G. Jesse Bender, MD “Simulation-Based Education” (faculty member leaving WIH by 7.7.17)

Simulation has entered the mainstream of medical education internationally. The Women & Infants Hospital simulation program has been active since 2009 across all specialties and departments. Simulation is used to test systems, introduce new protocols, and provide essential education without putting patients at risk. During your fellowship, you will experience simulation in so many arenas you’ll no longer notice the camera, practicing code leadership with high fidelity simulators, refining your delivery of Difficult Conversations, as well as teaching basic resuscitation and transport stabilization skills. Two elements are important for the aspiring academician: 1) mastering the art of simulation as a teaching tool, and 2) contributing to the literature. The art of simulation, a core tool for our generation of educators, can be learned with deliberate practice, from scenario development through the negotiating the thorny debriefing. The literature is open to fellow-accessible projects, from development of assessment tools to measuring the impact on patient and resident outcomes. Our publications include “Impact of High Fidelity Delivery Room Simulation on Resident Education and Retention” among our pediatric and family practice residents, “Emergency Medicine Residents taught Neonatal Resuscitation using Simulation Enhanced Training have Improved Resuscitation skills”, and “Transportable Enhanced Simulation Technologies for Pre-Implementation Limited Operations Testing in the Neonatal Intensive Care Unit (TESTPILOT-NICU)” from our experiences prior to moving into the new NICU. My most active research protocol is “Generalizing TESTPILOT” across other transitioning healthcare delivery models. (updated: 5/15)

Joseph Bliss, MD, PhD “Molecular Pathogenesis of Candida Infections”

My laboratory studies the pathogenesis of and host defense against Candida in the neonate. This organism is a leading cause of serious, systemic infections among the immunocompromised, particularly the premature infant. Despite treatment with available antifungal agents, the morbidity and mortality related to infections with this organism remain unacceptably high. My research program involves the study of three fundamental processes: [1] The morphological change from yeast to filament that C. albicans undertakes in disease states: As a commensal colonizing mucosal surfaces in humans, the organism grows primarily in the yeast form, while in disease states, it converts to a filamentous (hyphal) form. Novel antigens are expressed on the hyphal surface that are important for pathogenesis. Very few of these antigens have been described at the molecular level. [2] The ability of specific monoclonal antibody to afford protection in systemic disease: Although the importance of the cell-mediated immune system in defense against fungal infections is well established, there is an accumulating body of evidence that antibodies also have an important role by augmenting host effector cell function. In animal models of candidiasis, both systemic and topical administration of polyclonal antibodies against Candida have been effective in preventing infection. However, polyclonal sera contain complex mixtures of antibodies that may be protective or non-protective, and may in some cases enhance infection. To circumvent these issues, monoclonal antibodies have desirable attributes such as epitope specificity and uniform isotype that allow more targeted research design. We have characterized a group of antibody fragments against Candida. Several of these are specific to proteins on the fungus that are known to be important for virulence. We have developed a model for neonatal candidiasis in the mouse, and have begun to explore how these antibodies or other treatments may act for the benefit of the host in vivo. [3] The maturation of anti-fungal host
defense as it develops from preterm infants to term infants to adulthood: The innate immune system is known to play a vital role in defense against fungal infections. The neutrophil is of particular importance, as patients with inherited or acquired deficiencies in neutrophil number or function are at particularly high risk for fungal infection. For the neonate whose previous exposure to pathogens is limited, the ability of these cells to act in a non-specific manner to defend the host is vital. Studies investigating neutrophil function in both term and preterm neonates have demonstrated deficiencies relative to adult cells. Much less is known regarding the function of neonatal effector cells against fungus, despite the importance of these pathogens in neonatal disease. We are interested in specific innate immune functions when confronting Candida as a target, and in defining ways in which neonates may be deficient. (no changes needed: 3.17)

Juan Sanchez-Esteban, MD “Mechanical Forces in Fetal Lung Development”

Lung underdevelopment secondary to extreme prematurity and pulmonary hypoplasia causes significant morbidity and mortality to children. It is well known that mechanical forces generated in utero by repetitive breathing movements and by fluid distension are essential to mammalian lung development. In fact, increase of intrapulmonary pressure by tracheal occlusion has been performed in human fetuses affected by severe pulmonary hypoplasia secondary to congenital diaphragmatic hernia in order to accelerate lung development. However, the mechanisms by which mechanical signals promote lung development are still being explored. Previous in vitro studies from our laboratory have identified HB-EGF, an EGFR ligand that is released by lung epithelial cells in response to physiologic mechanical strain and promote type II cell differentiation. Currently, and using in vivo models of tracheal occlusion and pulmonary hypoplasia induced by oligohydramnios and nitrofen, we are investigating the role of HB-EGF in fetal lung development. Another area of interest is to investigate the role of exosomes in fetal lung development. Given that stretch-induced lung development is mediated via soluble factors, we hypothesized that mechanical forces promote lung development via release of exosomes. For these experiments, fetal lung epithelial cells will be exposed to physiologic stretch; exosomes will be isolated from the supernatant and processed by mass spectrophotometry and microRNA to identify protein, genes and pathways activated by stretch. These pilot studies will generate hypotheses to be tested in vivo in the tracheal occlusion and hypoplasia models described above. Ultimately, the translational implications of these studies would be whether administration of exosomes to fetal lungs accelerates lung development. Lastly, and in collaboration with the dean of the medical school, we are interested in investigating the role of chitinase 3-like-1 in fetal lung development, chorioamnionitis and bronchopulmonary dysplasia. (no update needed 3/17)

Justin R. Fallon, PhD “Congenital Muscular Dystrophy: Mechanisms and therapies”

The Fallon lab is developing a novel therapy for neuromuscular disorders. In addition to our long-standing interest in Duchenne Muscular Dystrophy, our recent work has also revealed novel approaches to CMD and ALS.

We are developing a novel therapy for DMD. One of the most attractive avenues for treating this disease is the up-regulation of utrophin, an autosomal homolog of the gene (dystrophin) that is mutated in DMD. We have shown that the extracellular matrix protein biglycan can be systemically delivered to dystrophin negative mice where it upregulates utrophin, counters dystrophic pathology and improves muscle function (Amenta et al., 2011). We are currently manufacturing biglycan in a form that can be used in humans and are determining its pharmacological and toxicological profile. Our goal is to test the efficacy biglycan for treating muscular dystrophy in patients affected by DMD. We anticipate that biglycan will enter the clinic in the coming year.

In recent work we have shown that biglycan also plays a role in stabilizing the neuromuscular junction (Amenta et al., 2012). This action is likely to be mediated through interaction with MuSK, a receptor tyrosine kinase that
Richard Freiman, PhD “Role Of A Specialized TFIID Complex In Reproduction And Development”

Transcriptional regulation is achieved by the coordinated interplay of numerous protein factors with regulatory control sequences coded in the genome. Mechanisms by which this complex machinery achieves precise control of cell and tissue-specific programs of gene expression observed in multi-cellular organisms are poorly understood. Our laboratory is interested in deciphering mechanisms of gene expression patterns critical for proper organ development and function in mammals. Using the mouse as a developmental system, we plan to probe the biological function of various components of the transcriptional apparatus to uncover novel pathways of cell type specification. In addition to characterizing basic mechanisms of differentiation and development, we will utilize developmental defects in the mouse to model human disease states as potential avenues of therapeutic intervention. Research in the laboratory will focus on two areas: The multi-protein complex TFIID is a general transcription factor at the core of the RNA polymerase II machinery. TFIID is composed of the TATA-binding protein (TBP) and several TBP-associated factors (TAFs). To test the hypothesis that TFIID functions in regulating tissue-specific programs of gene expression, we have characterized the biological role of a tissue-restricted subunit of TFIID called TAFII105 (also called TAF4b). Strikingly, female mice lacking TAFII105 are infertile due to developmental defects in the ovary that prohibits proper oocyte development. Male knockouts also display reduced fertility. Based on these initial findings, we plan to characterize reproductive phenotypes of the TAFII105-null mice, describe the normal expression patterns and function of TAFII105 in reproduction and development, and characterize underlying mechanisms controlling gonadal gene expression in the mouse. In addition, we will probe the function of TAFII105 in humans as a potential model of premature reproductive failure. Chromatin modification in development and disease: In addition to using the TAFII105-null mice as an inroad to studying reproductive development, our laboratory will study functional roles of the chromatin modifying and remodeling machinery in mouse development and human disease. Histone modifications known to be important for the control of gene expression are phosphorylation, acetylation and methylation. In addition, ATP-dependent chromatin remodeling complexes are critical for executing regulated gene- and tissue-specific programs of gene expression. We will identify and characterize the function of selective components of the cellular machinery that modify and remodel chromatin in a tissue-specific manner. Such tissue-specific modifiers of chromatin will be targeted for disruption in the mouse and associated phenotypes, and gene expression pathways will be dissected.

Susan A. Gerbi, PhD. “Developmental Regulation of DNA Replication”

Initiation of DNA synthesis is the major check point in the cell cycle: the cell is committed to divide once the genome has been replicated. Normally each replication origin fires just once per cell cycle to ensure that the entire genome will be replicated once and only once and therefore passed on as a perfect duplicate to the daughter cells. In rare cases, including in cancer cells, certain regions of the genome are amplified in copy number relative to the rest of the genome. How does DNA amplification override the controls that ensure that an origin fires once and only once per cell cycle? We are exploring the mechanism of DNA amplification in human breast cancer and also using the fly Sciara which undergoes DNA amplification of its “DNA puffs” on the salivary gland giant polytene chromosomes as a normal part of its development. Sciara is the only model system available to elucidate the mechanism of hormonally regulated DNA amplification. To understand the regulation
of initiation, we developed Replication Initiation Point (RIP) mapping to identify the start sites of DNA synthesis at the nucleotide level. We have shown that in yeast and in metazoa (Sciara) the site of initiation of replication is directly adjacent to the Origin Recognition Complex (ORC) binding site. Preliminary data suggests that the steroid hormone, ecdysone, induces DNA amplification, providing the first example of hormonal regulation of DNA replication, and may provide a useful paradigm for understanding certain cancers in humans. We are using genomic approaches to identify sites of DNA amplification in breast cancer cells and to analyze their proximity to estrogen receptor binding sites. In parallel, we are using molecular and genomic approaches to investigate the underlying molecular mechanism of DNA amplification in Sciara DNA puffs. (no updates needed 3/17)

Martin Keszler, MD. “Optimizing respiratory outcome in preterm infants through the use of novel respiratory support modalities”

Advances in neonatal intensive care have improved survival of extremely low birth weight infants, but the immature lung is extremely vulnerable to injury due to a combination of mechanical injury (volutrauma), inflammatory response to biophysical forces and/or infection (biotrauma), and oxygen toxicity. As a consequence, chronic lung disease remains high. Much of my clinical research has focused on investigation of volume-targeted ventilation as a means of reducing adverse consequences of mechanical ventilation and on the complex interactions between spontaneously breathing infant and the ventilator.

We have defined optimal tidal volume targets in a variety of clinical situations with different types of lung pathology that may influence physiologic dead space. My clinical and bench research challenged the accepted dogma that anatomical dead space gas does not mix with fresh gas during the respiratory cycle; we showed both in the clinical setting and on the bench that it is possible to ventilate effectively with tidal volume below dead space even at conventional ventilator rates and recently showed that flow characteristics of the mechanical breath affect the efficiency of this phenomenon. Currently, I am evaluating the capabilities of the newest state-of-the-art neonatal ventilator in relation to other available devices in a series of bench studies with a focus on how the devices react to a variety of simulated perturbations using an active lung simulator. This will be the first series of studies to evaluate the dynamic interactions between a ventilator and an actively breathing subject, in contrast to the passive lung models used to date.

I am a Co-PI of an NIH funded multinational clinical trial (the SAIL trial) exploring the hypothesized benefits of a sustained inflation immediately after birth in extremely preterm infants. The study is currently underway and offers an opportunity for some ancillary studies. I am the overall PI for a multicenter clinical trial of the high-frequency oscillation (HFO) feature of the Draeger VN 500 ventilator, which is unique in its ability to deliver volume-targeted HFO. This study is underway and provides an opportunity for a variety of ancillary studies. One such study is comparing the noise produced by the new ventilator to that of the FDA approved device.

Other clinical trials underway include studies of surfactant delivery by aerosol during CPAP, comparison of CPAP and NIPPV at equal mean airway pressure and a study to evaluate the ability of the combination of the Spontaneous Breathing Test combined with heart rate and respiratory rate variability to predict extubation readiness in ELBW infants (the APEX study).

I am the Brown University Alternate PI in the NICHD Neonatal Research Network (NRN) and the Site PI responsible for several active NRN studies. A multicenter randomized study of volume-targeted vs. pressure-limited ventilation has been approved by the NRN and is awaiting funding. A number of ancillary studies are planned in conjunction with this project. The NRN has a large database with extensive data on infants <1000g and this provides an opportunity to propose a variety of hypothesis-generating exploratory studies in this
vulnerable population. I can serve as a mentor in this endeavor and guide the interested Fellow in this process.

Finally, I am the WIHRI representative in the BPD Collaborative group, a ten-center multidisciplinary consortium that has begun to collect longitudinal data on infants with severe BPD and plan a variety of prospective collaborative studies. The group is committed to promoting junior faculty and fellow to take a lead in these projects. (updated 3.12.17)

**Abbot Laptook, MD “Brain Injury in Term and Premature Infants”**

The focus of my research efforts has been to function as the Principal Investigator for the Brown site of the NICHD Neonatal Research network (located at WIHRI). The network is a consortium of 15 large academic neonatal centers from across the US that pools their patients to test important concepts which typically cannot be performed in a single center. My role has been to contribute and conduct clinical studies of the Network in our NICU and Follow-Up clinic. My primary area of research within the multiple studies conducted by the Network has been hypoxic-ischemic brain injury in the term infant. This interest stems from more than 20 years of animal working investigating brain ischemia since this injury remains an important cause of neurodevelopmental deficits during childhood and cognitive impairment at school age. To date the Neonatal Research Network has completed 3 randomized trials of hypothermia for newborn encephalopathy. Currently there is an ongoing 4th randomized trial of hypothermia for preterm infants with encephalopathy.

Separate from the Network studies, I have strong interests in thermal regulation, use of the amplitude integrated EEG (aEEG) to increase our understanding of the pathogenesis of brain injury, moderate and late preterm infants, non-invasive methods to measure brain temperature an placental pathology among infants with encephalopathy. Former fellows have been involved in the following studies: associations between placental expression of cytokines and development of BPD, a small randomized pilot clinical trial of nasal ventilation, a cross-sectional study among infants with and without BPD to determine abnormalities on aEEG, a prospective evaluation of the extent of agreement between measured end tidal CO$_2$ and arterial pCO$_2$, a cohort study using the aEEG as a predictor of morbidities/length of stay for infants born at 34 weeks gestation, the association between early pulmonary hypertension and the development of BPD and the temporal sequence of changes in pulmonary artery pressure during the neonatal course for extremely preterm infants. Current work involves the evaluation of placental pathology among infants with and without encephalopathy. (updated 3.30.17)

**Beatrice Lechner, MD, “The Development of a Mouse Model of Gene-Environment Interaction in Premature Birth”**

Preterm delivery is the leading cause of newborn morbidity and mortality in the United States. Preterm premature rupture of membranes (PPROM) is the underlying cause of about one third of premature deliveries. The causes of PPROM are largely unknown. However, it seems likely that certain genetic factors predispose certain women to PPROM.

Infants that are born with Ehlers-Danlos Syndrome, a connective tissue defect caused by a variety of genetic abnormalities, including that of the proteoglycans biglycan and decorin, are more likely to be born prematurely secondary to PPROM than are their unaffected siblings. Mice deficient in biglycan and/or decorin are a model of Ehlers-Danlos Syndrome. Our lab has demonstrated that these mice display preterm birth and abnormal fetal membrane morphology and signaling.
Thus, the long-term aims of the lab are to use this novel genetic model of preterm birth to uncover the mechanisms by which biglycan and decorin are involved in the maintenance of intact fetal membranes and successful birth, as well as to ultimately develop therapeutics for PPROM and preterm birth. We are using embryo transfer techniques to transplant mouse embryos of one genotype into female mice of another genotype, as well as video monitoring of mice during labor and delivery.

We have multiple subprojects that interested fellows can choose from. A good strategy is to choose some experiments that are descriptive and very likely to work, as well as some that are mechanistic and require more troubleshooting. This can lead to some “safe” data to ensure meeting fellowship requirements, as well as to more “fun” data that will satisfy the adventurous researcher in you. Given that our lab is small, we can offer a personalized learning experience. The lab is located in Women and Infants’ Hospital’s research building, the Kilguss building.

“Palliative Care in the NICU” Within the NICU Palliative Care Committee we are currently working on and developing multiple projects aimed at guiding our practice to better support babies and their families in the NICU who are experiencing a challenging clinical course. Current research projects include a study of the quality improvement in the palliative care we provide since the development of our palliative care program; a study assessing bereaved parents’ views of the end-of-life care we provide; a study assessing the self-care of care providers in the NICU after an infant death; and finally, two studies assessing various aspects of counseling families in difficult situations, e.g. at the threshold of viability (one of these studies uses simulation as a tool). (no update needed 3/17)

Barry Lester, PhD and The Brown Center for the Study of Children at Risk

Children can be at risk for non-optimal developmental outcome because of biological factors, because of social factors, or often because of both biological and social factors. The study of these children enables us to learn about developmental processes in typically developing and atypically developing children and to develop treatment strategies to meet the individual needs of the child and family. Research at the Center includes studies of mechanisms in a variety of areas that explain developmental outcome with an emphasis on at-risk children. Six signature themes characterize our research areas: **Prenatal Substance Exposure**: multi-site longitudinal studies of prenatal cocaine exposure and prenatal methamphetamine exposure in the United States and New Zealand. Special initiatives in this research program include neuroimaging, sleep and obesity studies. **Preterm Infant Development**: studies involve a 17-year longitudinal of infants born in the Neonatal Intensive Care Unit (NICU), neuroimaging and kinematics and a study of the effects of the single room NICU model on infant medical and neurobehavioral development. In the area of **Maternal Depression** studies we study fetal and neonatal neurobehavior, prenatal antidepressant exposure and depression in mothers of preterm infants. Studies of **Autism Spectrum Disorder (ASD)** include behavioral and physiological reactivity during social interaction, ASD and social anxiety and fetal and newborn neurobehavioral responses in fetuses of siblings of children with ASD. **Epigenetics** involves genomic methylation and newborn neurobehavioral. Work in **Intervention** includes buprenorphine treatment for opioid addicted pregnant women, evaluation of programs for drug exposed infants and their families, the role of Family Treatment Drug Court, and the effects of Parent-Child Interaction Therapy (PCIT). (updated 6/22/09)

Yow-Pin Lim, MD PhD “Role of Inter-alpha Inhibitors in Systemic Inflammation and Neuroinflammation”

My research focuses on a family of natural blood proteins, inter-alpha inhibitor proteins (IAIIP) that have been
demonstrated to play crucial roles in modulating the body’s response toward severe systemic inflammation accompanying sepsis, infection, intoxication (anthrax toxin), trauma and injury. IAIP are inhibitors of serine proteases involved in a wide variety of physiological and pathological processes including coagulation, inflammation, wound healing, preterm labor and cancer metastasis. IAIP serve as a broad spectrum biological response modifier to regulate circulating levels of secreted inflammatory substances such as immune response regulators (cytokines), proteins that attract leukocytes to sites of injury or inflammation (chemokines) and destructive proteases that cause severe morbidity and excessive mortality in affected patients. Moreover, IAIP have been also reported to inhibit complement activation, an important element of inflammatory processes. During severe inflammatory processes, the circulating level of IAIP is consumed and rapidly depleted resulting in an uncontrolled disease process. The level of plasma IAIP is inversely proportional to survival in adult sepsis and might be potentially useful as a prognostic or a theranostic marker. Significant decrease of plasma IAIP level is also found in newborns with clinically proven sepsis and necrotizing enterocolitis (NEC) suggesting the clinical utilization of IAIP as a sensitive and specific marker to detect these life threatening conditions. Furthermore, administration of purified IAIP improves survival in several experimental adult models of systemic inflammation/sepsis as well as in neonatal sepsis models. We hypothesize that a replacement therapy with IAIP will reduce morbidity and mortality associated with severe systemic inflammation and will provide time to treat the underlying disease condition or infection in human. Current efforts are focused on human testing of IAIP as an effective and safe therapeutic agent in acute lung injury such as in severe Community Acquired Pneumonia. An on-going collaboration with the Dept. Pediatrics at WIH is focused on the study of IAIP as a useful biomarker in neonatal sepsis and NEC as well as an effective therapeutic agent in reducing neuroinflammation in hypoxia-ischemia (HI) brain injury in newborns. Neonatal HI remains a major cause of acute perinatal brain injury, leading ultimately to neurologic dysfunction manifesting as cerebral palsy, mental retardation, and epilepsy. There are no currently available therapies to prevent/treat and/or attenuate brain damage in premature infants other than supportive care and the only available therapeutic intervention for full term infants is hypothermia, which is only partially protective. Our recent investigations strongly demonstrate that IAIP reduced neuroanatomical injury in the brain of neonatal rats and improves long-term behavioral indices of learning and memory tasks. Current efforts are focused on the translational studies of IAIP to prevent/attenuate brain damage in full term as well as in preterm infants at risk for mental retardation (updated 03/17).

Kimberly L. Mowry, PhD “Localized gene expression in the Xenopus oocyte”

Establishment of polarity in the egg can be viewed as the earliest step in embryonic patterning. Thus, differences in cell fate among the early cleavage cells are a consequence of asymmetric distributions of informational molecules in the egg cytoplasm before fertilization. The basis for such polarity can be provided by localized maternal determinants in the form of mRNA. While there is evidence for the existence of localized determinants in many systems, the molecular mechanisms underlying control of spatially restricted gene expression are only now being unraveled. Among vertebrates, Vg1 mRNA is a prominent example of a localized mRNA that plays a role in embryonic patterning. Vg1 mRNA encodes a growth factor, and is localized during oogenesis to the vegetal cytoplasm of Xenopus oocytes. Restricted expression of Vg1 protein in the vegetal hemisphere of the egg is critical for correct patterning of the embryo, making localization of Vg1 mRNA an important model for understanding how maternal molecules are localized to influence pattern and polarity. The goal of our research is to investigate how RNA molecules can be targeted to specific regions of the cell cytoplasm to generate cell polarity through spatially restricted protein expression. To gain mechanistic insight, we have studied the molecular interactions that govern this process. We have identified and characterized key components of the localization machinery, including molecular motors that carry the RNAs to their cellular
destinations, and we have uncovered distinct steps in the transport pathway. A second mechanism to ensure spatially correct expression of localized mRNAs is through translational control, and we have also found that Vg1 mRNA is translationally repressed until after its localization. Our ongoing and future studies are designed to dissect the interconnected mechanisms and regulatory steps of RNA localization and spatially restricted protein expression in order to provide insight into how developmental signals are spatially distributed in the vertebrate embryo. (no changes as of 6/18/09)

James F. Padbury, MD.

Our interests in placental biology relate to the unique aspects of intrauterine development. Fetal endocrine systems are almost exclusively autonomous from the maternal endocrine systems and the extraterine environment. The placenta serves as a barrier to transplacental passage of nearly all the peptide and steroid hormones, catecholamines and other bioactive molecules. Thus, the placenta is crucial to maintaining neonatal homeostasis. We have identified DNA methylation and changes in histone and chromatin marks as alterations that reflect intrauterine challenges. We are using the Neonatal Network Neurobehavioral Scales (NNNS) to examine the impact of intrauterine challenges on changes in placental gene expression and DNA methylation on newborn neurobehavioral outcomes. We are extending these studies to examine the effects of the postnatal environment in the new Neonatal Intensive Care Unit on DNA methylation profiles of the tiniest infants who experience their critical period in the new NICU rather than in utero.

The genome era has led to the opportunity to examine complex, non-Mendalian diseases using high throughput genome assays. This includes DNA array-based studies of single nucleotide polymorphisms, copy number variations and other genetic variants that are associated with diseases like schizophrenia, diabetes and hypertension. We are conducting a modified, genome-wide association study on the genetics of preterm delivery. Using a novel bioinformatic approach to identify a set of candidate genes, we are examining the pathways and genetic variants that lead to preterm birth. We have recently received support for targeting re-sequencing of candidate regents in the human genome that may identify the rare variants associated with preterm birth. We have built a comprehensive database for the genes and variants associated with preeclampsia. We are enrolling and carrying out whole exome sequencing on patients with severe preeclampsia. Our novel bioinformatic approach is generalizable and is also being applied to other complex phenotypes. Similar approaches have been developed for preeclampsia and other conditions. (updated 3.15.17)

Peter Quesenberry, MD “The New Stem Cell Biology”

Our research program involves studies of bone marrow stem cell biology focusing on the fluctuating phenotype of the stem cell as it traverses cell cycle and its engraftment potential. In addition, we are studying approaches to induce tolerance in marrow engraftment of H2 mismatched mice and we have a program evaluating the circadian rhythm of the engrafted stem cells using a transgenic mouse model which expresses luciferase at certain points in the circadian cycle. Another part of the laboratory focuses on the ability of adult marrow stem cells to convert to skeletal and cardiac muscle, lung cells and skin cells. These studies hold the promise of new therapeutic interventions.

Surendra Sharma, MD, PhD “Biology of Pregnancy Complications and Health Risks Later in Life”
Surendra Sharma is a Professor in the Department of Pediatrics at Women & Infants Hospital of Warren Alpert Medical School of Brown University, Providence, Rhode Island and Director of Women and Infant Hospital’s Centers of Biomedical Research Excellence for Reproductive Health. He received his medical and PhD degrees from India. He also received an honorary MD degree from the Linkoping University in 2006. Dr. Sharma is a long-term member of the American Society for Reproductive Immunology (ASRI). He served as President of the ASRI (2010-2012). He has chaired and has been the presiding officer of several ASRI meetings, as recently as the 36th annual ASRI meeting held in Baltimore on November 12-16, 2016. He currently serves on the Executive Boards of the ASRI and the International Society for Immunology of Reproduction (ISIR). He has been funded by the NIH, ADA and other foundations and organizations and continues to garner funds from the NIH. He served as an Associate Editor of the Journal of Immunology and currently serves on the editorial board of the American Journal of Reproductive Immunology. Dr. Sharma is a highly sought after investigator for the review roster of grants and manuscripts.

His research focuses on the question of how some pregnant women are prone to developing devastating pregnancy complications such as preterm birth, preeclampsia and gestational diabetes. Most importantly, he investigates how these complications influence or are programmed by innate and adaptive immunity at the maternal-fetal interface. His recent work on preeclampsia has provided novel paradigms for mechanistic and therapeutic insights for preeclampsia, albeit using the Alzheimer’s insights. His long-term studies have involved both animal and human pregnancy models. Dr. Sharma has an extensive track record as a funded investigator by NIH and other agencies. He is PI/Director of an NIH-funded Center of Biomedical Research Excellence for Reproductive Health. He has published 113 research papers in peer-reviewed journals of reasonably high impact factor. (updated 4.10.17)

Jennifer Sanders, PhD “Regulation of Fetal Liver Development”

The mechanisms that control liver development are important to several diverse areas of human health and disease. The growth and functional differentiation of the liver is critical to fetal metabolism, the perinatal transition and metabolic adaptation by the newborn. Perturbation of these processes contributes to disorders of the newborn and to the fetal origins of adult metabolic disorders. The biology of fetal liver development also has implications for hepatic carcinogenesis, the regulation of fetal somatic growth, and cell-based therapy for liver disease. Our laboratory has been focused on the pathways that regulate late gestation liver development in the rodent. We have identified a fetal hepatocyte phenotype defined by the signaling pathways that regulate fetal hepatocyte growth, proliferation and gene expression. These pathways are distinct from those regulating adult hepatocyte proliferation and growth.

One of our current projects focuses on the nutrient regulation of fetal somatic growth. The overall goal of the project is to elucidate the mechanisms by which intrauterine growth restriction mediates changes in hepatic gene expression and liver mitochondrial metabolism. Another project in the laboratory is focused on cell-based therapy as an alternative strategy to liver transplantation. We have shown that fetal hepatocytes have the ability to engraft and repopulate an injured adult liver. This is a characteristic not shared by adult hepatocytes. This suggests that the unique signaling and proliferative phenotype of late gestation fetal liver cells provides a selective growth advantage. We hypothesize that the expression of histone variants and histone posttranslational modifications (PTMs), acting through effects on chromatin structure, account for the signaling phenotype of late gestation fetal rat hepatocytes and the persistence of this phenotype following
transplantation into the adult liver microenvironment. Studies are underway to characterize chromatin structure and gene expression in the fetal cells before and after transplantation to the adult liver.

Barbara S. Stonestreet, MD “Developmental Aspects of Brain Maturation and Injury in the Ovine Fetus and Neonatal Rodent”

Our laboratory is focusing on three main projects: [1] Cytokines and the blood-brain barrier in the Ovine Fetus: Perinatal brain injury often results in cerebral palsy and neurodevelopmental disability. Consequently, developmental disabilities place a huge burden on society, emphasizing the paramount need for improved prevention/treatment strategies to reduce individual and societal burdens related to perinatal brain damage. Therapeutic strategies are currently not available for infants with brain damage, except for hypothermia, which can only be used in full term infants for hypoxic-ischemic encephalopathy and is only partially protective. Recent studies suggest that fetal inflammation is a strong predictor of perinatal brain injury. Inflammatory processes in utero are likely antecedents of brain damage in preterm infants as cytokine elevations predict neonatal brain damage. The blood-brain barrier (BBB)/neurovascular unit (NVU) is a privileged site consisting of brain microvascular endothelia, glia, and neurons that regulate the microenvironment for neural functioning. A novel approach to prevent perinatal brain injury would be to target the BBB with agents to preserve its function to limit entry of toxic substances into brain. This proposal is based on the central hypothesis that cytokines cross the BBB in the fetus to damage the brain and that blockade of cytokines attenuates damage to the BBB/NVU and the brain parenchyma. Our published data show that blocking effects of pro-inflammatory cytokines with systemic infusions of cytokine neutralizing monoclonal antibodies (mAb) attenuate ischemia-related increases in BBB permeability in the ovine fetus. The novel overall goal of this continuation of an R01 is to elucidate the effects of systemic inflammation on BBB/NVU function to determine whether blocking cytokines with systemic antibody infusions attenuates inflammation-related damage to the BBB/NVU/brain parenchyma in the fetus. A multidisciplinary approach to address the specific aims includes physiological, biochemical, pathological, immunohistochemical, and molecular methods. Aim 1 tests the hypothesis that systemic inflammation (induced by LPS) results in increases in BBB permeability measured both with α-aminoisobutyric acid and radiolabeled cytokines, as a function of gestational age, and a result of changes in the NVU. Aim 2 tests the hypothesis that cytokine-specific neutralizing mAbs prevent inflammation-related increases in non-specific BBB permeability, cytokine transport into brain by attenuating damage to the NVU, and, thus, reduce parenchymal brain damage. Aim 3 tests the hypothesis that inflammation-related increases in BBB permeability are prostanoid dependent by determining whether cyclooxygenase inhibition prevents LPS-induced BBB dysfunction. Understanding how the BBB/NVU responds to inflammation and mechanism(s) responsible for damage to BBB/NVU will facilitate development of innovative therapeutic approaches. Treatment of neonates with anti-cytokine mAb would be feasible, as humanized anti-IL-1β antibody is currently in use to treat inflammatory conditions and shows beneficial effects in pediatric trials. This project could provide new insights into novel strategies to prevent brain injury in the human fetus and/or full term and premature infant.

[2] Inter-Alpha Inhibitor Proteins, Hypoxic-Ischemic Brain Injury: Neuroprotection in Fetal Sheep. Perinatal brain injury resulting in mental retardation (MR) and cerebral palsy (CP) represents one of the most severe disabilities in childhood. The incidence of CP is 40-148 in preterm and 1-2/1,000 in full term infants. Developmental disabilities place a huge burden on society (lifetime costs per person: ~1 million dollars), emphasizing the urgent need for improved treatment strategies to reduce perinatal brain damage. Unfortunately, hypothermia is the only approved therapy for brain damage in infants and is only partially protective. It can only be used to treat hypoxic-ischemic (HI) encephalopathy in full term infants, and cannot be
used in preterm infants in whom supportive care is the only ‘therapy’ to attenuate brain damage. Cytokines represent a final common pathway, which cause/augment fetal/neonatal brain damage. Inter-alpha inhibitor proteins (IAIPs) down-regulate pro-inflammatory cytokines in sepsis and inhibit destructive serine proteases. Little information is available about IAIPs in brain. Recent data suggests that bikunin, a fragment of IAIPs, isolated from urine, attenuates stroke-related brain injury and experimental autoimmune encephalomyelitis-related white matter loss in adult rats. However, the half-life of bikunin is very short (3-10 min), compared with the complexed form isolated from blood (18 h in neonatal rats), requiring large quantities of protein and continuous intravenous infusions. The potential neuroprotective effects of IAIPs isolated from blood have not been examined except for our recent data. We will examine the neuroprotective effects of the blood-derived IAIPs. We anticipate that this form will be a more viable neuroprotective agent for clinical use. Our preliminary studies with blood-derived IAIPs suggest that this agent has remarkable neuroprotective effects in HI neonatal rats and fetal sheep after ischemic injury. Our overall goal is to develop a novel effective therapy to treat ischemic brain damage using the preclinical fetal sheep model in which IAIPs can be given by the clinically relevant intravenous route. We hypothesize that systemic IAIP administration attenuates the development of ischemic-reperfusion related injury in the immature brain. The purpose of this proposal is to provide a strong biological basis to support the use of IAIPs as therapeutic agents to treat ischemia-related brain injury in the immature brain. The aims are: (1) To establish the dose of IAIPs with the greatest neuroprotective efficacy in the fetus; (2) To examine the neuroprotective efficacy of delayed treatment with IAIPs on brain injury; (3) To determine the pharmacokinetics of intravenously administered IAIPs in the ovine fetus. Fetuses will be surgically prepared; brain ischemia induced by carotid occlusion and injury measured by a multidisciplinary approach using ECoG, physiological, biochemical, pathological, immunological, immunohistochemical, and molecular methods. Results of the studies could yield novel information that will accelerate the use of IAIPs as neuroprotective agents to treat brain injury in the fetus/neonate potentially to prevent MR and CP in infants.

[3] Inter-Alpha Inhibitor Proteins, Hypoxic-Ischemic Brain Injury, and Neurobehavioral Outcomes. Hypoxia-ischemia (HI) is the leading cause of neurodevelopmental morbidities in preterm and full term infants. The only therapeutic strategy to treat HI encephalopathy (HIE) is hypothermia for full term infants, which is only partially protective, and treatment does not exist for HI exposed preterm infants except for supportive care. Based upon the Stroke Therapy Academic Industry Roundtable (STAIR) criteria, sufficient dose-response and therapeutic time windows, adequate histological and behavioral outcomes, and understanding mechanism(s) of action for neuroprotectants are necessary for preclinical drug development to facilitate translation of neuroprotective strategies from animals to humans. The basis of this proposal is the identification of novel immunomodulatory proteins, Inter-alpha Inhibitor Proteins (IAIPs) that are currently in development as effective therapeutic agents in systemic inflammation/shock syndromes. IAIPs are novel anti-inflammatory molecules that broadly inhibit destructive serine proteases, robustly block pro-inflammatory cytokines, augment anti-inflammatory cytokine production, and block complement activation during systemic inflammation. Our exciting preliminary studies using the blood derived IAIPs suggest IAIPs have remarkable neuroprotective properties in HI-neonatal rats. However, information regarding mechanism(s) of neuroprotective efficacy of IAIPs on HI-brain injury in neonates has not been elucidated. The purpose of this proposal is to fill this gap of knowledge: (1) To determine the optimal dose-response and therapeutic time windows for IAIPs in neonatal rats; (2) To assess short/long term neurobehavioral outcomes, and establish the greatest neuroprotective efficacy using optimal doses and time windows for IAIPs administration in neonates; (3) To examine molecular mechanism(s) of IAIPs (neuroinflammation/apoptosis) and targets (pro-inflammatory cytokines) on HI brain injury. The well-characterized Rice-Vannucci neonatal HI model will be used. The neuroprotective efficacy with various doses and time windows of IAIP treatment will be determined by comparing brain injury (Luxol fast blue/H&E), apoptosis, neuronal injury (Fluoro Jade B), and brain inflammation (cytokine production), astrogliosis, microglia activation, oligodendrocyte injury, etc. Short/long-term behavioral outcomes will be determined at different stages of brain development. The mechanism(s) of action and molecular targets of IAIPs will also be examined.
using primary neuronal cell co-cultures with and without exposure to oxygen-glucose deprivation (OGD). Mechanism(s) will be studied by determining neuroinflammation and apoptosis, along with neuronal/glial and microglial interactions. IAIP targeted pro-inflammatory cytokines will be determined by a Bio-Plex™ cytokine assay. The current proposal will establish drug efficacy, optimal dose-responses and time windows, with which IAIPs attenuate HI brain injury, reduce neuronal/glial cell death, and improve short/long term behavioral performance in neonatal HI rats. Results should yield novel preclinical information to accelerate IAIPs use as neuroprotective agents to treat HI-related brain injury in human premature and full term infants. (updated 3.13.17)

Marc Tatar, PhD “Neuroendocrine and Genetic Controls on Aging”

Our lab combines genetics, demography, evolutionary biology and physiology of Drosophila to understand the basic mechanisms of aging. Aging is the continuous decline in somatic function that leads to progressive increase in mortality. We are particularly interested in how hormones coordinate the components of life history (growth, reproduction, and aging) by mediating nutrient allocation and components of cellular or systemic stress response. We are currently exploring how insulin-like peptides respond to nutritional modifications and thereby mediate secondary endocrines such as neuropeptides, juvenile hormone, and ecdysone, and how these hormones affect reproduction and aging. Many stress response systems are of interest to our lab, including heat shock proteins, cellular anti-oxidant enzymes, and innate immunity. (no changes as of 6/19/09)

Yi-Tang Tseng, PhD “Signaling Pathways Regulating Cardiomyocyte Proliferation”

One of the dogmas of cardiovascular research states that adult heart, highly proliferative during fetal and neonatal development, permanently loses the ability to divide. In rodents, cardiomyocytes exit the cell cycle irreversibly after the fourth to fifth day of life, after which growth of cardiomyocytes shifts from hyperplasia to hypertrophy. The molecular mechanisms responsible for the switch from hyperplasia to hypertrophy are not completely known. Recent advancement in cardiac research, however, indicated that there may indeed be proliferative capacity in adult heart. Can post-proliferative heart be activated to reenter cell cycle by stimulating signaling pathways critical for cardiac proliferation? We have shown that the β-adrenergic receptors (βAR) are involved in regulation of neonatal cardiomyocyte proliferation, and that this mitogenic control may be mediated via the p70 ribosomal protein S6 kinase (p70S6K) pathway. Our recent studies showed that the β1-subtype of βAR alone may account for this control. We have further demonstrated that ontogeny of type-1 p70S6K (p70S6K1) and phosphoinositide 3-kinase (PI3K) is synchronized and highly regulated throughout development in the heart. The high activities of both kinases occur when cardiomyocytes are undergoing hyperplastic growth. We are testing the hypothesis that the p70S6K/PI3K signaling pathway under the control of β1AR is critical for the regulation of cardiomyocyte proliferation. Our approach is to characterize p70S6K/PI3K signaling pathway in existing mouse models, including β1AR-, β2AR- and β1.β2AR double knockout (KO). We are also utilizing a tetracycline transactivator conditional transgenic system to generate a mouse model with cardiac-specific overexpression of PI3K at desirable developmental points. A critical question to answer is whether timely over-expression of cardiac PI3K at post-proliferating stage can impact cardiomyocyte proliferation. Cardiomyocyte populations showing proliferation will be identified, separated by laser-capture and analyzed with mass spectrometry to identify phospho-active proteins. These studies will contribute to our understanding of regulation of cardiac development during perinatal transition and to impact cardiac pathophysiology of adult life.

The scope of the second main research project in this laboratory is studying the cross talk between βAR and PI3K signaling pathway. Recently, we have used in vitro approaches to study the intracellular signaling factors
involved in β2AR-mediated protection against doxorubicin-induced apoptosis in cardiomyocyte under normal and high glucose conditions.

The third project of the lab is to study the effects of angiotensin II receptor in high glucose-induced nephropathy. We have used in vitro approaches to delineate the signaling cascades responsible for this clinically pertinent complication. (updated: 6/4/09)

**Alper Uzun, PhD “Computational Genomics of Complex Diseases: Pre-eclampsia and Preterm birth”**

In the post genome era, biological research and genomic medicine have been transformed by high-throughput technologies. New techniques have enabled researchers to investigate biological systems in great detail. Nonetheless, the extraordinary amount of information in the large number of emerging high-dimension datasets has not been fully exploited. Increasingly, pathway analysis and other a priori biological knowledge based approaches have improved success in extraction of valuable information from high-throughput experiments and genome-wide association studies.

Preeclampsia and Preterm birth are the two complex diseases that I work on my current research. I’m applying bioinformatics techniques and computational biology methods by using next generation sequencing methods to understand the genetic architecture of these two diseases. Preeclampsia is a complex disease and one of the most common causes of fetal and maternal morbidity and mortality worldwide. It is one of the great but enigmatic health problems. Despite many studies, there has been little fundamental improvement in our understanding in decades. It is a multi-system hypertensive disorder of pregnancy, characterized by variable degrees of maternal symptoms including elevated blood pressure, proteinuria and fetal growth retardation that affect 2-8 % of deliveries in the US. Many clinicians believe there is a difference between preeclampsia and severe or early and late preeclampsia. However, to date there is little direct evidence that they represent different genetic etiologies. We perform a case/control study using whole exome sequencing and restricting our enrollment to patients with early, severe preeclampsia. The working hypothesis is that this will provide better power, lower heterogeneity, and higher genetic effect for this complex phenotype. We are developing new bioinformatics approaches to identify the gene networks and causal variants that contribute to severe preeclampsia.

The other complex disease that I am working is Preterm birth. We are developing bioinformatics approaches to understand the gene-gene interactions and working on identifying the preterm birth associated genes. For that purpose we are both using the existing data sets (e.g. GWAS, RNA-seq, targeted sequencing) and collecting blood samples to sequence from preterm birth and term mothers. We have built a web-based tool to aggregate the published medical literature, information from public databases, linkage analyses and finally pathway analysis to the genes involved in preterm birth. We made The Database for Preterm Birth-(dbPTB) publicly available at http://ptbdb.cs.brown.edu/dbPTBv1.php. This computational approach allows us a much more robust evaluation of GWAS data on preterm birth. Using this tool, we have demonstrated three logs greater statistical inferences for genes involved in preterm birth compared to the conventional genome-wide approach. We also employed a similar approach to mine published literature from PubMed and manual curation to authenticate preeclampsia associated genes and the research articles and built a database. The Database for Preeclampsia (dbPEC) consisting of the clinical features, concurrent conditions, published literature and genes associated with Preeclampsia. We included gene sets associated with severity, concurrent conditions, tissue sources and networks. The collection is now freely accessible at http://ptbdb.cs.brown.edu/dbpec/ (added 3.30.17)
Betty R. Vohr, MD “The Investigation of the Language, Neurodevelopmental, Behavioral, and Growth Status of High Risk Populations of Infants”

Our research interests are focused on clinical longitudinal outcome studies of high risk populations. The Neonatal Follow-up Program provides longitudinal assessments and clinical management of high risk extremely low birth weight NICU infants from birth to 16 years of age conducted by a multidisciplinary team. We are the Coordinating Center for NICHD neonatal research Network studies with neurologic and developmental outcomes. Our studies have shown that enhanced transition care and education of parents, results in decreased re-hospitalization rates of very preterm infants. We completed 3 years of a CMS funded innovation challenge grant, Partnering with Parents in October 2015 and are currently looking at longer term outcomes of these infants. The overarching goal is to improve care, improve outcomes and decrease costs. The CMS grant is a longitudinal study to investigate the effects of biologic and environmental factors which are associated with post-discharge emergency room visits and re-hospitalizations and the impact of a pre-discharge intervention combined with home visits by Neonatal nurse practitioners on outcomes. Over 1250 infants and their mothers were enrolled. We are investigating the environmental and psychosocial factors impacting on our outcomes and have identified that 37% of our mothers have adverse mental health histories. Another area of investigation is the impact of a NICU admission on father’s stress and depression.

A fourth interest is investigating early language development of very preterm infants and late preterm infants and the effects of a language curriculum intervention with linguistic feedback on infant language. Language data is collected in the NICU using a Language Environment Analysis digital language processor (LENA). LENA records 16 hours of infant vocalizations, adult speech conversation turns, silence and background noise in the NICU. Our findings to date show that very premature infants make vocalizations as early as 32 weeks, the vocalizations increase by 36 weeks (8 weeks and 4 weeks prior to mothers due date), and infants respond preferentially to their mothers rather than nurses. In addition, the adult word count and conversation turns between mother and infant in the NICU predict the 18 month language and cognitive scores. Currently there are two clinical trials in progress. One is the provision of a language curriculum intervention with linguistic feedback within a cohort of adolescent mothers and the second trial is with very preterm infants in the NICU. We are also examining, outcomes and language data collected in the current single room NICU will be compared with data collected in the open bay NICU. (updated 3.17.16)

Gary M. Wessel, PhD, “PRIMO lab interests, Multipotent cells”

As embryogenesis proceeds from a totipotent zygote into a multicellular organism, diverse cell types are specified by a progressive loss of potency. By contrast, germ cells retain the potential for totipotency and give rise to the next generation.

(A) Model 1: PGCs are specified from set-aside cells in the adult rudiment  (B) Model 2: PGCs are specified during embryogenesis

![Diagram](image-url)
The juvenile sea urchin is patterned from cells that are set-aside during embryogenesis and shuttled into the larval coelomic pouches, the site of adult rudiment formation. Cells that contribute to the coelomic pouches come from various early embryonic lineages, including the small micromeres lineage. We are testing the role of Vasa, Nanos, and Piwi, three conserved germ line markers, in sea urchin development. We find that Vasa, Nanos, and Piwi are selectively expressed in the small micromere lineage during embryogenesis. As larval development proceeds, the domain of Vasa expression expands to the entire developing adult rudiment. Thus, Vasa is not a strict germ line marker in the sea urchin, but may instead indicate multipotency. Functional analysis revealed that Nanos is required to maintain the identity of small micromeres as “set-aside” cells. Nanos-depleted small micromeres ectopically divide and are not incorporated into the larval coelomic pouches. Thus, the small micromeres are likely multipotent cells that give rise to many tissues, which probably includes, but is not limited to the germ line. We hypothesize that the germline is segregated late in larval development, rather than during embryogenesis. Furthermore, we propose that the sea urchin uses a 2-step germline determination mechanism: first a long-term multipotent precursor is specified from which later the primordial germ cells (PGCs) are segregated. Our work suggests a broader role for the traditional germline genes Vasa, Nanos, and Piwi in establishing and maintaining the fate of multipotent cells in 2-step germline determination.

Vasa has multiple roles

Vasa, an ATP-dependent RNA helicase, is broadly conserved among various organisms from cnidarians to mammals. It has a rich history of utility as a germline marker, and is believed to function as a positive translational regulator in the determination and maintenance of germline cells. Studies in non-model organisms including our favorite animal sea urchins, however, revealed that Vasa is also present in somatic cells of many tissues. In many cases these cells are multipotent, are non-germline associated, and give rise to a variety of different tissue types. Our recent work demonstrated that Vasa functions in the regulation of the cell cycle during early embryogenesis of the sea urchin. Additional evidence from our ongoing research suggests that sea urchins utilize this molecule Vasa in multiple cell lineages (embryonic cell, germ cell, and adult stem cell) for multiple functions (cell cycle, germ cell specification and potentially regeneration).

Post-transcriptional regulation in small micromeres

Vasa, Nanos, and Piwi are proteins that accumulate in the sea urchin small micromeres. We have begun to test if post-transcriptional mechanisms are involved in this specific localization. These 3 proteins were also shown to be involved in translational control in different organisms: Nanos was shown to be a translational repressor, Vasa and Piwi were shown to be translational activators. We want to understand the translational functions of these 3 proteins in sea urchin small micromeres. We are now using a luciferase assay in sea urchin cell-free cap-dependent translation system to understand these mechanisms.

Translational repression by Nanos in echinoderms
Nanos is a RNA-binding protein which was first described as a translational repressor in Drosophila. The translational repression is mediated through interaction between nanos and pumilio, which binds RNAs such as hunchback and cyclin B. These target RNAs contain a conserved motif, the Nanos Response Element, in their 3'UTR. Nanos is required for the survival and maintenance of primordial germ cells during embryogenesis. Its expression is highly regulated at the RNA and protein level, any misexpression induces cell cycle and developmental defects. Nanos has been found in all animals tested. In the sea urchin, three nanos homologs are present in the genome of *Strongylocentrotus purpuratus* (*Sp*), and all of them are expressed, with differential timing, in the small micromeres (SMM). Morpholino injection against *Sp nanos1* and *Sp nanos2* indicates that in the sea urchin, these homologs are required for adult rudiment formation. Now, using two sea urchins, *Sp* and *Hemicentrotus pulcherrimus* (*Hp*), our results show that a combination of selective RNA retention, translational control, and protein stability mechanisms instills selective nanos expression in the SMM lineage.

**cADPR and NAADP Ca2+ signaling and the role of ARCs and TPCs in echinoderm eggs**

Calcium signals play key roles in regulating diverse physiological processes. Metazoans have three main intracellular Ca2+ mobilizing messengers: IP3, cADPR and NAADP. Both cADPR and NAADP, which are generated by the same enzymes (ADP-ribosyl cyclases, ARCs), were identified as Ca2+ mobilizing messengers using a sea urchin egg homogenate, and this system continues to be a vital tool for investigating the mechanism of action of these two messengers. Despite cADPR and NAADP having been implicated in diverse physiological processes, and the demonstration that loss of the ARC enzyme in the mouse leads to disease states including diabetes and metabolic disturbances, the molecular mechanisms underlying ARC action and regulation has remained enigmatic. Dr. Wessel's group has recently made two important steps forward in the understanding of these issues by firstly demonstrating that in urchin eggs the endogenous active ARC forms are located within the lumen the cortical granules; and secondly identifying the two pore channel (TPC) proteins as integral components of the NAADP-targeted channel. In this context, the aim of this project is to gain key insights into the mechanisms of action of these two protein families, ARCs and TPCs. This project addresses two main questions: 1) How is the NAADP signaling output mediated amongst the three TPCs isoforms? and 2) How does interfering with ARC and TPC expression affect calcium signaling events in the egg and embryo and key physiological processes during fertilization and development? The first question is based on the fact that TPCs in sea urchins and starfish are present as three isoforms, and that they are differentially located and expressed in the eggs, and therefore might have different functional roles. We are will use reporter tagging of mRNAs encoding the TPCs and antibodies against the TPCs isoforms to identify the specific organelles populated by these isoforms. We expect to identify the specific organelles in which the TPC isoforms are located, and to determine how these TPCs are targeted differentially. In the second question, we intend to determine which ARCs and TPCs mediate physiological processes in the egg in response to physiological stimuli. We are currently using several approaches to interfere with ARC and TPCs function, such as commercially available NAADP antagonists and morpholinos. By studying the abnormalities in developmental processes caused by these enzyme malfunctions, we expect to gain vital insights into the link between cADPR and NAADP induced Ca2+ signals and the regulation of physiological processes in all cells.
Detecting variation between single oocytes and polar bodies

Much of early animal development is driven by mRNAs, proteins, mitochondria and other maternally deposited materials in the oocyte. This maternal endowment is essential in most cases for success of an embryo in early development. We are interested in what mRNAs are present in oocytes, and the variation found between individual oocytes because those differences may be able to predict the range of possible developmental outcomes even when considering the oocytes from the same female. We measure the transcriptomes of single cells (oocytes or polar bodies biopsied from the oocyte), within the same genotype (in the case of mice) or between genotypes (different mouse backgrounds or human IVF/ART patients). The biological variability of transcriptomes can be quantified between single cells within a genotype and the comparison between genotypes can reveal genes that are differentially expressed in a robust manner. We also demonstrated that detection and quantification of mRNA in human polar bodies is possible and reflects the transcript profile of the MII oocyte. The quantification of mRNAs is of particular importance since some transcripts have highly variable expression between oocytes, this variance is reflected in the polar body, and these variations may explain differences in developmental outcome.

Multidrug resistant (MDR) transporters and oocytes

Multidrug resistant (MDR) transporters are cell membrane proteins that belong to the ATP binding cassette (ABC) efflux transporters that are evolutionarily conserved for their ability to protect cells from toxins. Our objective is to determine whether immature oocytes’ MDR transport activity increases as oocytes mature in vitro. We demonstrate in our experiments that MII oocytes have improved mechanisms of effluxing transporter-dependent fluorescent dyes. Our work has also shown that susceptibility to ootoxic chemotherapeutic agents is mediated by MDR transport.
(updated 6/20/12; no further updates 4/14)

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