Advances and Challenges in Marine Cell Biology

Identification of Novel Egg Activation Circuits Revealed By Integrative Network Analysis of the Sea Urchin Egg Phosphoproteome, **HONGBO GUO[1], ANTHONY MORADA[2], ANDREW LUGOWSKI[1], ANA ELISA GARCIA-VEDRENNE[3], RUTH ISSERLIN[1], ANDREW EMILI[1], and KATHY FOLTZ[2]*** ([1]Donnelly Center for Cellular and Biomolecular Research, 160 College Street, University of Toronto, Toronto, ON, Canada M5S3E1; [2]Department of MCD Biology and Marine Science Institute, University of California Santa Barbara, Santa Barbara, CA 93106; [3]Department of EEM Biology, University of California Santa Barbara, Santa Barbara, CA 93106; kathy.foltz@lifesci.ucsb.edu).

At fertilization, an egg rapidly launches a coordinated series of metabolic, cytoskeletal and biosynthetic programs that initiate the egg-to-embryo transition. Studying the complex signaling circuitry that regulates these processes requires model systems that exhibit exquisite synchronicity of fertilization and are amenable to both single cell and biochemical analyses. As such, echinoderms are uniquely suited to probing the mechanism of egg activation. Relying primarily on post-translational modifications to maternal proteins, especially phosphorylation, the echinoderm egg establishes polyspermy barriers within seconds and commits to development within minutes. Using a high throughput phosphoproteomic enrichment platform, we have characterized over 4000 distinct protein phosphorylation events that occur in the *Strongylocentrotus purpuratus* egg at fertilization. The phosphorylation status of a subset of the proteins was carried out as validation of the approach. Computational integrative network analyses comparing the phosphoproteomes of unfertilized eggs and those at 2 and 5 min post-fertilization revealed many proteins and regulatory circuits that had not been implicated previously in egg activation, including those with similarity to mammalian immune cell activation. An index of these candidate proteins provides a road map for testing specific hypotheses about the mechanism of egg activation. Analyses of the phosphorylation status and experiments designed to test the function of specific proteins are in progress.

Comparative Genomes Emphasizing Echinoderms, **R. ANDREW CAMERON** (Division of Biology, California Institute of Technology, Pasadena, CA 91125; acameron@caltech.edu).

Echinoderms especially sea urchins and sea stars have long been excellent models for studies in cell and developmental biology. In this genomic era, they present new challenges for sequencing but also rich insights through comparative genomics into features conserved over deep evolutionary time. Not only do genome sequences reveal the entire gene catalog for an organism but they also expose the non-coding information that controls the way genes are regulated. The first echinoderm genome sequence assembly was that of the purple sea urchin. With the advent of next generation sequencing techniques two more genome assemblies have been completed and several more are rapidly becoming available. The time is now ripe to compare these genomes among themselves and with higher deuterostomes to identify conserved functions and structures. I will examine the extent and quality of the genome sequences being decoded. Then look at how comparative genomics has been used to understand how cellular and developmental processes work.

Life's Wonderful Solutions: Convergent Molecular Evolution Underlies Origins of Bioluminescence in Marine Animals, **TODD OAKLEY** (Department of Evolution Ecology and Marine Biology, University of California Santa Barbara, Santa Barbara, CA 93106; oakley@lifesci.ucsb.edu).

Despite contingency in life's history, the similarity of evolutionarily convergent traits like bird and bat wings may represent predictable solutions to common conditions. However, the extent to which the molecular changes underlying convergent traits are themselves convergent remains largely unexplored. I will discuss the convergent origins of bioluminescence and bioluminescent organs. In two different cases, convergent molecular changes underlie convergent phenotypic changes. I first argue that bioluminescent organs of two different squid are convergent, yet their overall levels of gene expression are predictably similar. I next argue that the enzymes responsible for catalyzing bioluminescence in a crustacean and a fish that eats them arose by co-opting similar genes convergently. These results point to widespread parallel changes in gene expression associated with convergent phenotypes. Therefore, nearly optimal and perhaps predictable solutions may drive not only the evolution of phenotypic traits, but also the evolution of overall gene expression levels that underlie those traits.

Dissecting Transporter Function in Sea Urchin Embryos, **AMRO HAMDOUN*, TUFAN GOKIRMAK**, **JOSEPH CAMPNALE, and LAUREN SHIPP** (Scripps Institution of Oceanography, University of California San Diego, La Jolla CA 92037; Hamdoun@ucsd.edu).

One quarter of the genome encodes membrane proteins, including ion channels, receptors and transporters. Despite extensive expression of these genes in development, understanding of their functions remains rudimentary. Here I review approaches and insights from our efforts to sort, localize and physiologically characterize ATP-binding cassette (ABC) efflux transporters expressed during early development of sea urchin embryos. I will present examples of the use of high-resolution, live-cell imaging of plasma membranes to map transporters to membrane physiological phenotypes and to determine their roles in control of cell motility. I will review how the results have revealed unanticipated functions for membrane transporters in intercellular signaling and tradeoffs between these signaling functions and the functions of transporters protection of the embryo.

Predicting Pollutant Transfer from the Oceans to Humans Using Biochemistry and Structural Biology, **SASCHA C. T. NICKLISCH[1]*, GEOFFREY CHANG[2] and AMRO HAMDOUN[1]** ([1]Marine Biology Research Division, Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92037-0202; [2]Skaggs School of Pharmacy and Pharmaceutical Sciences, Department of Pharmacology, School of Medicine, University of California San Diego, La Jolla, La Jolla, CA 92093-0657; ahamdoun@ucsd.edu).

Small toxic molecules, such as marine natural products and anthropogenic pollutants, can persist in the environment, bio-accumulate in tissue and then transfer through the food chain to humans. Many of these compounds have implications for human health and can be carcinogenic, endocrine disrupting, mutagenic and/or teratogenic. The physicochemical properties, such as vapor pressures, octanol- and air-water partition coefficients, that govern

their fate are well known, but less understood are their interactions with membrane proteins that govern chemical uptake and elimination. ATP Binding Cassette (ABC) multidrug resistance (MDR) transporters are present in cell membranes of all organisms and have been extensively studied in the context of rational pharmaceutical design. The overall goal of our work is to apply the same principles to understanding the molecular mechanisms by which natural and man made compounds move through marine cells. Using approaches from biochemistry and protein structural biology, we determined the extent to which there are predictable, evolutionarily-conserved patterns of molecular recognition of persistent organic pollutants (POPs) by MDR proteins. We determined the "real-world" levels of more than 320 POPs in muscle of 120 wild-caught yellowfin tuna (*Thunnus albacares*) worldwide and tested the most abundant compounds for pollutant/transporter interaction kinetics using purified tuna and mouse ABCB1 protein. The majority of the highly abundant pollutants found in tuna muscle tissue are inhibitors of both mouse and tuna ABCB1, including the flame retardant PBDE-209 (~4.1 μM) and the dielectric and coolant fluid PCB-145 (~4.4 μM). In addition, stereoisomer specific interaction kinetics for compounds (i.e. Dieldrin, 9.2 μM and Endrin, 0.9 μM) were conserved in mouse and tuna proteins, reaching IC_{50} values in the range of the pharmaceutical and model ABCB1 inhibitor cyclosporine A (~1.2 μM). The molecular mechanisms underlying this conserved interaction are the basis for a predictive understanding of small molecule bioaccumulation, and have implications for understanding the behavior of marine natural products and for rational design of low persistence industrial chemicals.

Sensing Acid/Base Conditions via the cAMP Pathway, **MARTIN TRESGUERRES** (Scripps Institution of Oceanography, University of California San Diego, 9500 Gilman Drive (0202), La Jolla, California, 92093, mtresguerres@ucsd.edu).

All organisms regularly experience variations in the levels of carbon dioxide, protons (~pH) and bicarbonate ions in their intra- and extra-cellular fluids. For example, feeding may induce blood alkalosis, exercising and environmental hypercapnia may induce acidosis, and photosynthesis and calcification may induce acidosis or alkalosis (depending on the fluid compartment considered). To maintain homeostasis, acid/base (A/B) stress must be readily compensated. Additionally, carbon dioxide, pH and bicarbonate levels are known to regulate many other biological functions not directly related to A/B regulation. Thus, all organisms must be able to sense A/B conditions. The cyclic AMP pathway can mediate multiple physiological responses through PKA-dependent phosphorylation, EPAC proteins, and channel gating. This presentation will discuss the potential roles of soluble adenylyl cyclase and transmembrane adenylyl cyclases in sensing carbon dioxide, pH and bicarbonate and triggering physiological responses in marine organisms.

Diversity in Germ Line Determination, **GARY WESSEL** (Department of Molecular and Cellular Biology, Brown University, Providence RI 02912; rhet@brown.edu).

A germ-cell lineage is formed during development of the embryo in most animals and eventually makes the eggs and sperm of the adult. This lineage is essential for reproduction, yet the mechanism of how these cells are initially formed is remarkably diverse amongst different animals. Some animals (flys, roundworms, frogs) determine their germ line by acquiring specially aggregated cytoplasmic factors in the early embryo that directs the resultant cell to a germ-line fate. Other animals (mice, primates) instead rely on cellular communication to induce a germ line fate. We are examining these cellular and molecular mechanisms in echinoderms (e.g. sea urchins, sea stars) to take advantage of the manipulations possible in these organisms, and in the context of significant developmental diversity. We find that sea urchins likely use an acquired mechanism to assign the germ-line fate to the four small micromeres that contribute to the germ line, whereas their sister group of sea stars do not have micromeres, and instead appear to use inductive mechanisms (cellular communication) later in development to accomplish the same task. These molecular mechanisms, and the evolutionary transitions that such animals undergo in forming the germ line, are informative to the process of stem cell formation, to the concept of germ line continuity, and to the evolutionary consequences in development for each mechanism in germ line formation.

Allorecognition in a Basal Chordate: A Simplified Model for Transplantation Tolerance, **ANTHONY W. DE TOMASO** (Department of MCD Biology and Marine Science Institute, University of California Santa Barbara, Santa Barbara, CA 93106; detomaso@lifesci.ucsb.edu).

The key event in adaptive immune function is the ability to discriminate between alleles of a highly polymorphic family of proteins called the MHC, by a diverse set of both adaptive and innate receptors. This process is often referred to as histocompatibility, and responsible for the rejection of transplanted tissues. While no orthologs of the MHC or cognate receptors exist in pre-vertebrates, the process of histocompatibility does, and there are well-studied system found throughout the metazoa, from the simplest (marine sponges) to humans. We are studying histocompatibility in the basal chordate, Botryllus schlosseri, and my talk will focus on recent results focused on dissecting this recognition event. Our data suggests that the core processes underlying the ability to discriminate between polymorphic ligands does not reside in the nature of the interacting molecules, but rather the ability of the cell to integrate multiple signaling events from the cell surface and make a decision on a response. These pathways are highly conserved throughout the metazoa, and integration underlies clinically important immune processes such as tolerance and education, of which we know little. Importantly, Botryllus is a simplified model to study these processes. Allorecognition occurs on an epithelial layer of a macroscopic extracorporeal vasculature which can easily manipulated; and to date only two inputs (one activating and one inhibitory) to this process have been shown. Current results will be discussed.