Molecular Reproduction and Development

How Sperm Learn to be Fertile, **KEITH SUTTON, MELISSA JUNGNICKEL, and HARVEY FLORMAN*** (Department of Cell and Developmental Biology, University of Massachusetts Medical Center, Worcester, MA 01655; Harvey.Florman@umassmed.edu).

No abstract was submitted.

Time to Make New Skin: Periodic Stem Cell Activation and Matrix Renovation in C. elegans, **ALISON FRAND** (Department of Biological Chemistry, School of Medicine, University of California, Los Angeles, 615 Charles Young Drive, Los Angeles, CA 90035; afrand@mednet.ucla.edu).

Nematode larvae shed and rebuild external skeletons (cuticles) through the process of molting. Their cuticles are composed mainly of collagens, which are secreted by underlying tissues similar to mammalian epithelia. The activation of skin stem cells is coupled to progression of the molting cycle, but the molecular mechanisms that orchestrate these two processes are not well understood. Related processes in humans are needed for homeostasis and repair of the body, but deregulated in various carcinomas and common disorders of skin and connective tissue.

This talk will describe recent studies of the fibrillin-like *fbn-1* gene, which emerged from a genetic screen for larvae unable to shed cuticles. The gene encodes several glycoproteins composed of tandem epidermal growth factor-like motifs, 2 integrin-binding sites, and a Zona Pellucida polymerization domain. Genetic and molecular biological analyses indicate that extracellular FBN-1 macromolecules are key components of apical matrices, including the epidermal stem cell niche. Further, FBN-1 macromolecules may confer resistance to mechanical forces at precise times in development, partly by interacting with integrins. Our findings reveal a significant role for biomechanical forces in completion of the molts, morphogenesis of the skin, and maintenance of body wall muscles. We propose that checkpoints in the stem cell cycle and the molting cycle survey and respond to the exchange of mechanical forces among epithelial cells and matrices.

Adaptive Co-evolution of Interacting Sperm-Egg Reproductive Proteins, **WILLIE J. SWANSON** (Department of Genome Sciences, University of Washington, Seattle, WA 98195; wjs18@uw.edu).

The identification of interacting male-female reproductive proteins is imperative for a molecular understanding of fertilization. Generally, the molecular descriptions have relied upon studies of male reproductive proteins. I will discuss characterizing interacting male-female reproductive proteins in abalone, a system with for which many of the molecular details of fertilization are well established and one that serves as a model for the study of reproductive molecules and evolution of species-specific fertilization. I will focus on evidence of direct functional interactions between sperm and egg molecules using our integrative approach, utilizing genomic, proteomic, biochemical, and computational methods. The functional specificity sperm–egg interaction will be linked to potential role in reproductive isolation (speciation). Our understanding of the molecular interactions between sperm and egg and the evolutionary dynamics of genes encoding sperm/egg proteins contribute to a broader understanding of the function and evolution of reproductive genes.

Activation and Regulation of Insect Sperm Motility, **CATHERINE D. THALER[1]*, HARUHIKO MIYATA[1,2], LEAH T. HAIMO[1], and RICHARD A. CARDULLO[1]** ([1]Department of Biology, University of California, Riverside, 900 University Ave., Riverside, CA, 92521; [2]Animal Resource Center for Infectious Diseases, Immunology Frontier Research Center, Osaka University, 3-1 Yamadaoka Suita, Osaka 565-0871, Japan; cathyt@ucr.edu).

Many insects produce sperm that have accessory fibers and microtubules surrounding the axoneme of the flagellum and these sperm reportedly undergo unusual motility behaviors. Sperm from the mosquito *Culex quinquefasciatus* initiate flagellar motility after mixing with male accessory gland components, and the sperm flagellum displays double wave (two superimposed waves propagate along the flagellum) and single helical wave forward motility patterns. Forward motility requires extracellular Ca^{2+} and the transition from the double to single wave is controlled by MAPK, since both MEK and ERK inhibitors block formation of the helical wave and sperm exhibit only the double wave. A MAPK substrate antibody stains the flagellum of accessory gland activated sperm and staining is most intense in the flagellum proximal to the head. In the absence of Ca^{2+} and the presence of a phosphatase inhibitor, sperm activate motility but swim backwards, suggesting that kinase activity is sufficient to generate a waveform and Ca^{2+} controls the direction of motility.

Trypsin is able to activate mosquito sperm motility without accessory gland components, suggesting that a trypsin-like protein is the endogenous activator. Sperm from the water strider *Aquarius remigis* are also trypsin activated and possess a PAR2-like trypsin receptor by antibody crossreactivity. Preliminary assays using the trypsin substrate BAEE suggest that water strider ejaculatory ducts contain a trypsin-like protease and we will examine the mosquito accessory glands for protease activity also. Protease activation of sperm is reported across many orders of insects, suggesting that this is an evolutionarily conserved mechanism of motility activation.

Elucidating the Molecular Mechanisms behind Female Choice, **KELLY KWAN, YIDING JIA, PETER CHANG, BRENT YOUNG and MATTHEW D. DEAN*** (Molecular and Computational Biology, University of Southern California, 304A Ray R. Irani Building, 1050 Childs Way, Los Angeles, CA 90089; matthew.dean@usc.edu).

Female choice, the ability of a female to bias paternity in favor of certain males, is a strong evolutionary force that has probably played an important role in shaping molecular evolution of reproductive genes. Although female choice can be demonstrated experimentally, its molecular basis remains poorly characterized. To begin dissecting the biochemical pathways that females deploy in response to mating, we compared whole genome RNA-seq data from four recently mated versus two unmated (but estrus) female brain and uterus tissues, using mice as an experimental model. The brain transmits important endocrine signals necessary for successful implantation of fertilized oocytes. From over 15,000 genes detected, we found 50 that were strongly were differentially regulated in response to mating in female brains and may represent the molecular basis for female choice. One of these genes, *prolactin releasing hormone receptor*, is responsible for priming a female's uterus for implantation. We discuss ongoing experiments testing whether males vary in their ability to induce upregulation of such genes.

Human Stem Cells from Single Blastomeres Reveal Pathways of Embryonic or Trophoblast Fate Specification, **TAMARA ZDRAVKOVIC[1], OLGA GENBACEV[1], LOUISE LAURENT[2], JEANNE LORING[2], and SUSAN FISHER[1]*** ([1]Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California San Francisco, 35 Medical Center Way, San Francisco, CA 94143; [2]Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA 92037; sfisher@cgl.ucsf.edu).

There are major mechanistic differences among species in how initial cell fate decisions are made in embryos. To gain insights into lineage allocation in humans, we derived ten human embryonic stem cell lines from single blastomeres of four 8-cell embryos and one 12-cell embryo from a single couple (UCSFB1-10). Compared to lines from blastocysts, they exhibited unique gene expression patterns and significant DNA hypomethylation. At a transcriptional level, UCSFB lines from different embryos were often more closely related than those from the same embryo. As predicted by the transcriptomic data, immunolocalization of Eomes and T showed differential expression among blastomeres of 8-12-cell human embryos. The UCSFB lines formed derivatives of the three germ layers and CDX2-positive progeny from which we derived the first human trophoblast stem cell line. Thus, the UCSFB lines mirror heterogeneity among early-stage blastomeres and have unique properties, suggesting a more immature state than lines derived from blastocysts.

Master Regulators of Early Lineage Formation in Mammalian Embryos, **JASON KNOTT** (Department of Animal Science, Michigan State University, East Lansing, MI, 48824; knottj@msu.edu).

The causes of preimplantation embryo failure and early miscarriage in women who have undergone assisted reproductive technologies (ART) are poorly understood. Research in the Knott laboratory is focused on elucidating the fundamental transcriptional and epigenetic mechanisms that facilitate preimplantation development and lineage formation in blastocysts. This talk will focus on a key regulator of transcription that controls blastocyst formation in mice.

Developmental Regulation of Heparan Sulfate Proteoglycan Synthesis, **LINE HOFMANN, DOUGLAS BORNEMANN and RAHUL WARRIOR*** (Department of Developmental and Cell Biology, 4219 McGaugh Hall, University of California, Irvine, Irvine, CA 92697-2700; rwarrior@uci.edu).

Heparan Sulfate Proteoglycans (HSPGs) are extracellular matrix and cell surface macromolecules found on virtually every cell type in all multicellular animals. They consist of a protein core covalently attached to long, unbranched glycosaminoglycan (GAG) sugar chains that are extensively modified. HSPGs play key roles in development and disease by affecting the transport, stability and signaling activity of multiple growth factors/morphogens. While HSPGs were believed to be expressed ubiquitously in metazoans, we have found that HSPGs are absent from early Drosophila embryos due to inhibited translation of GAG synthetic enzyme mRNAs. Transcripts for the Drosophila GAG chain co-polymerase Tout velu (Ttv; EXT1 in vertebrates) and the N-deacetylase/sulfotransferase (NDST) Sulfateless (Sfl) contain Internal Ribosome Entry Sites (IRESs) that are negatively regulated during the first three hours of development. Relief of this inhibition leads to expression of the proteins and HSPG chain synthesis. Homologous transcripts in other species have similarly complex 5'UTRs, and we find that human EXT1 can also mediate temporally regulated translation in the early fly embryo. This exciting finding argues that these regulatory mechanisms may be evolutionarily conserved, and that *cis*-elements and *trans*-acting factors identified in Drosophila could be relevant to understanding the basis of the human diseases Multiple Hereditary Exostoses that result from loss of a single copy of the human EXT1 or EXT2 genes.

A Role for Dynein in Germline Stem Cell Maintenance in C. elegans, **XIAOBO WANG[1], EKATERINA VORONINA[1]*, DOMINIQUE RASOLOSON[2], and MARIAH MALEY[1]** ([1]Division of Biological Sciences, University of Montana, 32 Campus Dr., Missoula, MT 59812; [2]Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine/HHMI, 725 N. Wolfe Street, Baltimore, MD, 21205; ekaterina.voronina@umontana.edu).

Germline stem cell maintenance in *C. elegans* depends on the activity of PUF domain RNA-binding proteins FBF-1 and FBF-2, which are similar but functionally distinct. Localization and function of FBF-2, but not FBF-1, depends on the integrity of P granules, perinuclear RNA granules of germ cells. Using a combination of mass-spectrometry and genetic screens, we identified a dynein component DLC-1 as a functional cofactor of FBF-2, but not FBF-1. DLC-1 is an LC8-type light chain, a cargo-binding component of dynein motor complex. Dynein traffics organelles, proteins, and mRNAs toward the minus ends of microtubules. Knockdown of DLC-1 by RNAi in the sensitized background leads to loss of repression of FBF-2 target reporter in the mitotic stem cell region and animal sterility. Localization of FBF-2 to P granules at nuclear periphery is lost after *dlc-1(RNAi)*, even when perinuclear P granules are not affected. By contrast, FBF-1 localization is not affected by DLC-1 knock-down. In vitro, DLC-1 interacts with FBF-2, but not FBF-1, suggesting that the specific contribution of DLC-1 to FBF-2 function is based on the selective protein-protein interaction.

We hypothesize that DLC-1 binds to FBF-2 and promotes its localization to P granules, which is important for FBF-2-mediated translational regulation and stem cell maintenance. Dynein motor complex has been implicated in formation, transport, and dynamics of RNA granules in other cell types. Our findings suggest that dynein may directly regulate recruitment of specific components to the RNA granules in addition to regulating RNA granule dynamics in a general way.

Developmental Robustness in the C. elegans Embryo, **MORRIS MADURO[1]*, HAILEY CHOI[1,2], CASSANDRA BENNETT[1], FRANCISCO CARRANZA[1], FARHAD GHAMSARI[1], GINA BROITMAN-MADURO[1], and GURJOT WALIA[1]** ([1]Department of Biology and [2]Graduate Program in Cell, Molecular and Developmental Biology, University of California, Riverside, Riverside, CA 92521; mmaduro@ucr.edu).

The 20-cell intestine of juvenile *C. elegans* nematodes is derived from a single cell, the embryonic blastomere E. The paradigm for E specification is that a cascade of transcription events, started by the maternal factor SKN-1, causes transient activation of the gut specification factors *end-1* and *end-3*. These reach a threshold of expression to activate *elt-2*, which maintains its expression

by autoregulation and drives the commitment to gut differentiation. We have created strains in which *end-1* and/or *end-3* are mutated for particular *cis*-regulatory sites. In such strains, embryos make variable numbers of apparently normal-sized gut cells, suggesting that specification has become subject to stochastic variation, and that commitment to a gut fate can occur later in the E lineage. Counting of embryonic *elt-2* transcripts by single-molecule FISH suggests that activation of *elt-2* is more graded in these strains, as opposed to an all-or-none mode as was previously reported for SKN-1-depleted embryos (Raj et al., 2010). As these effects are confined to the E lineage due to the nature of the strains constructed, we are also able to evaluate adults derived from embryos in which functional guts were made. We find that such adults store lipids at significantly higher levels and display other variable pleiotropic phenotypes, suggestive of primary defects in gut function. Together, these results build a picture in which specification of gut is not an all-or-none event, and that in animals that do make an intestine, the endoderm differentiation network is not fully self-correcting for partially compromised specification.

Deciphering the Role of EMP2 in Trophoblast Invasion and Placental Vascular Remodeling, **MADHURI WADHERA[1]*, CARMEN J. WILLIAMS[2], WENDY N. JEFFERSON[2], DEEPTHI SUDHAKAR[2], ELIZABETH PADILLA-BANKS[1], and NEVIL KHURANA[1]** ([1]Department of Pathology and Laboratory Medicine, 4525 MacDonald Research Laboratories, Geffen School of Medicine at UCLA, Los Angeles, CA 90095; [2]Reproductive Medicine Group, Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709 USA; mwadehra@mednet.ucla.edu).

In pregnancy, it has been shown that the interaction between uterine natural killer (uNK) cells, the dominant leukocytes in early human and mouse decidua, and trophoblasts have essential roles in the initiation of decidual spiral arterial modification as well as in regulating trophoblast invasion. One novel protein that may play an important role in this cross-talk is the tetraspan protein epithelial membrane protein-2 (EMP2). EMP2 has high expression in human cytotrophoblasts as well as interstitial trophoblasts, and it is expressed in all subsets of murine trophoblasts. Previous studies have shown that EMP2 has an important role in receptor-mediated cellular behavior, and it is believed to curate molecules at the cell surface that are brought into engagement with specific signaling complexes. In human trophoblasts, EMP2 physically associates with and regulates the activity of integrin-FAK signaling complexes. This coalescing of molecules at the surface has a number of consequences including an increase in trophoblast invasion as well as a concomitant release of stimulatory angiogenic factors and subsequent vascular remodeling. Consistent with this finding, preliminary studies analyzing the expression of EMP2 in pregnancy associated disorders show a marked reduction in protein levels in IUGR placentas. Pathological processes such as IUGR and preeclampsia are believed to be the ultimate manifestations of a disease process which can begin in trophoblasts or in the regulation of uNK cells. While EMP2 knockout mice are able to produce viable offspring, these mice reveal a novel regulation of uNK cell function influenced by EMP2 in the trophoblast.

Germline Development in the Colonial Ascidian, Botryllus schlosseri, **ADAM LANGENBACHER, ALESSANDRO DE MAIO, DELANY RODRIGUEZ, SUSANNAH KASSMER, and 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).

Botryllus schlosseri is a colonial ascidian that undergoes a 14-day budding cycle during which all somatic and germline tissues are regenerated, a process called blastogenesis. We are studying the source and mechanisms of germline regeneration during blastogenesis, and have found that long-lived germline stem cells (GSCs) are specified during embryogenesis and contribute to germline formation each week for the life of a genotype (6 mo- >2 yrs). However, following metamorphosis, colonies undergo 6-12 budding cycles prior to the appearance of gonads, and in addition adults often cycle between fertile and infertile states in both laboratory and natural populations. We are prospectively isolating these cells using FACS and have defined an enriched population based on cell surface phenotype and gene expression profiles. GSCs isolated from juvenile or non-fertile colonies can contribute to germline formation immediately following transplant to a sexually mature recipient, suggesting that GSCs are always present, and fertility is based on a colony-wide cue which causes these precursors to differentiate into mature gonads. To characterize these cues, we have carried out mRNA-seq on each stage of blastogenesis, identifying a number of differentially expressed genes. One of these is a TGF-b family member that is expressed in cells associated with GSCs in both fertile and infertile colonies, and also during early development of both testes and eggs. TGF-b expression may define a population of cells that act as a mobile niche, and have allowed us to characterize the dynamics of GSC migration during blastogenesis. Current results will be discussed.

Neonatal Estrogen Exposure Alters Global Epigenetic Marks in the Female Reproductive Tract, **CARMEN J WILLIAMS*, WENDY N. JEFFERSON, ELIZABETH PADILLA-BANKS, H. KARIMI KINYAMU, TIANYUAN WANG, and WEICHUN HUANG** (National Institute of Environmental Health Sciences, PO Box 12233, MD E4-05, Research Triangle Park, NC 27709; williamsc5@niehs.nih.gov).

Neonatal exposure to the phytoestrogen genistein or diethylstilbestrol (DES) results in abnormal reproductive tract morphology, female infertility and uterine cancer in mice. These treatments also cause altered gene expression in the female reproductive tract that persists into adulthood. The permanent upregulation of sine oculis-related homeobox 1 homolog (*Six1*) is of particular interest because this important developmental gene is upregulated in numerous cancers. The mechanisms underlying *Six1* upregulation were explored by performing chromatin immunoprecitation (ChIP) analysis to determine whether specific modified histones were associated with regulatory genomic regions of *Six1*. Mice were treated with genistein or DES on neonatal days 1-5 and uterine tissues were collected either on day 5, on day 22 (prepubertal), or in adulthood following ovariectomy. ChIP was then performed using antibodies against modified histones. Acetylation of histone H3 at lysine 9 (H3K9Acet) and methylation of histone H3 at lysine 4 (H3K4me3), both generally associated with active transcription, were increased at several regions of *Six1* on day 5 in treated female mice, and some of these histone marks were permanently altered in adult mice. These findings indicated that epigenetic modifications likely play a role in the permanent upregulation of *Six1* expression. ChIP-seq analyses of DES-treated and control mice on

day 5 and day 22 also revealed global changes in histone H3 lysine 27 methylation and H3K4me3 marks. These findings indicate that neonatal estrogenic chemical exposure permanently alters the epigenetic landscape of the adult uterus.

Molecular Determinants of Oocyte Competence, **GEORGE W. SMITH (**Laboratory of Mammalian Reproductive Biology and Genomics, Departments of Animal Science and Physiology, Michigan State University, East Lansing, MI 48824; 517-432-5401 smithge7@msu.edu).

Oocyte developmental competence is a limiting factor in pregnancy success in livestock species and humans, but inherent phenotypic characteristics of competent oocytes are not well understood. Oocytes gradually and sequentially acquire developmental competence during folliculogenesis by synthesizing and accumulating transcripts and proteins critical for successful meiotic maturation, fertilization, and early embryogenesis. We have conducted fundamental studies using the bovine model to elucidate differences in oocyte transcriptome associated with poor oocyte competence and the functional significance and therapeutic utility of such results. One finding was a positive association of follistatin mRNA abundance with oocyte competence in two distinct bovine models. Follistatin treatment of bovine embryos during initial stages of in vitro culture increased proportion of embryos developing to the blastocyst stage and numbers of blastocyst trophectoderm cells. Comparative studies in the rhesus monkey model demonstrated stimulatory actions of exogenous follistatin on rates of blastocyst development and support potential clinical relevance of results in the bovine model. Complementary loss of function studies in early embryos established a functional role for follistatin in bovine blastocyst development and cell allocation. Current studies are focused on understanding the mechanism of action of follistatin in mediating it's above described embryotropic actions and impact of follistatin treatment during embryo culture on pregnancy rates following embryo transfer. Such studies are critical to understanding the functional significance of follistatin to early embryos, and translation to improvements in clinical and applied reproductive technologies.

The Terra Incognita of Male Fertility: Flagellar Ion Channels and Their Function, **POLINA V. LISHKO*, MELISSA MILLER, STEVEN MANSELL, and SARA S. A. CHOO** (Department of Molecular and Cell Biology, Life Sciences Addition 221A, University of California, Berkeley, CA, USA 94720; Lishko@berkeley.edu).

On its route to the egg mammalian spermatozoa encounter multiple barriers: viscous mucus, the narrow lumen of the uterotubal junction, the sticky and complex maze formed by the epithelial folds of the Fallopian tubes, and finally the protective shields of the egg. In order to overcome these numerous barriers, the sperm cell must sense the cues released by the egg and change its swimming behavior. Sperm can achieve this by increasing the amplitude and driving force of their tail beating, changing their direction of movement, and releasing special enzymes to dissolve the egg's protective vestments. Such sperm responses depend upon electrical activity of the sperm ion channels that open in response to environmental cues within the female reproductive tract. This in turns changes conductance of the sperm plasma membrane and sperm behavior. In addition, ion channels are organized in clusters and are positioned on the flagellum to provide fine-tuned regulation of its motility. It is possible that many cases of idiopathic male infertility can be attributed to malfunctioning of sperm ion channels. Infertility affects ~ 4 million men in the United States alone, and the cause of male infertility can be diagnosed and treated in less than 20% of those cases. Here we present electrophysiological and pharmacological characterization of three main ion channels of human sperm: the proton channel Hv1, the potassium channel KSper and the calcium channel CatSper. We will also discuss how these channels work in a concerted manner and what are their differences and similarity between sperm of different species.

Molecular Pathways Involved in Oocyte Developmental Competence, **MARCO CONTI*, FEDERICA FRANCIOSI, HAKAN CAKMAK, and SHILA MANANDHAR** (Center for Reproductive Sciences, University of California, San Francisco, 513 Parnassus Avenue, HSW1656, Box 0556, San Francisco CA 94143-0556; contim@obgyn.ucsf.edu).

In mammals, profound changes in both somatic and germ cell compartments of the ovarian follicle are induced during the periovulatory period. The cells of the somatic compartment prepare for ovulation by acquiring new endocrine and paracrine functions. At the same time, the oocyte undergoes nuclear and cytoplasmic maturation. Whereas the events associated with nuclear maturation are well described and quantifiable, little is known about the molecular changes associated with oocyte cytoplasmic maturation. This transition is essential for the oocyte competence to develop as an embryo upon fertilization, and this developmental competence is critical for successful assisted reproduction technologies. Here we have tested the hypothesis that developmental competence is acquired through a program of mRNA translation executed during oocyte maturation. Polysome-array analysis of oocytes at different stages of maturation revealed a highly reproducible pattern of mRNA association and dissociation with the translation machinery in synchrony with the different stages of meiosis. Although qualitatively this program is executed when oocytes are no longer in contact with the surrounding somatic cells, quantitative analysis shows that somatic inputs are necessary for optimal translation of key oocyte transcripts. Mouse models of compromised developmental competence also show defects in the oocyte translation program. Developmental competence and enhanced translation require activation of the AKT/mTOR pathway in the gamete. Thus, these findings demonstrate that this translation program is required for oocyte cytoplasmic maturation and provide a novel and quantifiable assessment of developmental competence.

Cracking the Eggshell: Assembly of Protective Barriers Following Fertilization of the C. elegans Embryo, **SARA K. OLSON** (Department of Biology, Pomona College, 175 West 6th Street, Claremont, CA 91711; sara.olson@pomona.edu).

In metazoans, fertilization triggers the assembly of an extracellular coat that protects the embryo from environmental dangers. In nematodes, this coat is the eggshell, which provides mechanical rigidity, prevents polyspermy, and is impermeable to small molecules. Using electron and fluorescence microscopy approaches, we recently demonstrated that the multi-layered eggshell is assembled in a step-wise and hierarchical manner that requires chondroitin proteoglycans and glycolipids. A subsequent RNAi screen identified several new proteins required for eggshell formation, and our current work investigates the role of these proteins in cellular processes as diverse as cortical granule trafficking, glycolipid

biosynthesis, and scaffolding of the extracellular matrix. A better understanding of nematode eggshell assembly not only provides insight into fertilization and the formation of protective barriers, but may also allow the identification of new therapeutic targets to combat parasitic nematode infections that impact more than 30% of the world's population.

How Attractive is the Fish Egg's Micropyle? **GARY N. CHERR[1]* and RYUZO YANAGIMACHI[1,2]** ([1]University of California Davis Bodega Marine Laboratory, PO Box 247, Bodega Bay, CA 94923; gncherr@ucdavis.edu; [2] Institute for Biogenesis Research, Department of Anatomy, Physiology, and Biochemistry, University of Hawaii Medical School, Honolulu, HI; yana@hawaii.edu).

Most teleost fish sperm initiate motility upon dilution in water and the mechanisms and duration of this activation differ depending on the salinity of the spawning environment. An exception to this is the Pacific herring (*Clupea pallasi*) where sperm are immotile upon dilution in water across a wide range of salinities. In herring, a chorion glycoprotein surrounding the micropyle region initiates sperm motility and is required for fertilization. An increase in intracellular Ca^{++} occurs in sperm from both freshwater and marine fish at motility initiation and this is also the case with herring sperm. Continued increases in intracellular Ca^{++} in fish sperm are known to result in asymmetric flagellar bending and may be involved in directed motility at the micropyle opening.

While there has been no evidence of sperm attraction to the micropyle at a distance, we have recently shown that once sperm are near the outer opening of the micropyle, they exhibit directed movement towards it. Selective staining of the micropylar region of the egg can be detected using protein and carbohydrate probes in eggs from a variety of fish, and sperm attraction to the micropyle can be removed by protease treatment suggesting that a glycoprotein may be responsible. Attraction to the opening of the micropyle appears to be species specific, is dependent on extracellular Ca^{++}, and induces increases in sperm intracellular Ca^{++} and directed motility for increasing fertilization efficiency. Insect egg micropyles also show a distinct staining of the region surrounding the opening, suggesting a there may be similar sperm attraction function.

Converging Calcium Waves Occur as Drosophila Oocytes Activate, **TARO KANEUCHI[1], CAROLINE V. SARTAIN[2], SATOMI TAKEO[3], VANESSA L. HORNER[2], TOSHIRO AIGAKI[1], and MARIANA F. WOLFNER[2]*** ([1]Department of Biological Sciences, Tokyo Metropolitan University, Tokyo 192-0397, Japan; [2]Department of Molecular Biology and Genetics, Cornell University, Ithaca NY 14853, USA; [3]Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki 305-8572, Japan; mfw5@cornell.edu).

Egg activation is the essential process by which a mature oocyte becomes capable of supporting embryo development. In vertebrates and most invertebrates, activation is induced by fertilization. Molecules introduced into, or turned on by, the fertilizing sperm trigger progressive release of intracellular calcium stores in the oocyte. The resultant calcium wave(s) that spread through the oocyte induce completion of meiosis, new macromolecular synthesis, and modification of the vitelline envelope to prevent polyspermy. However, arthropod eggs activate without fertilization: in the insects that have been examined, egg activation occurs as the oocyte moves through the female's reproductive tract. Here, we show that a calcium wave is, nevertheless, characteristic of egg activation in Drosophila. This wave is caused by influx of calcium from the external environment and is induced as the egg is ovulated. Pressure on the oocyte (or swelling by the oocyte) can induce this wave through the action of mechanosensitive ion channels. Interestingly, the wave initiates at both poles of the oocyte and moves towards the center, prefiguring the way that waves of mitosis traverse the embryo during the syncytial stages of embryogenesis. Our data demonstrate that although a fertilizing sperm is not necessary for egg activation in Drosophila, the characteristic of increased cytosolic calcium levels persists. Additionally, many downstream signaling effectors are conserved in Drosophila and other species. Taken together, Drosophila offers a unique perspective of egg activation events due solely to maternal components that may also be applied to the study of this process in other organisms.

Sugar Coated Genomes – Sperm Sialome and Sexual Selection, **PASCAL GAGNEUX** (Department of Cellular and Molecular Medicine, and Glycobiology Research and Training Center, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093; pgagneux@ucsd.edu).

Why does it take millions of sperm to fertilize one egg? The answer to this simple question remains elusive. Spermatozoa face the unique task of shuttling a haploid genome towards the ovum they encounter in the reproductive tract of another individual. As allogeneic cells, sperm face substantial threats along the female reproductive tract. Female cellular and humoral immunity exert strong selection on sperm in mammals, allowing just a few out of millions of spermatozoa to reach the fallopian tubes. The mammalian sperm glycocalyx includes copious decorations with tens of millions of terminal sialic acid molecules. These are acquired during spermatogenesis, epididymal maturation, and from seminal fluid components during ejaculation. Sperm sialic acids act as self associated molecular patterns (SAMPs) promoting tolerance by female immune factors including antibodies, neutrophils, macrophages, and complement. For unexplained reasons, before fertilization sperm undergo dynamic remodeling of their sialome, mediated by sperm sialidases. Lack of sialidase activity and mismatches between sperm sialic acids and female immune response can affect fertility in mouse model systems and possibly in humans.