigate dynamic changes in the architecture of the GRN that underlies skeletogenesis in euechinoids. Early in development, this network is controlled cell autonomously, but during gastrulation, regulation becomes dependent upon extracellular signals. We know much about the first regulatory phase, which is evolutionarily derived, but little about the second, which is likely to reflect ancestral control mechanisms. Using the Tet-On system, we showed that Ets1 and MAPK signaling play essential roles in the signal-dependent regulation of the GRN, providing a potential link between one key ligand (VEGF) and the network. We also used the Tet-On system to examine the spatial regulation of gene expression within the skeletogenic mesenchyme during late embryogenesis. The skeleton is secreted within a syncytium, and local patterns of skeletal growth are associated with distinct sub-domains of gene expression. We expressed fluorescently-tagged forms of transcription factors and biomineralization proteins in sub-domains of the skeletogenic syncytium and found that both classes of proteins have highly restricted mobility. We propose that this limited mobility allows for the generation and maintenance of sub-domains of gene expression within the syncytium and local regulation of skeletal growth.

Actomyosin remodeling regulates biomineral formation, growth and morphology during eukaryote skeletogenesis

Eman Hijaze[1], Tsvia Gildor[1], Ronald Seidel[2], Majed Layous[1], Mark Winter[3], Luca Bertinetti[2], Yael Politi[2] and Smadar Ben-Tabou de-Leon[1]

[1] Department of Marine Biology, Leon H. Charney School of Marine Sciences, University of Haifa, Haifa 31905, Israel.
[2] B CUBE Center for Molecular Bioengineering, Technische Universität Dresden, 01309 Dresden, Germany.
[3] Department of Electrical Engineering, Computer Science and Mathematics, Technische Universiteit Delft, Delft 2628CD, Netherlands

Biomineralization had apparently evolved independently in different phyla, using distinct minerals, organic scaffolds and gene regulatory networks (GRNs). However, diverse eukaryotes from unicellular organisms, through echinoderms to vertebrates, use the actomyosin network during biomineralization. Specifically, the actomyosin remodeling protein, Rho-associated coiled-coil kinase (ROCK) regulates cell differentiation and gene expression in vertebrates' biomineralizing cells, yet, little is known on ROCK's role in invertebrates' biomineralization. Here we reveal that ROCK controls the formation, growth and morphology of the calcite spicules in the sea urchin larva. ROCK expression is elevated in the sea urchin skeletogenic cells downstream of the Vascular Endothelial Growth Factor (VEGF) signling. ROCK inhibition impairs the organization of F-actin around the forming spicules, disrupts skeletogenic gene expression and leads to skeletal loss. ROCK inhibition after spicule formation reduces spicule elongation rate and induces ectopic spicule branching. Reduced skeletal growth and enhanced branching are also observed under direct perturbations of the actomyosin network. Similar skeletogenic phenotypes are observed when ROCK is inhibited in a skeletogenic cell culture, indicating that these phenotypes are due to ROCK activity specifically in the skeletogenic cells. We propose that the actomyosin network was employed independently, downstream of distinct GRNs, to regulate biomineral growth and morphology across Eukaryotes.

A cephalopod molecular approach of evolving the largest invertebrate nervous system

Ali Murat Elagoz, Astrid Deryckere, Ruth Styfhals, Daniela Sofía Aparicio Quiñonez, Sofia Maccuro, Marie Van Dijck, Benjamin Sannen, Dries Janssen, Mark Lassnig, Eve Seuntjens

Department of Animal Physiology and Neurobiology, Katholieke Universiteit Leuven, Leuven, Belgium

The cephalopod nervous system is comparable to the nervous system of a small mammal in terms of neuronal number and richness in the behavioural repertoire it controls. Nevertheless, the last common ancestor between cephalopods and mammals was a worm-like marine organism that existed approximately 600 million years ago. Studying cephalopods presents an opportunity to understand the genetic drivers of neural development that evolved convergently with vertebrates. Octopuses have a centralized nervous system with circumesophageal brains and axial nerve cords passing through the center of each arm. Their nervous system consists of 500 million neurons. Approximately one-third of these neurons are found in the brain, and the rest is located mainly in the arms. While the morphological characterization of the octopus nervous system has been substantially carried out, molecular mechanisms driving the neurogenesis remain unclear. Our recent findings showed that there is a neurogenic zone located outside the brain adjacent to the eyes in lateral lips. We also discovered that newly born neurons display long-distance migration into the centralized brain, reminiscent of vertebrate neurogenesis. Our present study elaborates on the molecular characterization of octopus neurogenesis. We have identified another important pool of progenitors located at the basolateral epithelium layer in the arms. Live imaging experiments suggest these progenitor cells also display migration, strengthening the idea that migration is fundamental for large nervous system development. We have further investigated the spatiotemporal expression of intrinsic and extrinsic factors essential for neurogenesis and gliogenesis with *in situ* Hybridization Chain Reaction. Finally, using small molecules to impede the evolutionarily conserved signaling pathways, we unravel its functional implication in the morphogenesis of the octopus nervous system.

The role of BMP signaling during neural development in the squid *Doryteuthis pealeii*

Jessica Stock and Caroline B Albertin

Marine Biological Laboratory, Woods Hole, MA

The animal kingdom harbors a variety of different nervous systems, the foundation of which is laid during embryonic development. Morphogens are key components of neural development that shape the nervous system. In particular, the BMP signaling pathway is considered to play an ancestral role during evolution due to its involvement in patterning diverse brains and bodies. While both vertebrate and invertebrate models demonstrate similar roles for BMP signaling in dorsoventral patterning and neural induction, functional studies outside of the common model systems remain sparse and the role of BMP signaling in many invertebrate systems unclear.

Among invertebrates, coleoid cephalopods, including octopus and squid, stand out due to their massive brains that rival those of vertebrates in size and complexity. While cephalopods have been a prominent model system to investigate the anatomical attributes of the adult nervous system, its embryonic origins have yet to be understood. Recent advances in tool development present the squid *Doryteuthis pealeii* as a unique model to investigate the evolution and development of massive brains outside of the vertebrate lineage. Here, we leverage new molecular tools to functionally investigate the role of the BMP pathway in squid neural development to elucidate the ancestral role of BMP signaling as well as the development of massive brains outside of the vertebrate lineage.

Insights into the cellular and molecular mechanisms of the highly regenerative tunicate *Polycarpa mytiligera*

Tal Gordon[1,5], Tal Zaquin[2], Noam Hendin[3], Omri Wurtzel[3], Lucia Manni[4], Ayelet Voskoboynik[1], Noa Shenkar[5]

[1] Institute for Stem Cell Biology and Regenerative Medicine, and Hopkins Marine Station, Stanford University School of Medicine, Stanford, California 94305, USA
[2] Department of Marine Biology, The Leon H. Charney School of Marine Sciences, University of Haifa, Haifa, Israel
[3] School of Neurobiology, Biochemistry & Biophysics, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel
[4] Department of Biology, University of Padova, 35121, Padova, Italy
[5] School of Zoology, George S. Wise Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv 6997801, Israel

Regeneration is widespread in the animal kingdom, and a variety of model systems are employed to better understand the principles and genetic programs underlying this process. Ascidians are remarkable for their regenerative abilities, and while the majority of regenerative studies focused on well-known model species, our recent work suggested a new model: the solitary ascidian *Polycarpa mytiligera*. *In vivo* experimental observations revealed this species extraordinary ability to regenerate all body parts following their removal, including the central nervous system (CNS). Our current study further describes *P. mytiligera's* impressive regenerative potential and presents the morphological, cellular, and transcriptomic dynamics that lead to entire CNS regeneration. Our results revealed the expression of key neuro developmental markers that are not otherwise present in the adult CNS. Removal of the entire CNS resulted in high cell proliferation in the regenerated area. Transcriptome analysis revealed enhanced stem- cell related gene activity, with high expression of P53 and piRNA pathways preceding the activation of Notch, Wnt, and Nanos pathways. Our new findings provide an in-depth characterization of *P. mytiligera's* regeneration process, presenting insights into the cellular and molecular aspects of CNS regeneration, further emphasizing the importance of this new model system in the study of the evolution of chordate regeneration.

TIKI is required for anterior neuroectoderm and skeletal patterning in sea urchin embryos.

Cheikhouna Ka, Katherine M. Buckley and Ryan C. Range

Department of Biological Sciences, Auburn University, Auburn, AL 36849

Three different, yet interconnected Wnt pathways govern anterior/posterior (A/P) axis specification and patterning during early sea urchin embryogenesis. Previous studies suggest that secreted and membrane-bound modulators play critical roles in regulating embryonic Wnt signaling in multiple organisms. Specifically, in vertebrates, the novel, Wnt-specific metalloprotease known as TIKI was shown to regulate A/P axis patterning. In this study, we describe how TIKI1/2 is integrated into the A/P Wnt signaling network in sea urchin embryos. Expression analyses indicate that maternal-and-later-zygotically expressed *tiki1/2* transcripts are distributed broadly during cleavage stages and then dynamically expressed throughout the rest of embryogenesis. These expression patterns often overlap with other known components of Wnt signaling pathways that regulate A/P patterning. Functional perturbations using Morpholino antisense oligonucleotides and overexpression experiments demonstrate that TIKI1/2 is required for proper sizing of the anterior neuroectoderm, likely through antagonism of the Wnt8-Fzd5/8-JNK signaling pathway. Furthermore, our functional analyses indicated that Fzd5/8 is required for *tiki1/2* expression in the anterior pole, which may serve as a signaling feedback loop. Notably, our data also indicate that TIKI1/2 is required for embryonic skeletogenesis by affecting PMC migration. Together, these results suggests that TIKI1/2 is critical in the regulation of Wnt/JNK signaling during A/P axis pattering and functions broadly to regulate the Wnt ligand activity during sea urchin embryogenesis.

Transcription of miRNAs is regulated by developmental signaling pathways

Malcolm Arnott[1], Nina Faye Sampilo[2], Jia L. Song[1]

[1] Department of Biological Sciences, University of Delaware, Newark, DE 19716, USA
[2] Invitae - Metropark, Iselin, NJ 08830, USA

In early embryonic development, the cross-regulation of transcription factors and signaling pathways are critical in mediating developmental and physiological processes such as body axis formation, cell fate specification and morphogenesis. Additionally, studies have shown the importance of post-transcriptional regulation of signaling and network components mediated by miRNAs; however, how miRNAs are transcriptionally regulated is poorly understood. miRNAs are critical fine-tuners of many biological processes and their dysregulation leads to numerous diseases and developmental defects. Previously, we have shown that miRNAs are dynamically expressed throughout development, suggesting that miRNAs themselves are transcriptionally regulated by developmental processes. Using inhibitors of signaling pathways, we identified key signaling pathways (Wnt, Delta/Notch, Nodal/Bmp, MAPK/ERK, Hedgehog, and Vegf) and transcription factors (Alx1, Ets1/2, and Tbr) to affect the levels of several miRNAs. The miRNAs we tested include evolutionarily conserved miRNAs in all metazoans (miR-1, miR-31, miR-92, miR-124), invertebrate-specific miRNAs (miR-71 and miR-2012), and sea urchin-specific miRNAs (miR-2002, and miR-2007). We used computational methods to identify potential transcription factor binding sites of these miRNAs. By comparing experimental data on miRNA expression upon signaling pathway perturbation and bioinformatic predictions, we identified several specific factors for each miRNA which may regulate miRNA expression. Overall, this study provides a deeper insight and understanding of how miRNAs are transcriptionally regulated by signaling pathways and transcription factors during embryogenesis and our overall understanding of development.

Marine heatwave driven bleaching and its impacts on the reproduction of the stenophagous nudibranch *Berghia stephanieae*

Ruben X.G. Silva, Diana Madeira, Paulo Cartaxana, Ricardo Calado

ECOMARE & Centre for Environmental and Marine Studies (CESAM), Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal

The rise in sea surface temperature has led to an increase in bleaching events of marine organisms throughout the ocean, during which animals are physiologically and nutritionally challenged. The cascading trophic impacts of this nutritional shift are still misunderstood, as well as their potential carryover effects to the next generations. To study such impacts, we chose the stenophagous nudibranch *Berghia stephanieae* that feeds upon the glass anemone *Exaiptasia diaphana*, exposed to combined impacts of a subpar diet, preying upon either symbiotic or bleached anemones, and thermal stress, a marine heatwave (32 °C) scenario for 7 days and control temperature (26 °C). After the exposure, we performed molecular analysis on the adults to infer their cellular stress response (CSR) and analysed the number of egg strings and weight of egg strings as a proxy of reproductive success. Our results suggest that under such an extreme weather event, nudibranchs can maintain reproductive output in terms of number of egg strings, but the weight of sea slugs' egg strings decreases, revealing lower parental investment. As for cellular stress it appears that both diet and the exposure to the marine heatwave interact to modulate CSR of nudibranchs. Significant differences on the CSR amongst the organisms fed with bleached anemones were found according to the temperature they were exposed. With this work we were able to understand that high temperature is the main driver of the cellular stress observed in these organisms, however, having a nutritionally balanced diet appears to favour the maintenance of homeostasis.

The mTOR pathway regulates a translational network stimulating lysosome biogenesis in the sea urchin early embryo

Sonia Dufour, Patrick Cormier, Sandrine Boulben, Julia Morales and Fernando Roch

Sorbonne Université, CNRS (Centre National de la Recherche Scientifique), Integrative Biology of Marine Models (LBI2M), Station Biologique de Roscoff, CS 90074, CEDEX, 29680 Roscoff, France.

In metazoan organisms, fertilization triggers the so-called egg-to-embryo transition, a developmental process in which the egg's molecular landscape is entirely reshaped to create a totipotent cell capable of generating a new individual. In the sea urchin, fertilization immediately triggers protein translation, which is essential for cell division and subsequent embryonic development. However, although it has been shown that activation of protein synthesis is under the control of the mTOR pathway, little is known about the specific contributions of translation and mTOR signaling to the many biological processes participating in the egg-to-embryo transition. Using lysosomotropic markers, we have shown that fertilization induces the formation of a large pool of acidic organelles. Our results reveal that these organelles correspond to lysosomes, and that both mRNA translation and mTOR activity are essential for their formation and maintenance. In addition, our translatomics data indicate that many mRNAs encoding lysosomal component are actively recruited into polysomes upon fertilization in an mTOR dependent manner. Our findings thus reveal that mTOR activity could orchestrate a downstream translational network controlling lysosome biogenesis. We are currently investigating whether this regulatory network is also involved in the regulation of autophagy. Our aim is to enlarge our understanding of the biological processes accompanying early embryonic life and provide new insights into the molecular mechanisms participating in mTOR signaling in a developmental context.

A translational network regulates the dynamics of cell division in sea urchin early embryo

Julia Morales[1], Sandrine Boulben[1], Sonia Dufour[1], Florian Pontheaux[1,a], Héloïse Chassé[1,b], Julie Aubert[2], Erwan Corre[3], Gildas LeCorguillé[3], Fernando Roch[1], Patrick Cormier[1]

[1] Sorbonne Université, CNRS (Centre National de la Recherche Scientifique), Integrative Biology of Marine Models (LBI2M), Station Biologique de Roscoff, CS 90074, CEDEX, 29680 Roscoff, France.
[2] Université Paris-Saclay, AgroParisTech, INRAE, UMR MIA Paris-Saclay, 91120, Palaiseau, France
[3] CNRS, FR 2424, ABiMS Platform, Station Biologique de Roscoff (SBR), Roscoff, France.

Present address
[a] Sorbonne Université, CNRS, Observatoire Océanologique 66650 Banyuls sur mer, France ;
[b] Université de Montpellier, CNRS, Institut de Génétique Humaine (IGH), 34396 Montpellier Cedex 5, France.

Translation of proteins in the sea urchin embryo is strongly stimulated upon fertilization, and is necessary for cell cycle progression and early development. The rise in translation activity depends on the mTOR pathway and involves the assembly of the canonical eIF4 complex on the cap structure of maternally stored mRNAs. Describing the full spectrum of translated mRNAs at fertilization in *Paracentrotus lividus* using polysome profiling, we showed that a subset of maternal mRNAs is selectively recruited onto polysomes following fertilization. To gain insights on the mTOR-dependent and independent translation at a genome-wide level, we performed a polysome profiling in the presence of the pan-mTOR inhibitor PP242. Interestingly, while translation of most mRNAs showed sensitivity to the inhibitor, indicative of recruitment via the canonical initiation step as expected, a fraction of the recruited mRNAs is still addressed to the polysomes in the presence of PP242. We observed an over-representation of cell cycle control genes in the translated mRNAs. Using morpholino-directed translation inhibition of specific mRNAs, we showed that in addition to the well-known mitotic cyclins, translation of other mRNAs controls the cell division dynamics following fertilization. Our finding provides new insights into the complex translational regulatory network, which orchestrates the egg-to-embryo transition in sea urchins.

Contribution of ER network to microtubule aster centration in early *Paracentrotus lividus* embryo

Jérémy Sallé, Javad Najafi, Héliciane Palenzuela and Nicolas Minc MOVE

Institut Jacques Monod, Centre National de la Recherche Scientifique and Université de Paris UMR7592, Paris, France

Microtubule aster positioning plays a central role in the determination of early embryonic cleavage geometry. However, how forces exerted on microtubules are generated and how they are translated into aster motion remains only partially understood. To date, several non-exclusive models of force generation have been uncovered including polymerizing microtubules pushing against cell cortex or microtubules pulling via membrane-anchored dynein motors. Here we use the early sea urchin embryo (*Paracentrotus lividus*) to explore the contribution of cytoplasmic pulling forces during microtubule aster motion. Right after fertilization, the microtubule aster starts to grow as its center travels toward the cell center defining the position of the zygote nucleus. This process of aster centration is very robust and is generally completed in 15 minutes. The ooplasm contains a large amount of endomembranes and the drag force produced by their displacement toward aster center by microtubule-associated motors has been proposed to drive aster motion. By optimizing fixed and live microtubule imaging we now have access to the detailed aster morphology which appears to be way more complex than a radial array of microtubules. In particular, we observed a population of long microtubules at the front of the aster that might be the support of cytoplasmic pulling. In addition, we found that endoplasmic reticulum, which is the only cytoplasmic compartment showing a global centripetal motion during aster centration, displays discrete pulling events along microtubule tracks and fragmentation of ER network severely impairs aster centration. Based on our current findings, it appears that the ER network has a crucial function as a cytoplasmic anchor during microtubule aster motion. This highlights the potential of the sea urchin model for exploring the properties of the cytoplasm and its relationship with the cytoskeletal networks.

Foundations for Understanding the Biology of the Echinoderm Ovary

Cosmo Pieplow, Nathalie Oulhen, Gerardo Reyes, Ellie Kim, Tarrin Dewberry, Madi Francouer and Gary Wessel

Providence Institute of Molecular Oogenesis (PRIMO), Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI 02906, USA

Due to their innate ability to produce millions of oocytes throughout their lifespan, echinoderms have historically been an indispensable model organism for studies of reproduction, fertilization, and embryogenesis. While Oogenesis itself is a fascinating and popular topic of study, the somatic components of echinoderm gonads remain poorly understood. Here we utilize three species: *S.purpuratus, L.variegatus* (echinoidea) and *P.miniata* (asteroidea), for several discovery-based studies on echinoderm ovary biology. First, we present a guide for molecular staging of echinoderm ovaries by integrating RNA-seq, scRNA-seq, in situ hybridization, and histology. These results provide hallmark gene expression profiles for stages of the ovarian cycle: I, II, III, and IV, respectively. Importantly, we have found that Stage I ovaries, the earliest stage of annual development, resemble an intermediate sex phenotype, expressing low levels of both male- and female- gonad transcripts. Oogenesis within sea urchin ovaries is further fit into this model by quantification of the spatial expression of key gene products involved in yolk sequestration and egg structure. We have also begun characterization work on the somatic cell subtypes with RNA in situ hybridizations and immunohistochemistry. Finally, we tested responses to three signaling pathways in ovaries: Activin, Wnt, and Estrogen. We learned that each signaling pathway has a full complement of players for pathway function present in the echinoderm ovary. Together, this study sheds new light on the molecular biology of the somatic gonad, and begins to contextualize how the signaling pathways of the ovary have evolved over time.

Molecular and cellular mechanisms controlling sea star reproductive longevity

Periklis Paganos and S. Zachary Swartz

Eugene Bell Center for Regenerative Biology & Tissue Engineering, Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543, USA

Animal reproduction depends on the creation and maintenance of oocytes, or egg cell precursors. In female mammals, including humans, there is a predetermined reproductive window, which is defined by a limited oocyte reserve established during fetal development. Apart from the limited number of oocytes set aside, their number and quality declines with age, as they must endure an extremely long quiescent arrest before being made available for fertilization. This reproductive potential depends on specific cell types within the ovary and their signaling interactions. Here, we shed light on these mechanisms with an evolutionarily extreme case of fecundity and reproductive longevity: the sea star *Patiria miniata*. Sea stars continuously produce hundreds of thousands of new oocytes throughout their 35+ year lifespan, making them an ideal model to understand why other animals lost this feature and whether it is possible to take advantage of this ability to improve human reproductive health and aging. To achieve this, we hypothesize that the sea star maintains an adult oogonial stem cell pool, however, the cellular identity and molecular signatures of this stem cell remain undefined. Moreover, from an evolutionary point of view, we aim to ask “What is an ovary?” To this end, we are determining the minimum conserved cell type repertoire shared amongst animals. We are combining high-resolution 3D imaging, single cell transcriptomics, and functional approaches to define the sea star ovarian cell types, including the putative stem cells and their somatic niche interactions. Overall, this study will advance our understanding of animal reproduction and development and contribute to the development of new strategies to improve reproductive health.

POSTER ABSTRACTS

An RNAseq analysis of isolated animal and vegetal halves from *Patiria miniata o*ocytes

Deema Abayawardena, Athula Wikramanayake

Department of Biology, University of Miami

The formation of a metazoan embryo from an egg is one of the most complex and amazing processes in biology. A critical event during embryonic development is the specification of the primary body axis, the anterior posterior (AP) axis in the early embryo. It is well-established that localized activation of Wnt/β-catenin (cWnt) signaling in posterior blastomeres plays a significant role in AP axis formation during embryogenesis. Studies in *Patiria miniata* have shown that Dishevelled (Dsh), a central regulator of cWnt signaling, is required for activation of this pathway in posterior blastomeres. Live imaging of Patiria oocytes showed that a Dsh-GFP fusion protein was localized to puncta throughout the cortex of the oocyte, and during oocyte maturation these puncta are lost from the cell cortex and new puncta are assembled at the vegetal pole. The mechanisms that regulate Dsh localization and activation at the vegetal pole are not known, but the elucidation of these processes is critical to understanding how the cWnt pathway is selectively activated in vegetal blastomeres to initiate AP axis patterning. To identify maternal factors that may play a role in regulating Dsh activity and cWnt activation in vegetal blastomeres, we carried out RNAseq of isolated animal and vegetal halves of Patiria oocytes. Preliminary analysis of this data indicated that there were coding and non-coding RNAs enriched in animal and vegetal halves of oocytes. The significance of these molecules to establishing the animal vegetal axis and their putative roles in activating cWnt signaling will be discussed.

The molecular basis of circadian rhythms in echinoderm larvae

Tanya Alessandro, Rossella Annunziata

Biology and Evolution of Marine Organisms (BEOM), Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Naples, Italy

Most living organisms on Earth have evolved circadian clocks to synchronize physiology and behavior to the daily alternation of light and dark cycles. These sophisticated adaptation systems have been studied in animals as well as in plants, fungi and bacteria and different genes have been found as components of the different clocks. In animals, all the circadian clocks investigated so far work through a conserved group of genes including positive (*Clock*, *Cyc*/*Bmal*) and negative (*Period*, *Tim*, *Cry*) regulators.

Exploring the available genomic and transcriptomic databases for 11 species belonging to the Ambulacraria clade, we identified homologs of all protostomes and deuterostomes canonical clock genes with the exception of *Period*, a widespread component of the negative loop in the animal circadian oscillators. Our results strengthen previous hypothesis suggesting that the Ambulacraria clock architecture might be organized differently from the circadian clocks so far investigated in metazoans.

To identify the components of the circadian clock in echinoderms, we will analyze the diel transcriptome of the sea urchin *Paracentrotus lividus* larvae entrained in 12L:12D and select all cycling transcripts. These data will be combined with scRNA-seq and ATAC-seq data, leading to the identification of the putative core components of the clock. Finally, their involvement in the regulation of circadian rhythms will be tested through functional analyses. Our study aims to elucidate for the first time the circadian clock architecture in a non-chordate deuterostome and promises to shed light on the evolution of fundamental timekeeping mechanisms in animals.

PFAS compounds PFOA and GenX are teratogenic to sea urchin embryos

Sophie Bodine[1], Alexandra T. Lion[1,2], Kelley M. McCutcheon[1], Cynthia A. Bradham[1,2,3,4].

[1]Biology Department, [2]MCBB Program, [3]Bioinformatics Program, and [4]Biological Design Center

Boston University, Boston MA 02215, USA

Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals used to make fluoropolymer coatings that are found in many consumer products, such as non-stick pans, clothing, cosmetics, and food packaging. These chemicals are known as “forever chemicals” due to their persistence in the environment. PFAS are highly water soluble and easily contaminate water sources and, as a result, PFAS molecules bioaccumulate in all animals including humans. PFAS are now recognized as hazardous pollutants with oncogenic and teratogenic effects, especially at higher concentrations that correspond with current environmental levels. We determined the effects of two PFAS molecules, perfluorooctanoic acid (PFOA) and the newer, "safe" hexafluoropropylene oxide dimer acid (GenX) on the development of *Lytechinus variegatus* embryos. We found that both PFOA and GenX are sufficient to perturb embryonic skeletal patterning and PMC migration. GenX is sufficient to significantly perturb the spatial expression of genes associated with dorsal-ventral (DV) specification. The temporal windows of effect for each chemical indicates that Gen X acts earlier during development than PFOA, consistent with its perturbation of DV specification. These results demonstrate that, despite being considered much less hazardous than PFOA, GenX has teratogenic effects on body axis and skeletal patterning in sea urchin embryos, and impacts development at an earlier time point than PFOA.

Single-Cell Transcriptomic Analysis Reveals the Molecular Profile of Go-Opsin Photoreceptor Cells in Sea Urchin Larvae

Maria Cocurullo*, Periklis Paganos*, Rossella Annunziata, Danila Voronov and Maria Ina Arnone

Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy
* These authors contributed equally

Light perception is a fundamental ability for animals since light not only provides spatial information on the surrounding environment but is also one of the main natural oscillators inducing rhythmic behavioral and physiological responses. At a mechanistic level, animals commonly utilize a set of GPCRs proteins called opsins to perceive the light. These opsins are expressed by specialized sensory cells called photoreceptors (PRCs). Sea urchin larvae have a set of PRCs deploying a Go-type opsin (Opsin3.2) but sharing transcription factors and morphology with PRCs of the ciliary type. This evidence raised new questions related to how the sea urchin larva Go-Opsin-PRCs are specified and whether they share a common ancestor with ciliary-PRCs. To investigate how the Opsin3.2 PRCs develop in the sea urchin Strongylocentrotus purpuratus from early to late larval stages, we combined immunohistochemistry and fluorescent in situ hybridization. Subsequently, we applied single-cell transcriptomics to investigate the molecular signature of the Go-Opsin3.2-PRCs and show that they deploy an ancient regulatory program responsible for PRCs specification. Finally, we also discuss the possible functions of these PRCs based on their molecular fingerprint, suggesting that they are involved in a variety of signaling pathways, including those entailing the thyrotropin-releasing hormone (TRH).

Tarrin Dewberr**y**

Providence Institute of Molecular Oogenesis (PRIMO), Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI 02906, USA

Sea urchins are famous for their ability to make large numbers of sperm and eggs throughout their lifetime. As such, their gonads may serve as important models for prolonged ovarian function and stem cell immortality. I am focusing on testing the role of the wnt signaling pathway in ovarian function. The Wnt pathway allows cells to communicate through cell surface receptors to change the transcriptional profile of a cell and its development. The Wnt pathway is known to be a critical part of embryonic development; however there's a lack of understanding of its role in the function of ovary development (and ovary regeneration). The sea urchin, *Lytechinus variegatus*, will be our model system in this project due to its regenerative capabilities in the production of oocytes. Here, sea urchin ovaries were treated with lithium chloride (an activator of the Wnt pathway) to identify by qPCR the genes that are affected by the Wnt pathway. In preliminary experiments, a qPCR experiment was conducted on stage IV ovary (after spawning) and the results showed that genes such as Wif (Wnt inhibitor factors) are highly upregulated in the ovary when the Wnt pathway is stimulated. I will now test other genes recently identified to respond to the Wnt pathway, and in different stages of ovaries to determine how broad this response may be. These results should be helpful to understand the prolonged, highly fecund gonad and how it may inform us in the biology of reproductive senescence in general.

Repressive interactions between GRNs: Alx1-mediated repression of NSM GRNs ensures skeletogenic specification

William B. Douglas and Charles A. Ettensohn

Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213, USA

The process of cell specification is controlled by dynamic gene regulatory networks (GRNs), which are composed of interacting regulatory (transcription factor encoding) genes and the downstream effector genes that provide specialized functions to different cell types. The major regulatory genes of GRNs have been well-studied for their positive transcriptional inputs; however, robust cell specification also relies on the negative regulation of potentially competing GRNs. Already, such negative regulatory interactions are understood to play an important role in development and direct somatic cell reprogramming, but the mechanisms of GRN repression are poorly understood. To study this phenomenon, I have used the sea urchin, a widely-studied model organism for understanding development, and focused on the sea urchin skeletogenic GRN, one of the best characterized developmental GRNs. Alx1, a lineage-specific transcription factor, provides direct positive inputs into many skeletogenic genes. At the same time, Alx1 appears to repress GRNs that are normally deployed in the adjacent non-skeletogenic mesoderm (NSM). Knockdown of Alx1 leads to an ectopic activation of NSM genes in the presumptive skeletogenic mesoderm and an increase in the overall expression levels of several known NSM regulatory genes. By further characterizing the effects of Alx1 knockdown, I demonstrate that Alx1 expression is necessary for proper cell patterning and boundary separation between the skeletogenic mesoderm and NSM. I also demonstrate that ectopic overexpression of Alx1 is sufficient to repress the specification of pigment cells, one of the two major derivatives of the NSM. These data have led me to hypothesize that the skeletogenic cells are primed to express an ancestral NSM GRN were it not for the regulatory intervention of Alx1. To investigate the mechanisms underlying this regulatory relationship, I explored possible direct and indirect mechanisms by which Alx1 or its downstream targets might repress NSM GRNs.

The Actin Cytoskeleton is Required for Normal Skeletal Patterning and PMC migration

Benjamin Fang[1], Abigail Glick[2,] James Huth[2], Alexandra T. Lion[3], and Cynthia A. Bradham[2,3,4,5]

[1]Biomedical Engineering, [2]Biology, [3]MCBB Program, [4]Bioinformatics Program, [5]Biological Design Center
Boston University, Boston MA 02215, USA

The larval skeleton is secreted by the primary mesenchyme cells (PMCs) that arise from the large micromeres, and which, following their ingression, migrate to locations within the blastocoel that collectively comprise a stereotypic ring-and-cords pattern that matches the initial skeletal pattern. Because skeletal pattern formation relies on PMC migration, we explored the role for the actin cytoskeleton in the patterning process by using the established inhibitor Nocodazole, which inhibits actin polymerization. We used dose-response experiments to define an optimal Nocodazole dose. Our results show that actin polymerization is required for normal PMC migration and for the formation of normal skeletal patterns.

Chromatin Remodeling Enzymes are Required for Normal Skeletal Patterning

Rachel I. Ferrigno, Alexandra T. Lion, and Cynthia A. Bradham

Biology Dept, Boston University

Chromatin remodelers regulate gene expression by dynamically altering the structure of chromatin. We characterized the temporal expression profiles for multiple chromatin remodelers in *L variegatus* embryos, and thereby identified a number of genes encoding chromatin remodelers that are expressed from fertilization through the pluteus stage. Next, we aimed to determine the specific roles these genes play in development. We performed morpholino microinjections to knock down several chromatin remodeler genes including MLL 1 and ISWI. The results show that each gene is required for normal skeletal patterning and for development. MLL 1 and ISWI are both expressed in the earliest stages of development. In embryos in which these early-expressed chromatin remodelers 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. Further experiments will continue to characterize the phenotypic consequences of chromatin remodeler perturbation.

Sea Urchins as Indicators of Seasonal Environmental Stress

Lydia Francis, Alana Acevedo, Abigail Kirkpatrick, Abigail Wilson and Michelle Roux-Osovitz

The University of Tampa. 401 West Kennedy Blvd. Tampa, FL 33606, USA

Lytechinus variegatus inhabits the sea grass and unvegetated benthos in and surrounding Tampa Bay. Seasonal fluctuations in this region include temperature, salinity, nutrients, and sea grass density. These fluctuations can introduce stress into the urchins' habitat, potentially affecting the health and reproductive potential of the population (Mantranga et al., 2005, Brothers 2016). Urchins serve an important ecological role in the benthos, contributing significantly to the food web via nutrient recycling and promotion of growth through grazing in protected sea grass beds (Eklof et al., 2008). The study presented here aims to test if *L. variegatus* can be used as an indicator species for the health of Tampa Bay benthic habitats and the reproductive success of the residing urchin populations during seasonal fluctuations. To do this, we took advantage of the well-studied immune coelomocyte population of the urchins to assess responses to seasonal stressors at two distinct ecological sites in Tampa Bay. Urchins were sampled weekly over 3 months (June-July-August) and diversity within the coelomocyte populations was determined by morphology (vibratile, red amebocytes, white amebocytes and phagocytes) and compared to echinochrome-A levels, gonadal/somatic index and egesta contents. We applied principal component analysis and regression modeling to assess the relationship between environmental fluctuation and innate immune response to predict reproductive success and health of the urchin populations living in sea grass and unvegetated habitats.

Sea urchin spines as a model specimen for innate immune cell chemotaxis

Aidan Furze, Nathalie Oulhen, Madison Francouer, and Gary Wessel

Providence Institute of Molecular Oogenesis (PRIMO), Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI 02906, USA

Immune cells are crucial for an animal's ability to respond to infection and injury. In sea urchins, it is well known that pigment cells arise from the embryonic veg2 lineage and begin to express a characteristic set of genes involved in pigment production as early as the gastrula stage. In the larvae, migratory pigmented immune cells are visible on the epithelium and respond to sites of damage, bacterial, and viral challenges. In the adult urchin, immune cells are known to be located in the coelomic fluid. Here, I present new data emphasizing the presence of migratory pigmented immune cells within the spines of the sea urchin *Lytechinus variegatus*. Interestingly, the pigmented coelomocytes show characteristic immune cell migration in the spines of the adult urchin, similar to what has been documented in pigment cells of the larvae. However, in order to study pigment cell migration in larvae the animals must be cultured for multiple days or weeks depending on the species. By using spines, ex-vivo experiments can be conducted at any time as long as adult animals are available. Our work aims to clarify the mechanisms of immune cell migration within the spines of adult sea urchins under control conditions and when exposed to stressors. I am currently conducting ex-vivo challenge experiments where I expose adult and juvenile *Lytechinus variegatus* spines to bacteria and chemical agents to better understand the mechanisms of their chemotaxis.

The role of heterochronic gene expression and regulatory architecture in early developmental divergence

Nathan Harry and Christina Zakas

Department of Biological Sciences, North Carolina State University, Raleigh, NC 27695, USA

New developmental programs can evolve through adaptive changes to gene expression. The annelid S. benedicti has a developmental dimorphism, which provides a unique intraspecific framework for understanding the earliest genetic changes that take place during developmental divergence. Using comparative RNAseq through ontogeny, we find that only a small proportion of genes are differentially expressed at any time, despite major differences in larval development and life-history. These genes shift their expression profiles across morphs by either turning off any expression in one morph or changing the timing or amount of gene expression. We directly connect the contributions of these mechanisms to differences in developmental processes. We examine F1 offspring— using reciprocal crosses— to determine maternal mRNA inheritance and the regulatory architecture of gene expression. These results highlight the importance of both novel gene expression and heterochronic shifts in developmental evolution, as well as the trans-acting regulatory factors in initiating divergence.

α-Actinin Localization and Myosin II Inhibition Indicate that the Early Assembly of the Cytokinetic Contractile Ring is Independent of Actomyosin Contraction

Maritriny Galvez-Ceron[1,3], Alexandra Hershey[1,3], Nina T. Lo[1,3], Karina Herrera[1,3], Gabriela Reyes[2,3], Quenelle McKim[1,3], Hannah Herzon[1,3], C. Bradley Shuster[2,3], and John H. Henson[1,3]

[1]Department of Biology, Dickinson College, Carlisle, PA
[2]Department of Biology, New Mexico State University, Las Cruces, NM
[3]Friday Harbor Laboratories, University of Washington, Friday Harbor, WA

We have investigated the cytokinetic contractile ring (CR) in early sea urchin embryos using advanced imaging and our results indicate that it initiates as a band of clusters containing myosin II, actin, septin and anillin, which then congress over time into a linearized array. Recently we have begun to examine the localization of the important actin crosslinking protein α -actinin using an anti-human peptide antibody which labels an appropriate 100 kDa band on immunoblots of sea urchin embryos and stains coelomocyte stress fibers in a characteristic periodic pattern. α-Actinin has long been known to be present in the CR, however questions remain about the details of its association. In first division embryos and isolated cortices α -actinin localizes with the late stage, linearized CR but is not present in the clusters and patches of the early CR – even when these two forms exist within the same embryo. In isolated cortices the linear actomyosin arrays contain periodic α -actinin labeling which is characteristic of actomyosin contractile assemblages. This is consistent with the finding in other cell types that α -actinin binding to actomyosin structures is enhanced under increased mechanical tension. In experiments using the myosin II ATPase inhibitor para-aminoblebbistatin (PAB) we demonstrated that myosin II clusters can assemble in the absence of myosin II motor activity. Embryos treated with PAB undergo nuclear divisions and can exhibit anaphase B elongation and shallow furrowing but ultimately fail to divide. We are currently using PAB treatment of coelomocytes to examine the impact of reduced actomyosin tension on α -actinin localization.

Plastic leachate-induced toxicity during sea urchin embryonic development

Eva Jimenez Guri

Stazione Zoologica Anton Dohrn

Microplastics pose risks to marine organisms through ingestion, entanglement, and as carriers of toxic additives and environmental pollutants. Plastic pre-production pellets are the raw material used in the manufacture of plastic items. In the producing process, these pellets are supplemented with different chemical additives that will act as plasticisers, stabilisers or antioxidants, among others. Accidental loss of these pellets during manufacture, processing and transport is not uncommon, and can lead to potential high concentration of toxic leachates. Therefore, it is very important to understand the short-term effect of these leachate additives for marine life. We investigate the effects of leachates from new and beach-collected plastic pellets on the embryonic and larval development of the sea urchin species *Strongylocentrotus purpuratus* and *Paracentrotus lividus*, and demonstrate that exposure of developing embryos to these leachates elicits severe, consistent and treatment-specific developmental abnormalities including radialisation of the embryo and malformation of the skeleton, neural and immune cells. We define the developmental pathways disturbed upon exposure to PVC leachates, and provide a mechanistic view that pinpoints cellular redox stress and energy production in the *S. purpuratus* larvae as drivers of phenotypic abnormalities following exposure to PVC leachates. Studying the effect of these leachates on adult *P. lividus* we see higher oxidative stress in coelomocytes, as well as changes in the immune cell profiling. Moreover, we find that even in highly polluted areas, sea urchins are fertile, but that effects seen in the adults may lead to transgenerational effects that reduce developmental robustness of the embryos.

Profiling the DNA damage response at the single-cell level in sea urchin coelomocytes

Riss Kell and Andrea Bodnar

Gloucester Marine Genomics Institute, Gloucester, MA 01930, USA

Coelomocytes, the innate immune cells of sea urchins and other echinoderms, have been shown to possess both a low susceptibility to DNA damage and some capacity for DNA repair *in vitro*. However, it is unknown if the extent of genotoxicity sensitivity and DNA repair capacity differs among short-lived vs. long-lived sea urchin species, or if specific coelomocyte cell populations (red and white amoeboid cells, vibratile cells, phagocytes) differ in their susceptibility to DNA damage. In this study, DNA damage was measured in coelomocytes of three sea urchin species with different lifespans (*Lytechinus variegatus*, 3-4 years; *Strongylocentrotus purpuratus*, 50+ years; and *Mesocentrotus franciscanus*, 100+ years) via the comet assay after exposure to UV-B (0—9999 mJ/cm^2). Coelomocytes from all three species demonstrated a high resistance to UV-B induced death (>60% viability after 24 hours at 9999 mJ/cm^2) in comparison to embryo-derived cell cultures of *L. variegatus*. Time course DNA damage measurements at 1hr, 6hr, and 24hr post UV-B delivery demonstrated some capacity for DNA repair in coelomocytes of all three urchin species. Use of the comet assay, which measures DNA damage at the single-cell level, further revealed the heterogeneous extent of DNA damage sustained by different cell types within the coelomocyte population. Overall, these results indicate differing DNA damage susceptibility and response systems among coelomocyte cell types and sea urchin species with a trend for longer-lived species to have a greater DNA repair capacity compared with shorter-lived species.

Estrogen is awesome

Ellie Kim and Gary Wessell

Providence Institute of Molecular Oogenesis (PRIMO), Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI 02906, USA

Sex steroids are essential in reproductive systems, having an involvement in development of secondary sexual characteristics, gonadal maturation, germ cell proliferation, and sexual behavior. In women, estrogen regulates the reproductive system along with neuroendocrine, skeletal, adipose, and cardiovascular systems. In contrast to humans who are born with a finite pool of oocytes, Echinoderms produce new oocytes throughout their lifespan. This phenomenon implies the existence of stem cells for eggs that are constantly replicating and developing. This continuous pool of new oocytes must require continual regulation by gonadal somatic cells, likely through intercellular signaling mechanisms. We are testing the hypothesis that echinoderms use an estrogenic signaling mechanism to regulate long-term stem cell function for oocyte development. First, we find that the genomes of sea urchins and sea stars have expressed genes in their ovaries required for estrogen biosynthesis. Second, we have conducted an estrogen challenge on ovary explants followed by RNA-seq experiments to test if exogenous estrogen can alter gene expression in this tissue. Preliminary data shows that estrogen affects the RNA expression of multiple genes in the ovary of the sea urchin *Lytechinus variegatus*. Third, I report here tests on the effect of the estrogen on different stages of ovary maturation. Ovary cultures from different stages of maturation were treated with estrogen and gene expression of potential estrogen responsive genes was tested by qPCR. Our results indicate that estrogen reduces the expression of genes specifically involved in hormonal biosynthesis (such as wwox) and increases genes regulating the Wnt pathway (such as Wif). We will next determine what cells are responsible for this response, and how they may interact with the germ line.

Dissecting the role of copper in sea urchin embryogenesis

Nick Kraieski, and Katherine Buckley

Department of Biological Sciences, Auburn University, Auburn, AL 36849, USA

Copper is an essential micronutrient in biological systems. It is necessary for the function of crucial oxidative metabolism proteins such as Superoxide Dismutase 1 (SOD1) and Cytochrome Oxidase (COX). Intracellular copper levels are tightly regulated. Mutations causing copper homeostatic dysregulation produce disorders such as Wilson's and Menkes diseases. Copper also acts as a metalloallosteric regulator in multiple cell processes including the MAP Kinase Pathway and ULK1 and ULK2-mediated autophagy. During MAPK, MAP Kinase Kinase (MEK) activates MAP Kinase (Erk) via phosphorylation. The physical interaction between these kinases is enhanced by copper in a dosage-dependent manner. Mek1 has high-affinity binding sights for copper with conserved residues. Copper's involvement in the MAPK Pathway has been observed in multiple models, including human and murine cell lines, mice, and fruit flies. It is also an area of research in cancer biology. Embryos of the purple sea urchin (*Strongylocentrotus purpuratus*) undergo skeletogenesis via a complex MAPK-dependent signaling cascade, making them an enticing model for studying the role of copper as a MAPK regulator. Here we characterize copper's regulatory role in cell differentiation and proliferation using a novel model. We use the copper chelator BCS to produce low copper conditions, perturbing development and producing skeletogenic defects. We use ICP-OES to measure chelation efficacy. We use U0126, a small molecule Mek inhibitor, for comparison. We use Western Blotting to quantify copper depletion's effect on Erk phosphorylation. We use sequencing to compare the transcriptomes of BCS-treated embryos to those of controls and U0126-treated embryos.

Improvement of scRNA-seq analyses by extending gene annotation to 3' UTR using the Iso-Seq technology

Che-Yi Lin[1], Han-Ru Li[1], Mei-Yeh Jade Lu[2], Jr-Kai Yu[1,3], Yi-Hsien Su[1]

[1] Institute of Cellular and Organismic Biology, Academia Sinica, 11529 Taipei, Taiwan
[2] Biodiversity Research Center, Academia Sinica, 11529 Taipei, Taiwan
[3] Marine Research Station, Institute of Cellular and Organismic Biology, Academia Sinica, 26242 Yilan, Taiwan

Single-cell RNA sequencing (scRNA-seq) has revolutionized transcriptomic studies by enabling analysis at the single-cell level. The adoption of commercial droplet-based scRNA-seq technology, such as the Chromium Single Cell 3' solution, has allowed identifications of major cell states in model and atypical model organisms. Given that the 3' end sequencing approach introduces a bias towards the 3' end in gene body coverage, correct annotations at 3’ ends of genes are crutial for valid scRNA-seq analyses. Nevertheless, gene models of atypical model organisms are not always comprehensively annotated, especially in the 3’ untranslated region (UTR) of a gene. For genes lacking 3' end annotations, the reads would be interpretated as intergenic, compromising the accuracy when estimating gene expression levels. To overcome this limitation, we employed the long-read RNA sequencing technology (Iso-Seq) to improve gene models of *S. purpuratus* sea urchins. Our results demonstrate a significant reduction in the proportion of intergenic reads, with a decrease from 29% to 7.9% of the overall mapped reads when estimating read distributions using the modified gene models. Several indicators of quality control in the scRNA-seq analysis are also improved. Additionally, the number of differentially expressed genes detected is increased. Overall, we have established a pipeline that utilizes Iso-Seq technology to improve gene annotations in 3' ends. This pipeline allows for a more precise estimation of gene expression while preserving original gene IDs when analyzing scRNA-seq data. Moreover, this methodology can be effectively applied to other atypical model organisms, increasing the accuracy of scRNA-seq analyses.

Cell type comparisons between hemichordates and vertebrates

Lauren Lubeck, Paul Bump, Bo Wang, Christopher J. Lowe

Stanford University

In the past decade, single-cell RNA-sequencing opened the possibility of understanding the cellular diversity of a broad range of organisms. With the increasing number of single cell transcriptomic atlases in many animal phyla, comparing cell types has the potential to better understand the origins of such cellular diversity. To compare possible homologous cell types between hemichordates and vertebrates throughout development, we use SAMap, an algorithm to identify homologous cell types with shared expression programs between species. We use SAMap to map cell type atlases between two different phyla, hemichordates and chordates. We use a cell type atlas of an indirect developing hemichordate, *Schizocardium californicum,* which includes five different developmental timepoints. We map this to a single-cell transcriptomic atlas of zebrafish at 10 days post fertilization. Across phyla, SAMap revealed broad cell type homology between these species, including cell types that map between each of the phyla including muscle, immune, and nervous system cell types.

Exploring sea urchin larval immunity in response to microbial isolates behind black spot disease in *Lytechinus pictus*

Jenna K Luc and Catherine S Schrankel

San Diego State University

Black spot disease (BSD), urchin balding disease, and lesion syndrome are diseases that affect adult echinoderms worldwide. Infected adult urchins display a progression of symptoms, starting with a small lesion on the test, loss of spines, and degradation of skin tissues. In severe cases, lesions can permeate into the skeleton and cause death. Literature has been sparse in identifying the sources of this disease. A strain of *Vibrio* bacteria is strongly associated with BSD lesions in the adult test, however, it is not completely known if bacteria are the original source of the infection. To identify possible bacterial origins of BSD that are culturable in the lab, samples of water and lesions of increasing stages of necrosis were swabbed and plated on marine agar. Single colonies of bacteria were sequenced for 16S ribosomal DNA, where a total of 12 unique colonies were identified. Bacteria of the *Vibrio* genus are prominent in both water and lesion samples. Pilot exposure experiments on larvae indicate a high level of pathogenicity of the lesion-specific *Flavobacterium spp*., a known fish pathogen. It is interesting to note that some of these bacterial species are found in the urchin commensal microbiome, and the induction of dysbiosis by bacterial exposure has been shown to cause strong immune responses. Future work will test the ability of the bacterial isolates to initiate BSD phenotypes in adult urchins. This will be in tandem with a metagenomic approach to identify additional candidate disease-causing agents, including viruses and phage.

Cell division during animal reproduction in a changing ocean

Jamie MacKinnon and Zak Swartz

Marine Biological Laboratory, Eugene Bell Center for Regenerative Biology and Tissue Engineering, Woods Hole, MA 02543, USA

Animal reproduction depends on a fragile developmental window: the transition of oocytes (egg cells) from fertilization through embryonic cell division. This requires complex coordination of cellular events, and for marine eggs, it must occur in fluctuating environments. Ocean temperatures are forecasted to increase 1-4°C across the next century, which raises an important ecological question: will marine eggs and embryos be able to adapt? We are using the sea star, *Patiria miniata*, as a research organism suited to cellular and biophysical experiments on thermal tolerance during early development. Using live imaging, we find that a 3°C increase from the normal physiological temperature in *P. miniata* embryos causes incomplete cleavage at the two-cell stage. However, some embryos recover in the subsequent cell cycle through a plastic, multipolar division. We hypothesize that this failure to complete normal cell division is dependent on either faulty biochemical signaling during regulation of cytokinesis, or altered biophysical properties including cytoplasm viscosity, cortex stiffness, and force generation by the contractile ring. We are combining cell biological, biophysical, and evolutionary approaches using related organisms which are adapted to different temperatures to define the potential failure points for cell division. The results of these experiments will yield predictive measures for embryonic responses to climate change, and more broadly, for predicting survivability of ecologically critical invertebrates in a warming ocean.

Pigment cell development in late larval stages of the painted sea urchin, *Lytechinus pictus*

Megan E. Maloney, Tyler K. Smith and Katherine M. Buckley

Department of Biological Sciences, Auburn University, Auburn, AL 36849, USA

Marine invertebrates rely on sophisticated systems of cellular immunity as protection from rapidly-evolving microbial pathogens. Like most echinoderm species, in sea urchins, fertilized eggs develop indirectly through a feeding planktonic larval stage prior to metamorphosis into a long-lived, sessile adult form that exhibits distinct anatomy and physiology. Adult sea urchin immune responses are mediated by circulating cells known as coelomocytes. This includes one subtype – red spherule cells – that have been implicated in wound healing and responses to bacterial infection. Morphologically, red spherule cells resemble the pigment cells that are involved in the sea urchin larval immune response. Each of these highly granular cell types contains echinochrome A, which confers their characteristic red color and has been shown to exhibit antibacterial properties. However, little is known about the potential developmental relationship between these cell types. Furthermore, it is well-known that, in later stages, larvae contain many more pigment cells than early-gastrulae. quantity and replication of these cells as larvae metamorphose into adults. We therefore set out to quantify pigment cell formation and location during early development through metamorphosis to better understand the source of adult pigment cells.

Emx is required for normal skeletal patterning and gut development in *Lytechinus variegatus* embryos

Stanley Marjenberg[1], Alexandra T Lion[1,2], and Cynthia A. Bradham[1,2,3,4].

[1]Biology Department, [2]MCBB Program, [3]Bioinformatics Program, and [4]Biological Design Center Boston University, Boston, MA 02215, USA

Emx is a conserved transcription factor that is broadly expressed during embryonic development and that is required for the specification and development of a range of tissues. In *L. variegatus* embryos, Emx expression occurs between thickened vegetal plate stage though mesenchyme blastula stage. To define the functional role of Emx in *L. variegatus* development, we designed an LvEmx-specific morpholino. We used dose-response experiments to optimize the dose, then assessed the resulting phenotypes. The morphant embryos exhibit abnormal development in both the PMCs and skeleton as well as in the gut, implicating a role for Emx expression in the normal development of each of these tissues. Thus, our results show that Emx is required for normal development of the primary mesenchyme cells, likely via perturbation of ectodermally expressed patterning cues, and development of the endodermal cells, indicating that, like in other organisms including vertebrates, LvEmx operates during the specification and/or development of multiple germ layers.

Investigating tropomyosin isoform localization and function during sea star development.

Valeria F. Montenegro Vazquez[1], John H. Henson[2] and Charles B. Shuster[1]

[1]Department of Biology, New Mexico State University
[2]Department of Biology, Dickinson College

Tropomyosin (Tpm) is an actin-binding protein whose differential effects on actin dynamics and myosin contractility may help define critical cell shape changes in both muscle and nonmuscle cells. In vertebrates, four genes generate 28+ different splice forms, which display different effects on actin organization, diverse expression patterns and differing levels of functional redundancy. However, the isoform-specific roles of Tpm during cell division and early development are largely unknown. In the sea stars *Patiria miniata* and *Pisaster ochraceus*, there is a single Tpm gene that gives rise to five (3 long, 2 short) and nine (3 long, 6 short) isoforms, respectively. In *Patiria*, mRNA for both short isoforms (X4 and X5) was detected in oocytes and throughout early development, with long isoforms not detectable until the early larval stage (96 hr). Fluorescently tagged X3 (long) and X4 (short) isoforms localized to the cortex of oocytes and early embryonic blastomeres and became increasingly restricted to the apical cortex and adherent junctions. Interestingly, neither long nor short isoforms localized to contractile rings, but could be detected on the nuclear envelop just prior to NEB and to the mitotic spindle. Injection of morpholinos against X3-X5 suggest that oocyte maturation, fertilization and early cleavage were normal in morphants, but depletion of either long or short Tpm isoforms failed to form a normal midgut, which normally expands during late gastrulation. Current efforts are focused on further characterizing the spatio-temporal expression patterns of long and short isoforms and determining their function during early development and morphogenesis.

Echinoderm ovaries are awesome

Nathalie Oulhen, Shumpei Morita, Madison Francoeur, Cosmo Pieplow, Stephany Foster, and Gary Wessel

Providence Institute of Molecular Oogenesis (PRIMO), Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI 02906, USA

In contrast to women, echinoderm ovaries have the amazing ability to keep producing functional gametes throughout their lifespan. The histology and ultrastructure of echinoderm ovaries has been described in the past but how these ovaries function and maintain the production of these high-quality gametes is still a mystery. Here, we present the first single cell RNA seq datasets of two sea urchin species (*Strongylocentrotus purpuratus* and *Lytechinus variegatus*) and one sea star species (*Patiria miniata*). We find 14 cell states for both sea urchins, and 12 cell states in the sea star. Ovaries from the sea urchins have very similar cell states and profiles of transcript expression, whereas sea stars are distinct in many of their cell states and profiles. So far, we have identified diverse cell clusters in each species and we are now mapping these clusters to their location in the ovaries using in situ RNA hybridization. This resource is essential to understand the structure and the biology of the ovary in echinoderms, to identify the cell signaling essential for their functions, to solve the mystery of this infinite gamete production, and to characterize the changes that happened throughout evolution. We are currently interrogating key gene activities by Cas9-targeted gene knock-out and knock-in approaches and in dissociated cell cultures to test the function of each cell type identified.

Getting Meta*more*phosis for your money: Studying Tailed and Tailless Tunicate Metamorphosis to Identify Neurodegenerative Gene Dkk3 Form and Function

Sydney Popsuj and Alberto Stolfi

School of Biological Sciences, Georgia Institute of Technology, 30332 Atlanta, GA, USA

Understanding the genetic regulation of neurodegenerative events has important implications for human health. Marine invertebrates that undergo indirect development can provide insight into these processes. In most tunicates, metamorphosis involves widespread and coordinated cell death of differentiated larval neurons but not of the neural progenitors set aside for the adult phase. In contrast, there are also tunicates in the *Molgula* genus that have evolutionarily lost the tailed (swimming) larval phenotype and instead undergo precocious metamorphosis in favor of direct development via a tailless (non-swimming) larva. By comparing such tailed and tailless species, we can identify genes upregulated during larval development to identify candidate genes underlying this evolutionary change. This might pinpoint genes specifically involved in neural remodeling during metamorphosis. From such transcriptomic comparisons, we have identified Dkk3 as a uniquely upregulated gene in the tailless species that might be promoting cell cycle arrest, differentiation, and even apoptosis of larval neurons. Dkk3, a member of the Dickkopf family of Wnt inhibitors, is an understudied gene implicated in human neurodegeneration, neuroprotection, and even in neuron differentiation but its status as a Wnt and/or LRP receptor antagonist has been disputed. Here we present evidence that Dkk3 can suppress Wnt signaling in a pigment cell differentiation assay in the model tunicate *Ciona*. We also investigated its expression and regulation in multiple different larval neurons, hinting at diverse Wnt-dependent and Wnt-independent functions in different contexts.

Inducible CRISPRi technology to investigate rapid diversification of embryonic immune cell lineages in the green sea urchin, *Lytechinus variegatus*

Zachary Pracher[1], Carl J. Manner[1,2], Esther Miranda[1], and David McClay[1]

[1]Department of Biology, Duke University, Durham, NC
[2]Cell and Molecular Biology, Duke University, Durham, NC

The developmental gene regulatory network (GRN) of the green sea urchin (*Lytechinus variegatus*) has been extensively investigated to illuminate the genetic interactions underlying embryogenesis and regeneration. Traditionally, functionally characterizing new GRN elements requires target knockdown using morpholino antisense oligonucleotides, which are costly, take a long time to synthesize, and can sometimes induce toxicity, thus confounding experimental results. To address these obstacles, we created the FIRE-KRAB system for gene-specific, inducible, and reversible transcriptional repression in the *L. variegatus* embryo utilizing a catalytically dead Cas9 fused to an inducible dimerizing pair and a KRAB repressor domain. In this investigation, we deploy FIRE-KRAB to investigate the rapid diversification of the blastocoelar cell lineage into four functionally distinct subpopulations during gastrulation. Although the blastocoelar subpopulations have been previously identified, the molecular mechanisms and signaling interactions underlying their individual specification and diversification remain unknown. Taking advantage of previously collected single-cell RNA-seq data, we computationally identify novel marker genes for each blastocoelar cell subpopulation, which we subsequently confirm by *in situ* hybridization. By perturbing GRN elements with FIRE-KRAB and tracking blastocoelar cell supopulations with these new markers, we aim to deepen our understanding of the molecular signaling dynamics which diversify the blastocoelar cell fates within a window of only several hours in the early embryo. With these findings, we hope to contribute to our knowledge of how the early embryo can specify such diverse lineages in a short time span, and begin to assess whether these rapid diversification circuits are conserved across the tree of life.

Using Polychrome Labeling to Study Skeletal Patterning

Marko Radulovic[1,2,3,4], Abigail Descoteaux[4,]5, and Cynthia Bradham[1,4,5,6]

[1]Biology Department, [2]Trustee Scholars Program, [3]STEM Pathways Internship Program,
[4]Biological Design Center, [5]MCBB Program, [6]Bioinformatics Program
Boston University, Boston MA

The larval skeleton of the sea urchin *Lytechinus variegatus* is an ideal model for studying developmental patterning; however, our understanding of the etiology of the pattern in this model is limited by the lack of live-imaging approaches. To study the temporal dynamics of skeletal patterning, we optimized labeling protocols for calcium-binding fluorochromes and used nested pulse-chase experiments to reveal that the initiation of skeletogenesis begins around 15 hours post fertilization. We also demonstrate that triradiate formation is delayed and asynchronous in embryos ventralized via nickel or chlorate treatment. Finally, we compare the extent of fluorochrome incorporation in triple-labeled embryos to determine that skeletal elements elongate more slowly in embryos with VEGF signaling and that inhibition is also sufficient to induce abnormal orientation of the triradiates. Of the five fluorochromes tested, only alizarin red (AZ) significantly perturbed skeletal patterning. Embryos exposed to AZ have abnormal anterior patterning and skeletal element rotation. Immunostains reveal delayed migration and ectopic clusters of PMCs, while polychrome labeling confirms the delayed initiation of biomineralization and spurious elements. We also find mild dorsal-ventral 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.

Physical association of the microtubule-based cytokinetic signaling protein MKLP1 with the nascent contractile ring in sea urchin embryos

Gabriela Reyes[1,3], Nina T. Lo[2,3], Karina Herrera[2,3], John H. Henson[2,3] and Charles B. Shuster[1,3]

[1]Department of Biology, New Mexico State University, Las Cruces, NM 88003, USA
[2]Department of Biology, Dickinson College, Carlisle, PA 17013, USA
[3]Friday Harbor Laboratories, University of Washington, Friday Harbor, WA 98250, USA

In the early embryo, the cleavage plane is determined by an overlapping array of astral microtubules, which position a signaling apparatus that leads to localized Rho activation and contractile ring assembly. Centralspindlin, a complex of MKLP1 (mitotic kinesin-like protein) and a GTPase-activating protein (Cyk4) accumulates on interdigitating astral microtubule ends and recruits the RhoGEF Ect2, which in turn stimulates Rho, which is required for activating actomyosin contraction. 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. Examination of isolated cortices revealed that MKLP1 strongly colocalized with early MII-containing nodes, but as ring assembly matured, MKLP1/MyoII colocalization diminished. In cultured cells, a positive feedback loop exists between the microtubule-based signaling apparatus and the CR, and to explore this notion, cells were treated with either Latrunculin A or para-aminoblebbistatin at anaphase onset to block actin assembly or MII contractility, respectively. MyoII inhibition did not prevent node assembly or MKLP1 recruitment, suggesting that MKLP1 did not require local tension for association with the anillin/septin/MyoII. 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.

Which came first? Red, Green, or White

Gerardo Reyes, Rushane Dunn, and Gary Wessel

Providence Institute of Molecular Oogenesis (PRIMO), Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI 02906, USA

Pigmentation plays several roles in nature. In plants, pigmentation is necessary during photosynthesis to absorb light energy for carbon capture and biosynthesis into sugar. In animals, pigments are often used for courtships and other behaviors such as camouflage, mimicry, and warning coloration. In echinoderms, such as the sea urchin, *Lytechinus variegatus* (Lv), spine color variation is due to pigment diversity. Some genes responsible for this diversity, such as PKS and FMOs, are linked to innate immunity, microbial interactions, and microbial load of the animal. What is responsible for these color changes within the same sea urchin species? Moreover, how are color changes linked to immunity? PKS is an essential gene in sea urchin pigmentation, PKS knockout animals become albino. In addition, Lv PKS KO adults died early on, while FMO3 KO mutants lived as long as the control, up to three years. FMOs have been suggested to modify the polyketide produced by the PKS protein to generate the diverse color morph observed in echinoderms. However, how the different FMOs impact pigmentation in sea urchins is still unclear. My initial analysis in spine FMOs, PKS and their relationship to pigment variation suggest that each pigment seen in the Lv's spines can be distinguished by a type of FMO transcriptional expression and PKS mRNA abundance. Further analysis will be needed to understand the mechanism by which these molecules interact with each other to create pigment diversity and how impacts on their immunity role.

Characterization of mesenchymal photoreceptors in *Paracentrotus lividus* larvae

Maria Lorenza Rusciano, Maria Cocurullo, Periklis Paganos and Maria Ina Arnone

Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy

For most animals, light sensing is a crucial mechanism of interaction with the environment as it is essential to synchronize physiology and behavior with the habitat. The molecular basis for animal photoreceptor cells (PRCs) to sense light are membrane proteins belonging to the family of G-protein coupled receptors, called "opsins". Sea urchin larvae exhibit many photosensitive behaviors and express various opsins. A few years ago, an echinoderm-specific opsin was discovered, named Echinopsin A or Opsin 2, whose domain of expression and function remain largely unexplored. We combined immunohistochemistry, fluorescent and chromogenic in situ hybridization, and Hybridization Chain Reaction (HCR) to define the domain of expression of Opsin2 and characterize the Opsin2^{+} PRCs in the Mediterranean sea urchin *Paracentrotus lividus*. We found that, in the pluteus larva, the photoreceptor cells expressing Opsin2 are localized at the tip of the oral and post-oral arms. Strikingly, such PRCs do not belong to a neuronal-cell type population, but rather to a mesenchymal one, and precisely to the pigment cells. Pigment cells are a mesodermal-cell population, derived from the secondary mesenchyme, and synthesize a pigment called echinocrome. Further investigation is required to unravel the function of the Opsin2^{+} PRCs in the Mediterranean species.

Delineating the gene regulatory network that controls blastocoelar cell differentiation in *Strongylocentrotus purpuratus*

Tyler K. Smith, Amelia G. Williams and Katherine M. Buckley

Department of Biological Sciences, Auburn University, Auburn, AL 36849, USA

In sea urchin larvae, immune responses are mediated, in part, by a heterogeneous suite of cells known as blastocoelar cells. Blastocoelar cells differentiate from a set of 10 – 14 precursors within the oral mesoderm. These cells maintain a pluripotent state through the co-expression of three transcription factors: Gata1/2/3, Scl, and Erg. The differentiation process is initiated when Gata1/2/3 transcription is downregulated and the cells ingress into the blastocoel. The early specification of oral mesoderm as well as the morphology and behavior of the terminally differentiated cells have been well-described in the purple sea urchin (*Strongylocentrotus purpuratus*). However, the genetic program that underpins this differentiation process remains unknown. Here, we investigate the regulatory interactions that occur downstream of Gata1/2/3 with an emphasis on the transcription factor IRF4. *SpIRF4* is orthologous to vertebrate IRF4, which has a role in early B cell development. In sea urchins, SpIRF4 is expressed in a subset of blastocoelar cells that respond to injury. Using hybridization chain reaction (HCR), we show that IRF4 and Gata1/2/3 are co-expressed during mid-blastula stage within the oral mesoderm. Functional perturbation of Gata1/2/3 using morpholino antisense oligonucleotides (MASOs) reveals that Gata1/2/3 is required for *IRF4* expression. Notably, perturbed embryos also exhibited a defect in larval skeletogenesis. Future work includes identifying direct targets of Gata1/2/3 using RNA-Seq and single-cell sequencing strategies.

Drug transporter knockout and gut microbiome analysis in *Lytechinus pictus*: Building a model for commensal interactions and disease mechanisms

Lauren Stoeltje and Catherine S. Schrankel

San Diego State University

Increasingly recognized as a global health challenge, Inflammatory Bowel Disease (IBD), which includes Crohn's disease and ulcerative colitis, remains enigmatic in its underlying causes. Recent insights point to dysregulated immune responses against the intestinal microbiota in genetically susceptible individuals. The transporter ABCB1 (known as P-glycoprotein) has emerged as a pivotal player for proper immune modulation and maintenance of microbial balance in the gut. However, the exact mechanisms leading to heightened IBD pathogenesis in the absence of strong ABCB1 activity remain unclear, and are difficult to quickly test and validate in mammalian models. Utilizing an established ABCB1 knockout sea urchin line (*Lytechinus pictus*), we seek to build a model for testing the function of ABCB1 in the context of commensal maintenance. Imaging of early gastrula and larval stages revealed inflammation within the gut epithelia of ABCB1-KO larva, resembling an IBD phenotype. Ongoing 16S amplicon Next-Generation Sequencing across various larval stages up to metamorphosis will be used to assess differences in the relative abundance and composition of commensal communities between wild-type and ABCB1 knockout larvae. These findings underscore the conserved role of the ABCB1 transporter in gut health regulation and its interplay with the gut microbiota. Our ongoing comprehensive analysis of gut microbiota dynamics will provide a deeper understanding of these intricate interactions. Further exploration of these mechanisms could offer novel insights into IBD pathogenesis, potentially paving the way for innovative therapeutic strategies.

Dynamic evolution of the Nod-like receptor (NLR) gene family within echinoderms

Yu Sun and Katherine M. Buckley

Department of Biological Sciences, Auburn University, Auburn, AL 36849, USA

Nucleotide oligomerization domain (NOD)-like receptors (NLR) are broadly conserved across metazoan lineages. The proteins encoded by this gene family are involved in innate immunity and play key roles in resistance to pathogens and regulation of the immune response. The function of these proteins has been well-described within vertebrates, but is less well-understood in other systems. Echinoderms represent the second largest group of deuterostomes and include sea urchins and sand dollars, feather stars and sea lilies, sea stars, brittle stars and sea cucumbers. Increasingly available genomic and transcriptomic data are available from a growing number of echinoderm species. However, NLRs have not been systematically described in echinoderms. We annotated the NLR gene repertoires from 14 echinoderm genomes, analyzed the domain organization, phylogenetic distribution, and molecular evolution. Notably, many of the identified NLRs appear to be partial or pseudogenes. Results show different abundances and structural complexities among echinoderm lineages. These data will help to shed light on our understanding of evolutionary pressures on genes involved in animal immunity.

Formation, and reformation, of the anterior - posterior axis in halved sea urchins embryos.

Haruka Suzuki, Junko Yaguchi, Koki Tsuyuzaki, Shunsuke Yaguchi

Shimoda Marine Research Center, University of Tsukuba, Shimoda, Shizuoka, Japan

It is well known that some animal embryos can develop complete individuals even though the embryo is cut in half. This ability is called “regulative development”, and sea urchins have such marked ability as revealed by Driesch more than 100 years ago. Since that seminal discovery, the molecular mechanisms related to regulative development remains elusive. Our recent observations may help explain such mechanisms. Halved embryos of the Japanese sea urchin, *Hemicentrotus pulcherrimus,* go through unique shape changes in forming a blastula. When blastomeres are separated at the 2-cell stage, subsequent divisions result not in a small spherical embryo, but in a flat layer of dividing cells that only later in development round up and form a normal looking blastula. We performed *in situ* hybridization to visualize the anterior and posterior regions in these halved embryos and find that while the presumptive anterior region and posterior region was always found at opposite sides in control full-size embryos, in halved embryos the presumptive anterior and posterior regions were transiently adjacent each other. Subsequently, the anterior and posterior characteristics are located in oppositely regions as in control. This result strongly implies that the anterior-posterior axis is re-organized in halved sea urchin embryos. In this presentation, we will discuss how the anterior-posterior axis is modified in halved embryos and how it is related to signal pathways involved in the formation of the anterior-posterior axis in normal embryos.

Epigenetic changes shaped by early environmental conditions in *S. purpuratus*

Amy Tan[1], Katherine M. Buckley[2], Marie Strader[3]

[1]Department of Biology, Texas A&M University, College Station, TX
[2]Department of Biological Sciences, Auburn University, Auburn, AL

In invertebrates, the role of epigenetic changes on transcription and developmental plasticity are still poorly understood. Additionally, little is known about how and when epigenetic changes occur and the downstream effects of such changes. It is likely that multiple epigenetic marks may interact to have causal effects on gene expression and phenotypes. We examined the influence of early developmental environments on the epigenetics and development of the purple sea urchin (*Strongylocentrotus purpuratus*). Embryos were reared in four water conditions, factorial combinations of temperature (14ºC or 18ºC) and microbial content (sterilized artificial sea water or filtered adult urchin tank water) and pluteus larvae were sampled to examine changes in epigenetic state associated with immune phenotype. We expect that early developmental exposure to microbes may prime the immune system through epigenetic change, although high temperature may stress embryos and larvae to the point where immune function is compromised. Overall, this experiment addresses how much of the epigenetic landscape is environmentally influenced in *S. purpuratus* and how the environment and epigenome shape developmental phenotype and plasticity. With the potential for the marine environment to dramatically shift in the future, these results will reveal key mechanisms behind how a keystone invertebrate species may be able to acclimate to changing environments.

Motility is required for the pathogenicity of *Vibrio diazotrophicus* in purple sea urchin larvae

Jake F. Tatum and Katherine M. Buckley

Department of Biological Sciences, Auburn University, Auburn, AL 36849, USA

Like most echinoderms, the larval stage of the purple sea urchin (*Strongylocentrotus purpuratus*) live for several months in the microbe-rich ocean where they swim and feed. They also have a well-characterized complex cellular and transcriptional immune system. In response to exposure to the marine bacterium *Vibrio diazotrophicus,* larvae mount a robust inflammatory response that includes rapid upregulation of the cytokine interleukin 17 (IL-17) within the gut epithelium and the migration of pigment cells from the ectoderm to the gut. The goal of this study is to identify the mechanisms by which *V. diazotrophicus* elicits this larval immune response. To interfere with bacterial motility, *V. diazotrophicus* was treated with sub-lethal concentrations of azithromycin, which inhibits normal levels of flagellar subunit synthesis. To generate fully non-motile strains, *V. diazotrophicus* isolates were genetically modified to eliminate key flagellar proteins. Strains were used to infect *S. purpuratus* larvae (7 days post-fertilization). Results show that larvae exposed to non-motile *Vibrio* exhibit reduced pigment cell migration. To assess bacterial invasion, *in situ* hybridization was performed using probes specific for 16S srRNA. IL-17 transcript levels were quantified to compare larval responses of non-motile strains to control strains. Together, results indicate that motility is required for pathogenesis of *V. diazotrophicus* in sea urchin larvae. Future studies will define the precise mechanisms involved in this interaction.

Echinobase: a community resource for echinoderm research.

Cheryl A. Telmer[1], Macie Chess[1], Kamran Karimi[2], Sergei Agalakov[2], Bradley I. Arshinoff[2], Vaneet S. Lotay[2], Peter D. Vize[2], Charles A. Ettensohn[1], Veronica F. Hinman[1]

[1] Department of Biological Sciences, Carnegie Mellon University , Pittsburgh, PA, USA
[2] Department of Biological Sciences, University of Calgary, Calgary, AB, Canada

Echinoderms have been used as model organisms for developmental biology and gene regulatory network research for over 100 years. 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 over 38,000 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 with source and product specifications. Tabs beyond the summary gene page provide gene specific literature, transcripts, expression data, protein sequences and interactants. Automated literature collection has retrieved over 18,000 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 will be used to label functional genomic data for S. purpuratus and expanded for use with other species. To support the community, collections of data, protocols and other resources are shared using EchinoWiki and a Download site. To enable interdisciplinary collaborative studies, descriptions and contact information of community members are available and searchable.
Echinobase is funded by NICHD P41 HD095831.

Elucidating the function of Fascin Isoforms in early embryogenesis

Michael D. Testa, Carolyn Remsburg, Jia L. Song

Department of Biological Sciences, University of Delaware, Newark, DE 19716, USA

Originally discovered in the sea urchin, Fascin is an evolutionarily conserved actin-bundling protein that has been studied in the context of cell migration, focal adhesion, and cancer biology. However, its function in development remains unknown. Using the sea urchin embryo, we identified two *Fascin* isoforms that have distinct spatial and temporal expression patterns. *Fascin A* is expressed in secondary mesenchyme cells (SMCs) which give rise to pigment cells, immunocytes, coelomic pouch germ cells, and muscle cells. In contrast, in the early cleavage stage embryo, *Fascin B* has a cell-cycle dependent localization where it is localized perinuclearly in interphase cells and associates with the mitotic spindle in dividing cells. Based on their expression patterns, we hypothesize that Fascin isoforms regulate distinct developmental processes. Specifically, Fascin B modulates the cytoskeleton through its conserved actin and microtubule binding domains to ensure proper mitosis. To test the function of Fascin isoforms, we used morpholino antisense oligonucleotide (MASO) against both forms of *Fascin* to examine their loss-of-function phenotypes. Results indicate that Fascin depletion leads to a significant decrease of tubulin and F-actin that correlated with developmental arrest and embryonic lethality in the early cleavage stage. Later in development, Fascin depletion also resulted in skeletal and gastrulation defects. Interestingly, Fascin knockdown larvae display a significant depletion in mature pigment cells compared to control. Taken together, these results provide insight into the novel and likely evolutionarily conserved functions of Fascin isoforms in early embryogenesis. This work is funded by NSF MCB to JLS.

Ethanol exposure perturbs sea urchin development and disrupts developmental timing

Nahomie Rodríguez-Sastre[1], Nicholas Shapiro[1], Dakota Y. Hawkins[1], Alexandra T. Lion[1], Monique Peyreau[1], Andrea E. Correa[2], Kristin Dionne[2], Cynthia A. Bradham[1]

[1]Boston University, Boston, MA
[2]Universidad de Puerto Rico-Recinto Aguadilla, Puerto Rico

Ethanol is a known vertebrate teratogen that causes craniofacial defects as a component of fetal alcohol syndrome (FAS). Our results show that sea urchin embryos treated with ethanol similarly show broad skeletal patterning defects, potentially analogous to the defects associated with FAS. The sea urchin larval skeleton is made up of calcium carbonate that is secreted by the primary mesenchymal cells (PMCs), while PMCs positioning results from their reception of cues from the ectodermal cells. Perturbations in RA biosynthesis and Hh signaling pathways are thought to be causal for the FAS phenotype in vertebrates. Surprisingly, our results indicate that these pathways are not functionally relevant for the teratogenic effects of ethanol in developing sea urchins. We found that developmental morphology as well as the expression of some ectodermal and PMC genes was delayed by ethanol exposure. Temporal transcriptome analysis revealed significant impacts of ethanol on signaling and metabolic gene expression, and a disruption in the timing of GRN gene expression that includes both delayed and precocious gene expression throughout the specification network. We conclude that the skeletal patterning perturbations in ethanol-treated embryos likely arise from a loss of temporal synchrony within and between the instructive and responsive tissues.

Temperature influences immune larval cell development in *Strongylocentrotus purpuratus*

Emily Wilkins[1], Audrey Anderson[2], Katherine M Buckley[1], Marie Strader[3]

[1]Department of Biological Sciences, Auburn University, Auburn, AL 36849, USA
[2]Department of Biological Sciences, University of Nebraska, Lincoln, Lincoln, NE 68588, USA
[3]Department of Biological Sciences, Texas A&M University, College Station, TX 77843, USA

Anthropogenic climate change has increased the frequency and intensity of marine heatwaves that may have broad impacts on the health of marine invertebrates. As ocean temperatures continue to rise, studies suggest that disease prevalence also increases. Thus, it is critical to understand how marine heatwaves impact immune responses in marine invertebrates. The purple sea urchin *(Strongylocentrotus purpuratus)* is an ecologically important, broadcast-spawning, omnivore that primarily inhabits kelp forests in the eastern Pacific Ocean. The life cycle of this species includes a relatively long-lived (~2 months), planktonic larval stage in which larvae are free-swimming and feeding. Importantly, larvae also have a well-characterized cellular immune system that is mediated, in part, by a subset of mesodermally-derived cells known as pigment cells. To assess the role of environmental temperature on larval immune cell development, embryos were generated from adult sea urchins conditioned at 14˚ C and cultured in either ambient (14˚ C) or elevated (18˚ C) seawater to pluteus larval stage. Results indicate that plutei raised in elevated temperatures had 1.3 times more pigment cells and were 1.1 times larger compared to those raised in ambient conditions. Notably, significant variation was observed between genetic crosses reared in identical conditions, suggesting that genotype also plays an important role in structuring immune system development in different environments. Overall, these results suggest that adverse environmental conditions play a role in shaping the development of the larval immune system and may adversely affect survival long-term.

Analysis of the sea urchin larval response to bacterial challenge using single cell nuclei RNA-sequencing

Amelia G. Williams and Katherine M. Buckley

Department of Biological Sciences, Auburn University, Auburn, AL 36849, USA

As invertebrate deuterostomes, echinoderms occupy an important phylogenetic niche to understand the evolution of animal immunity. One of the best-characterized echinoderm model systems to understand immune response is the pluteus larval stage of the purple sea urchin (*Strongylocentrotus purpuratus*). These planktonic larvae are free-swimming and feeding, which allows for the potential introduction of pathogenic microbes through diet. In response to exposure to the marine bacterium *Vibrio diazotrophicus* challenge, *S. purpuratus* larvae differentially regulate a multitude of immune genes. To analyze the transcriptional changes at single-cell resolution, we have performed single cell nuclei RNA-Seq on larvae challenged with *V. diazotrophicus*. Extracting nuclei allows us to specifically quantify genes that are actively transcribed during this immune response. This work sheds light on how the system-wide larval immune response is coordinated and how specific cell types contribute to this response.