

2009 Grant Recipients

Matthew Call, Ph.D.

Harvard Medical School

"Assembly, Structure and Function of Activating Immune Receptors"

The cells of the mammalian immune system constantly survey their environments for molecular changes reflecting cellular stresses, such as infection or transformation, which require a protective response from the organism. Many of the immune receptors that monitor cellular surfaces for stress-related signals exhibit a common molecular architecture in which receptor subunits couple to dimeric transmembrane (TM) signaling modules through non-covalent interactions within the lymphocyte membrane. A great deal is known about the extracellular receptor-ligand interactions and intracellular biochemical cascades that initiate immune responses through these receptors, but precisely how a signal is propagated across the lipid bilayer remains unclear. Much of the uncertainty surrounding the mechanisms of receptor triggering stems from the lack of detailed knowledge about the structures of intact receptor complexes. In my previous studies of the T-cell antigen receptor (TCR)-CD3 complex, I identified a conserved structural motif that organizes the assembly and spatial arrangement of subunits within receptor complexes around triads of polar amino acids in the membrane-spanning regions of receptor subunits. Subsequent studies revealed that this membrane-embedded basic-acidic-acidic triad is the essential unit organizing the assembly of a diverse group of activating immune receptor complexes. The highly focused nature of these intramembrane contacts raises the interesting possibility that reorientation of TM helices around these intermolecular contacts could play a role in the propagation of activating signals across the cellular membrane. We have performed extensive biochemical studies of this motif in previously published studies, but a full understanding of the key molecular interactions and their functional consequences will require a comprehensive structural analysis. The goal of this proposal is a high-resolution NMR structure of the membrane-embedded portions of an assembled activating immune receptor complex. Dr. James Chou's laboratory has a well-established record of solving difficult membrane protein structures using multi-dimensional liquid-state NMR techniques. The methods I have developed for producing covalently linked peptide constructs comprising the TM domains of a representative immune receptor have yielded promising results, and preliminary NMR data have confirmed feasibility. This research will produce significant new insights into the structure of activating immune receptors and lay the groundwork for future structure-based functional studies.

Frauke Drees, Ph.D.

Massachusetts Institute of Technology

"Role of the MRL Protein Lamellipodin in Neuronal Migration and Axon Guidance"

The development of the nervous system involves extensive migration of axons and dendrites to establish the intricate synaptic network found in the mature nervous system. During neuronal development axons are guided to their respective targets in response to specific molecular cues. Axonal growth cones encounter a diverse array of guidance signals that must be integrated and transduced to the cytoskeleton to

enable them to migrate to the appropriate target. Although much progress has been made in identifying the guidance factors and their receptors, less is known about how these signals are converted into changes in the direction and rate of axonal migration. Members of the MRL (MIG-10/RIAM/Lpd) protein family are known regulators of cell motility, lamellipodial dynamics and adhesion that are highly expressed in the developing nervous system. Genetic evidence implicates MIG-10, the *C.elegans* orthologue of the mammalian proteins RIAM and Lpd, in neuronal migration and axon guidance downstream of both attractive and repulsive guidance factors.

We hypothesize that Lpd plays an important role in the migration and guidance of neurons. The aim of this proposal is to elucidate the requirement of Lpd in the developing nervous system through the analysis of conditional Lpd knockout mice. I will examine loss of function phenotypes for Lpd using histological and immunohistochemical analysis to investigate defects in neuronal proliferation, migration, neurite initiation and polarization, and axon guidance. I further propose to study the effect of loss of Lpd on growth cone response to Netrin in vitro using cultured Lpd null neurons.

In addition, I propose to characterize the interaction between, Lpd and Ena/VASP proteins with SHIP-2, a 5'phosphatidylinositol phosphatase and investigate its functional relevance. We have identified SHIP-2 as a novel Ena/VASP binding partner found in a ternary complex with Lpd. Depletion of SHIP-2 or Lpd by siRNA induced an increase in filopodia formation in an Ena/VASP dependent manner. I will test the hypothesis that relative levels of phosphoinositides at the leading edge of a cell function as a switch to favor filopodial vs lamellipodial modes of migration and investigate its role in chemotaxis.

Sophie Dumont, Ph.D.

Harvard Medical School

"Linking Mechanical Force to Kinetochore Chemistry and Motility"

Cell division is fundamental to life. Without cell division we cannot grow or develop, we cannot reproduce, and we cannot repair damage. The main task of cell division is to accurately segregate chromosomes, moving one copy of the newly replicated DNA into each daughter cell. Even small errors in this process can be devastating, leading to cancer and birth defects such as Down's syndrome. How does the cell coordinate the movement of its chromosomes and accurately deliver the two copies to different daughter cells? This problem is fundamentally a mechanical one, and our understanding of how mechanical forces regulate kinetochore motility and checkpoint chemistry is still poor. Chemical and genetic spindle perturbations have provided us with a long list of molecules involved in chromosome movement and segregation, but we do not know how the molecules come together to generate and detect forces in vivo. In large part, this is because the experimental systems available have been either mechanically or molecularly tractable, but not both. I have developed a novel method, 'spindle flattening', to apply externally controllable forces to the spindle and kinetochores of mammalian cells (Ptk2) that will allow me to combine mechanical perturbations with molecular ones. Preliminary data show that spindle size responds dramatically to mechanical force and that microtubule bundles attaching to

kinetochores are inextensible without new tubulin addition, allowing us to effectively pull and push on kinetochores. I propose to examine kinetochore motility, structure and chemistry under both internal and external mechanical perturbations.

1. I will image oscillating sister kinetochores at high resolution and track their movement to determine how the motion of one kinetochore depends on the motion of its sister and the tension between them, and use spindle flattening to probe how externally applied pushing and pulling forces affect kinetochore motility.

2. I will examine how the tension on a kinetochore changes its protein architecture, composition and the chemical state of key signaling molecules. This approach promises to provide significant new insight into a longstanding, central problem in cell biology, the question of how kinetochores detect and respond to tension.

Jesse Goldberg, M.D., Ph.D.

Massachusetts Institute of Technology

"Basal Ganglia-Thalamic Interactions in Behaving Songbirds during Learning"

Listening to a toddler babble is fascinating-she is trying to communicate, but cannot yet coordinate her vocalizations. To learn such a complex action sequence, she must formulate a goal, vocalize, listen to herself, and evaluate her sound. How do neural circuits carry out these basic functions? It has been proposed that the basal ganglia (BG) brain circuit implements such trial and error motor learning, which is impaired in BG-related diseases such as Parkinson's and dystonia. But how BG output signals implement learning and how they go awry in disease is poorly understood. It is known that the BG output, the inhibitory pallidal projection to thalamus, is tonically active and exhibits brief pauses during movement. A dominant model posits that these pallidal pauses constitute the main BG output signal, allowing thalamic neurons to burst when disinhibited. However, this pause-burst model has not been tested in freely moving animals, and it remains unknown how BG output signals contribute to learning. The songbird is an ideal model system to address these questions. First, songbirds have a discrete BG circuit dedicated to song learning that contributes to vocalizations in real time, providing an opportunity to record the BG circuit in its natural context. Second, the BG output in songbirds is unusually large and accessible. In preliminary results, I found that by implanting electrodes into motor thalamus, I could record both resident thalamic neurons as well as large pallidal axon terminals that originate in the BG. Surprisingly, pallidal terminals and the thalamic neurons they are supposed to inhibit simultaneously increased their activities as the bird sang. My goal is to use the songbird system to examine BG-thalamic signaling, and to clarify how these signals contribute to learning and disease. 1) I will record from the motor thalamus in singing juvenile songbirds, to test the hypothesis that the BG-thalamic signals contribute to trial and error learning. 2) I will record from connected pallidal-thalamic pairs during singing, to test the pause-burst model of BG output. 3) I will develop deep brain stimulation in birds, to examine how controlling BG output affects singing and downstream thalamic signals.

Dominique Helmlinger, Ph.D.

Harvard Medical School

"Regulation of Gene Expression by Coactivator Complexes in Eukaryotes in Schizosaccharomyces Pombe"

How a cell responds to developmental or environmental changes by altering gene expression is one of the most fundamental and widely studied biological questions. One critical level of regulation is transcription initiation. This step involves the coordinated activities of several multiprotein complexes, including transcription coactivators. Coactivators possess multiple different activities and little is known about how these activities integrate signals from the environment and contribute to the fine tuning of gene expression in eukaryotic cells. My work has established the SAGA coactivator complex from the fission yeast *Schizosaccharomyces pombe* as an excellent model to address this aspect of coactivator function. We have discovered that, in *S. pombe*, SAGA regulates the switch from proliferation to sexual differentiation in response to a change in environmental conditions. In addition, we have initiated a comprehensive biochemical and functional analysis of the *S. pombe* SAGA complex and found that some its subunits have different *in vivo* roles between *S. pombe* and *S. cerevisiae*.

The overall objective of this proposal is to address key issues in transcriptional control in eukaryotes by focusing on the different roles of the SAGA complex *S. pombe*. This proposal contains two sets of experiments. The first set addresses the mechanisms by which different components of SAGA regulate the expression of differentiation genes and how distinct SAGA activities are regulated by changing environmental conditions. One important outcome of these studies will be the identification and characterization of novel, non-histone acetylation targets of the SAGA subunit Gcn5, an acetyltransferase. In the second set, we will follow up on initial observations suggesting marked differences between *S. pombe* and *S. cerevisiae* in the biological roles of two SAGA subunits, Spt3 and Tra1. Biochemical and genetic approaches will be used to identify these roles and are likely to illuminate new mechanisms for the regulation of transcription initiation by multifunctional coactivators.

Stacey Kenfield, Sc.D., M.Sc.

Harvard School of Public Health

"Diet, Lifestyle, Biomarkers, and Prostate Cancer Survivorship"

Over 2 million men in the United States are prostate cancer (PCa) survivors. Many studies have examined dietary and lifestyle factors in relation to PCa incidence, but few have assessed their impact on PCa progression and survival. PCa has a very heterogeneous course; in some men, the disease rapidly progresses to metastases and death despite aggressive therapy, but in many, the course is indolent even with no treatment at all. Intriguing evidence suggests that diet and lifestyle after PCa diagnosis can affect prognosis. To address this important gap, I propose to evaluate three promising factors: fish intake, statin use, and physical activity and integrate these exposure data with biomarker data to explore relevant biologic pathways. These factors have been related to risk of advanced PCa, and have anti-angiogenic, anti-inflammatory, anti-proliferative, and pro-apoptotic effects, which may delay disease progression and improve survival. The specific aims are to examine the relation of PCa

recurrence and mortality with: 1) high fish intake (both before and after diagnosis); 2) fish intake and markers of angiogenesis in the tumor tissue; 3) statin use and markers of angiogenesis, apoptosis, proliferation, and inflammation in the tumor tissue; and 4) high levels of physical activity (both before and after diagnosis). I will use data from approximately 3,500 participants in the Health Professionals Follow-up Study (HPFS) diagnosed with PCa who are followed every 2 years since diagnosis for recurrence outcomes. The HPFS is an ongoing prospective cohort study of 51,529 men that began in 1986, and dietary and other covariate data are collected every two to four years. Cox proportional hazards regression will be used to analyze the associations between diet and lifestyle factors and time to PCa recurrence and PCa-specific death. The findings from this research will be useful in determining whether these three promising factors may be beneficial for the growing population of men diagnosed with PCa; the biomarker analyses will determine potential mechanisms for those effects.

Angela Leung, M.D.

Boston University Medical Center

"Breast Milk Iodine and Perchlorate Concentrations: Effect on Infant Thyroid Function"

Context: Breastfed infants are reliant on maternal iodine for the production of thyroid hormones required for normal neurodevelopment. Recent studies have demonstrated a decrease in the dietary iodine intake of U.S. women of childbearing age.

Environmental exposure to perchlorate, a competitive inhibitor of the sodium/iodide symporter (NIS), is ubiquitous in the U.S. Perchlorate exposure could potentially decrease NIS-mediated uptake of iodine into breast milk and infants' thyroid and could directly affect infant thyroid function. Thiocyanate, a byproduct of cigarette smoke and another inhibitor of NIS, may also decrease breast and thyroidal iodine utilization.

Objective: To cross-sectionally examine the relationships between iodine sufficiency of lactating women and their 1-2 month old infants, environmental perchlorate and cigarette smoke exposure, and infant thyroid function.

Participants: 275 healthy 1-2 month old breastfed infants and their lactating mothers in the Boston area.

Measurements: Concentrations of infant urinary iodine and perchlorate; infant serum TSH and free T4; maternal urinary iodine, perchlorate, and thiocyanate (as a marker for cigarette smoke exposure); and breast milk iodine, perchlorate, and thiocyanate.

Significance: The present proposal will be the first study to specifically examine relationships between iodine concentrations, perchlorate and cigarette smoking exposures, and thyroid function in breastfed infants. These data, far larger than any previous U.S. survey, will help to define adequate dietary iodine intake for breastfed infants and their mothers, and will also determine whether environmental perchlorate and cigarette smoking exposure have deleterious effects on infants' thyroid function.

Weikai Li, Ph.D.

Harvard Medical School

"Structural and Biochemical Basis of the Vitamin K Cycle"

Vitamin K epoxide reductase (VKOR) is a membrane embedded enzyme and the target of warfarin, the most commonly used oral anticoagulant. Warfarin is a coumarin drug used to treat and prevent thrombosis diseases including deep vein thrombosis, pulmonary embolism, stroke, and myocardial infarction. Warfarin has a narrow therapeutic window due to the high risk of hemorrhage and the design of safer VKOR inhibitors is prohibited by the complete absence of structural knowledge of VKOR.

We will use structural and biochemical approaches to understand the mechanism of VKOR catalysis and warfarin inhibition. 1) We have obtained crystals of a VKOR homolog that diffract to 3.6Å and have solved the structure by multiple isomorphous replacement. The phases and the resolution of the current VKOR structure will be further improved to obtain an unambiguous model. We will solve the VKOR structures in complex with vitamin K substrates and with warfarin and other coumarin drugs. We will make cysteine mutants in VKOR and its reducing partner to determine structures of reaction intermediates. 2) We will use purified VKOR proteins to study the biochemistry of VKOR catalysis and warfarin inhibition, which will complement the knowledge from the VKOR structures. Mutations will be designed to identify interactions essential for VKOR catalysis and warfarin inhibition. Finally, mutagenesis experiments, combined with the structural information, will elucidate the exact pathway by which electrons are transferred by VKOR.

Since the VKOR structure is the first of its kind, we believe that this will lead to the determination of a series of related structures. We will combine the structural information with biochemical studies to elucidate the mechanism of vitamin K catalysis and coumarin drugs as inhibitors. These studies will be the basis to design better anticoagulation drugs. The structural information of warfarin-resistance mutations can be combined with pharmacogenetics check of individual patients' genotypes to predict proper warfarin dosing and reduce the risk of hemorrhage.

Karen Lienkamp, Ph.D.

University of Massachusetts Amherst

"Nanotechnology for the Fight Against Multiple Resistant Bacteria - Self-cleaning, Cell-selective Antibacterial Surfaces for Medical Devices"

With multiple resistant bacteria spreading in hospitals and the community, there is an ever-increasing demand for materials that help contain and eradicate these pathogens. 2 million people are infected with these bacteria in US healthcare facilities every year; 100,000 of them die. The continuous increase of bacterial resistance to traditional antibiotics and the resulting nosocomial infections also have serious economical consequences, adding 5 billion US \$ per year to the nation's healthcare costs. Infected catheters contribute 45% to these figures. Only a few bacteria that contaminate the surface of a medical device can develop a biofilm in less than 24 hours, causing infection and inflammation.

Thus, effective antibacterial surfaces that prevent biofilm formation comprise an immediate need. The aim of this project is to develop highly active antibacterial polymer surfaces which selectively kill bacteria, but are benign to mammalian cells. By incorporation of surface components that prevent cell and protein adsorption, these surfaces will be self-cleaning and long-term active. Previous studies have shown that, because the particular polymers used do not target specific cellular receptors but the cell membranes, resistance build-up is significantly retarded compared to traditional antibiotics. The surfaces will be obtained by texturing a substrate with covalently attached nanometer-scale patches of antibacterial polymer clusters within an antibiofouling polymer matrix. The resulting surface properties will be analyzed using diverse physical techniques (e.g. electron and atomic force microscopy, ellipsometry, infrared spectroscopy). Various in-vitro tests will be used to investigate their antimicrobial properties (Kirby-Bauer-assay, bacteria spraying experiments, live-dead stain), their compatibility with mammalian cells (erythrocyte hemolysis and adhesion) and their antibiofouling properties at the cellular and protein levels (fluorescence methods, reflectometry, lateral microscopy). Surface-bacteria interactions will be studied with and without the presence of background amounts of leukocytes, platelets, and erythrocytes.

Once we understand how surface texturing on the nano-scale affects antimicrobial activity and biofouling, robust materials that reduce or prevent the infection of patients with resistant bacteria can be obtained. If successful, these materials will significantly improve the quality of life of post-operation catheterized and long-term bedfast patients.

Alexander Loewer, Ph.D.

Harvard Medical School

"Dynamics of the DNA Damage Response in Individual Cells"

A major goal of systems biology is to understand the control of signaling pathways. This requires precise quantitative information about the dynamics of cellular responses. I focus on studying the dynamics of the p53 signaling pathway. Our lab has recently used long-term time-lapse microscopy studies on single cells and discovered that p53 levels show a highly unexpected pulsatile response to specific types of DNA damage. These repeated pulses had been masked in previous studies that measured p53 levels in populations of cells. I now plan to combine quantitative dynamic measurements in single living cells, mathematical modeling and manipulation of the p53 circuit to ask how, and why, the p53 signaling pathway generates this series of uniform pulses.

In my first aim I will examine whether the amount of DNA damage affects the number of pulses. I have developed a novel system for quantifying DNA double-stranded breaks (DSBs) in living cells and will use this system in parallel with tracking p53 pulses to ask whether the initial number of DSBs affects the number of p53 pulses, and whether a threshold of damage exists for the activation of p53. I will then examine how the repair rate affect p53 dynamics and how p53 dynamics feedbacks on repair. Next, I will determine how p53 pulsatile behavior is connected with specific cellular outcomes and with the activation of specific downstream programs such as apoptosis, cell cycle arrest and DNA repair. I will track p53 dynamics in parallel with marker proteins for

downstream programs in single living cells, and identify the fate of each imaged cell. I will manipulate the control circuit to alter or eliminate p53 pulses, and ask how these changes affect the outcome for the cell. I will quantitatively measure protein dynamics with high temporal resolution in single living human cells using time-lapse microscopy and combine the resulting data with mathematical models.

The p53 network is perhaps the most important pathway preventing the initiation of cancer. Understanding it in a quantitative, predictive way will help analyzing the effects of therapeutic interventions in cancer, and may also suggest entirely new therapeutic approaches.

Michelle Longworth, Ph.D.

Massachusetts General Hospital

"Rb Dependent Mechanisms of Transcriptional Regulation by CAP-D3"

The retinoblastoma protein (pRB) was the first identified tumor suppressor protein, and its mutation is the rate limiting step in the genesis of retinoblastoma. pRB, p107 and p130, the three members of the human pRB family of proteins, and their Drosophila homologs, RBF1 and RBF2, are best known for their ability to bind to E2F/DP complexes and repress transcription. However, pRB has been suggested to bind to over 100 different proteins, and the characterization of many of these interactions has provided us with the current knowledge of pRB's role as a tumor suppressor. My previous research has uncovered a new interaction for the pRB family of proteins with the Condensin II subunit, CAP-D3, which is conserved in both Drosophila and human tissue culture cells. RBF1/pRB promote the localization of CAP-D3 to DNA. Importantly, in Drosophila, this newly discovered role for RBF1 is independent of its ability to repress dE2F/dDP mediated transcription, and promotes the uniform condensation of chromosomes in prometaphase of mitosis. However, the reason why RBF1/pRB facilitates the localization of CAP-D3 to DNA remains unknown. Preliminary data shows that decreased expression of dCAP-D3 in flies results in a significant upregulation of a number of genes previously shown to be regulated by RBF1. Combined with data that dCap-D3 mutants suppress Position Effect Variegation, it is quite likely that RBF1 interacts with dCAP-D3 to regulate transcription, and that this role might also be conserved in human cells. Therefore, the hypothesis to be tested in this proposal is that CAP-D3 complexes regulate transcription in an RB dependent manner which is conserved from Drosophila to humans.

In Specific Aim 1, I will perform microarray analyses in both Drosophila and human cells to identify and characterize genes/ gene families which are regulated by CAP-D3 in an RB dependent manner. The actual gene promoters and DNA loci that dCAP-D3 binds to which are dependent on the presence of RBF1 will be studied in Specific Aim2 through CHIP on chip analysis. In Specific Aim 3, I will determine which proteins associate with the CAP-D3/RB complex throughout the cell cycle in both Drosophila and human cells.

Justine Melo, Ph.D.

Massachusetts General Hospital

"Metabolic and Endocrine Control of Appetite in C.elegans"

Our understanding of the internal surveillance of metabolic circuits and how those circuits control appetite is extremely limited. In my research, I hope to identify the metabolic signals that regulate food-seeking behavior. I have conducted an RNAi screen of all essential and metabolic genes in order to identify gene inactivations that stimulate appetite in *C. elegans*. The rationale behind this strategy is that inactivation of endogenous metabolic pathways can be used to mimic dietary deficiencies. So far, I have identified ~400 genes which, when inactivated, cause animals to forage in search of alternative food sources. This list includes genes involved in basic lipid, carbohydrate and amino acid metabolism, sterol metabolism, oxidative phosphorylation, ribosome biogenesis, G protein-coupled receptors (GPCRs), neuropeptides and other secreted signaling molecules.

The appetite screen I've conducted has ultimately provided me with an extremely rich data set with which to start my own lab. My long-term goals are to study the mechanisms by which identified genes act to suppress appetite. In the final years of my post-doc, I hope to publish 2-3 papers validating this approach to identification of physiological pathways controlling appetite. The first paper will describe the screen itself, and will provide the first comprehensive anatomical map of nutritional & metabolic signaling in an animal (Aim 1). The next paper will provide a detailed follow-up of specific pathways identified in my screen, in which I hope to develop a functional connection between a metabolic signal and its signaling apparatus (Aim 2). In my proposal, I describe two potentially exciting examples -- metabolite signals resulting from glycolysis, and novel endocrine signaling involving components of the canonical hedgehog/patched developmental pathway. My third aim describes the identification of common transcriptional responses to stimulation of a foraging signal -- these responses are likely to act at the level of endocrine signaling to the nervous system or the genetic targets in the nervous system whose expression controls foraging behavior directly. In these follow-up papers, I hope to make the mechanistic connections originating in primary metabolic signals, relayed through endocrine signals, and terminating in the nervous system by activation of food-seeking behavior.

Soyeon Park, Ph.D.

Harvard Medical School

"A Novel Pathway for Proteasome Biogenesis and Its Regulation"

The proteasome is essential in eukaryotes, and regulates many fundamental cellular processes, including the cell cycle, transcription, and apoptosis. In the proteasome, the proteolytic core particle (CP) is associated with the regulatory particle (RP), which in turn consists of the base and lid. The 10-subunit base is responsible for the recognition, unfolding, and translocation of substrates into the CP to be degraded. Six ATPases (Rpt1-6) form a heteromeric ring, which is central to base function. The Rpt ring sits directly atop the CP, bridging lid to CP. I have begun to investigate how the Rpt ring assembles. I have found that two specific Rpts initiate Rpt ring assembly by using the heteroheptameric outer ring of the CP as a template. During these events, two

precursor complexes form; BP1 and BP2 (Base Precursor 1 and 2), each containing a subset of Rpts. Base assembly is regulated by three novel chaperones, which bind specific Rpts. Upon correct Rpt-CP binding, two chaperones are released from BP2, allowing for BP1 to join the nascent complex and to complete the Rpt ring, with release of the third chaperone from BP1. The base assembly pathway is conserved between yeast and mammals. I will employ both systems. Base assembly will be studied in "real time" by pulse-labeling cells with ¹³C-methionine. ¹³C-methionine incorporation into precursors will be quantified by mass spectrometry based on the ¹³C/¹²C mass difference. This method will be coupled with native PAGE, which resolves precursors from mature base. Interestingly, I found BP2 levels to be linked to metabolic regulation, suggesting that BP2 defines a key control point for base assembly. I will attempt to identify the regulatory mechanisms that mediate metabolic control of base assembly. One of the two BP2 chaperones, Gankyrin/Nas6, is an oncoprotein. The basis for Gankyrin's oncogenicity remains uncertain. In the proteasome, Gankyrin/Nas6 specifically binds to Rpt3. During base assembly, Nas6 is released from Rpt3 upon Rpt3 binding to CP, which marks a critical step in proteasome biogenesis. I will investigate whether the function of Gankyrin in proteasome assembly contributes to its oncogenic properties.

Kirthi Reddy, Ph.D.

Massachusetts Institute of Technology

"Genetic Analysis of Innate Immune and Behavioral Responses to Pathogens in C. elegans"

The innate immune system is critical for survival, as it functions to recognize and respond to invading microbes in a generalized manner. Studies of innate immunity in mammals and invertebrates have revealed that diverse organisms use similar mechanisms to defend themselves against microbial pathogens: the key signaling pathways of the innate immune system are evolutionarily conserved. I am carrying out studies of immunity in the nematode *Caenorhabditis elegans*, in which it is known that conserved innate immune signaling pathways are activated in response to bacterial infection. *C. elegans* also responds to pathogenic bacteria with the induction of pathogen avoidance behaviors. I am using a genetic approach to characterize the mechanisms that affect the ability of *C. elegans* to survive bacterial infection by the human opportunistic pathogen *Pseudomonas aeruginosa*. Many genes involved in the innate immune response of *C. elegans* have been identified through genetic screens using either mutagenesis or RNAi. Here, I propose a complementary approach to identify new immunity genes by studying naturally occurring polymorphisms that lead to variation in resistance to pathogen infection. To date, my research has identified and characterized one such polymorphism that affects pathogen susceptibility through changes in behavior. In addition, I have identified a neuronally-expressed gene that is required for pathogen resistance in *C. elegans*. I will define the mechanisms underlying the pathogen susceptibility caused by mutation of this gene and will test the hypothesis that this gene is involved in the neuroendocrine regulation of *C. elegans* immunity. I anticipate that the genetic dissection of pathogen resistance in *C. elegans* will enhance our knowledge of pathogen recognition and defense, with implications for the understanding of the evolution and function of mammalian innate immunity. These studies may contribute to our understanding and treatment of a variety of disorders

such as septic shock and chronic inflammation as well as immunomodulatory therapeutics that might improve the efficacy of vaccines.

Przemyslaw Mike Sapielha, Ph.D.

Harvard Medical School

"Influence of Omega-3 Long Chain Polyunsaturated Fatty Acids and Cyclooxygenase Inhibition on the Progression of Retinopathy"

Ischemic proliferative retinopathies such as diabetic retinopathy (DR), are the leading cause of blindness in middle age in the industrial world. They are characterized by an initial phase of vascular dropout followed by a compensatory and deregulated neovascularization which can ultimately culminate in retinal detachment.

DR is increasingly thought to involve an inflammatory component. Relevantly, lipid based molecules act as effectors of inflammation and angiogenesis; particularly potent are certain eicosanoids, derived from the 20 carbon long chain omega-6 polyunsaturated fatty acids (LCPUFAs), arachidonic acid (AA, C_{20:4n-6}) via the cyclooxygenase (COX) pathways. Conversely, the omega-3 LCPUFA eicosanoic acid (EPA) is the substrate for anti-inflammatory mediators and suppresses the production of pro-inflammatory eicosanoids. As LCPUFA tissue status is modified by and dependent on dietary intake, these nutrients are reasonable choices for interventions to prevent DR with foods that are not readily consumed in the Western diet.

We hypothesize that moderate physiological dietary doses of omega-3 LCPUFA in conjunction with COX-2 inhibitors will have synergistic effects on preventing retinopathy and will influence expression profiles of genes associated with the disease.

Aims: Here we will: 1) Evaluate the inhibitory effect of omega-3 LCPUFA rich versus omega-3 LCPUFA deficient diets in a hypoxia-induced proliferative retinopathy mouse model, alone and in combination with COX inhibition. 2) Use a systems biology molecular mapping approach to determine gene expression profiles of specific factors related to lipid metabolism, inflammation and angiogenesis. 3) Determine interventions to suppress retinopathy, complementary to omega-3 LCPUFA by investigating the contribution of 4 pathways known to promote retinopathy and against which exist FDA approved drugs (antagonists of TNF-alpha, iNOS, VEGF, and MMP).

Although much effort has been invested in investigating the role of growth factors in retinopathies, considerably less is known of the influence of lipids. We expect the translational research proposed in this study to form the foundation for a clinical trial to evaluate prevention or delayed progression of DR with omega-3 LCPUFA intake and COX inhibition. The potential impact of this work on DR is great since nutritional interventions are safe, inexpensive and readily put into practice.

Yifeng Zhang, Ph.D.

Harvard University

"Genetic Dissection of Neural Processing in the Mouse Retina"

Vertebrate retina carries out a considerable amount of processing and compression of the visual signal through a network of a large variety of interneurons. Much has been

done to identify and classify the different neuronal types in the retina by neuroanatomical and molecular approaches, yet there is only limited understanding of the functions these different types of neurons perform. We propose to use a combination of molecular genetics and electrophysiological approaches to dissect the visual processing performed by the mouse retina.

In Specific Aim 1 of this proposal, we will study the synaptic mechanism underlying the function of a novel type of direction selective retinal ganglion cells, the J-RGCs. These cells have been genetically labeled with a fluorescent protein, and can be targeted specifically for whole cell voltage and current clamp recordings. We will study the spatial and temporal properties of the synaptic inputs these cells receive under different conditions to understand the mechanisms that give rise to their direction selectivity. In Specific Aim 2, we will develop genetic tools for manipulating the activity of retinal neurons to study their functions. We will establish a transgenic mouse system to target the expression of the "effectors" into subsets of retinal neurons. We will take advantage of the Cre recombinase mediated expression control via removal of transcription STOP cassettes. Expression of ligand-gated effectors will allow manipulation of the neuronal activity with temporal control, and in a dose-dependent manner. Using multi-electrode array technique, we will record and analyze the response of the retinal neurons at a population level to any visual input, before and after the activation of the effectors. We will be able to infer the function of the targeted neuronal subsets by studying the response properties of such retina under different conditions.

This research will contribute to a better understanding of the mouse retinal circuitry and the mechanisms underlying the encoding of the visual information.

2008 Grant Recipients

Vikas Bhandawat, Ph.D.

Harvard Medical School

"Probing the circuit determinant of sensory detection-threshold using specific cellular lesions"

Our ability to perform varied and complex tasks is a result of computations performed by neurons in our brain. A striking feature of the brains of most animals is that it contains an astronomical number of neurons. Furthermore, each neuron receives signals from a large number of other neurons. It would seem that the large number of neurons is necessary because of the complexity of the computations our brains perform. But, it turns out that even for simple problems the brain uses a large number of neurons. Also, many neurons carry essentially redundant information. It is believed that pooling of information from many neurons increases the reliability of computations performed by our brains. Whether these gains are achieved in an actual brain is an important, yet largely unexplored problem in neuroscience. This question is also of central importance to understanding the pathology of neurodegenerative diseases where specific pools of neurons are depleted. Here, we propose to use a simple, genetically tractable neural circuit to address this issue. Our model circuit is the *Drosophila* antennal lobe (a part of the brain that processes olfactory information), which offers the experimental

advantages of genetic accessibility, an organized anatomy, and a quantifiable pool of input neurons. Fruit flies have neurons in their antenna (called olfactory receptor neurons or ORNs) that bind to odors and report these binding events to projection neurons (PNs) in the antennal lobe. Each PN receives input from a homogenous ("redundant") population of ~60 ORNs, all of which share the same odor – response profile. The specific aims of this project are:

1. Since ~60 ORNs are connected to a single PN, we expect that PNs will be more reliable in their response to odors than ORNs. We will record from ORNs and PNs and measure odorant detection threshold based on the responses of these neurons.
2. We will genetically reduce the number of ORNs and determine how reducing the number of first – order neurons affects neural and behavioral thresholds.

These experiments will yield quantitative predictions for how pooling of information from redundant neurons increases the reliability of computations performed by the brain. This project would provide insight into fundamental questions that are relevant to understanding neurodegenerative diseases where specific pools of neurons are depleted.

Melanie Brinkmann, Ph.D.

Whitehead Institute for Biomedical Research

"Regulation of TLR signaling by UNC93B and herpes virus"

The human body's defense against invading pathogens such as viruses and bacteria is mediated by two components of the immune system: innate and adaptive immunity. Both components recognize microorganisms as "non-self" and efficiently lead to their elimination. Upon infection of the host by invading pathogens the innate immune system constitutes the first line of defense, and members of a protein family named Toll-like receptors are essential players in it. They specifically recognize "patterns" of bacteria or viruses and set the infected cell and surrounding cells into an alert state by inducing the production of messenger substances. In order to recognize the intruders, the Toll-like receptors need to travel to distinct locations within the infected cell, where they meet and bind the "patterns" of pathogens such as nucleic acid or proteins. How the traveling of Toll-like receptors is initiated is largely unknown. With this project I am going to address how specific Toll-like receptors travel to the location where they meet invading pathogens which is the prerequisite for their efficient elimination.

Herpes viruses establish lifelong persistent infections by employing mechanisms to evade the host's immune system to prevent their elimination. Multiple ways to evade the adaptive immune response have been described, but little is known about viral strategies to escape the innate immune response mediated by Toll-like receptors. My preliminary data suggests that herpes viruses can interfere with Toll-like receptors, preventing them from setting the cell on alert upon an infection. I will address the viral factors and mechanisms by which herpes viruses prevent their recognition by Toll-like receptors. For that, I will infect cells with viruses that carry deletions in the viral genome and screen for a virus that is no longer able to block Toll-like receptors.

Knowing how the trafficking of Toll-like receptors is regulated and how herpes viruses interfere with Toll-like receptors will help design strategies that may counteract the maneuvers used by herpes viruses to escape immune destruction. The aims of this project are designed to achieve that goal.

Craig Ceol, Ph.D.

Children's Hospital Boston

"Identifying events and genetic regulators of melanoma progression using the zebrafish *Danio rerio*"

Melanoma is the most aggressive and lethal form of skin cancer, accounting for nearly 8,000 deaths per year in the U.S. alone. I am using the zebrafish *Danio rerio* to characterize the effects of known and identify new genetic alterations that cause melanoma. Most human nevi (moles that are sometimes precancerous) and melanomas have mutations that overactivate the BRAF gene, suggesting that BRAF overactivation is an important but insufficient step in tumorigenesis. Expression of the human overactive BRAFV600E mutant gene in zebrafish causes nevi formation and, in combination with a mutation in the p53 tumor suppressor gene, causes melanoma. The progression of normal melanocytes to melanomas will be explored. Preliminary data have shown that overactive BRAF causes melanocytes in zebrafish and mammalian cells in culture to become binucleate. The mechanisms by which these cells become binucleate and whether binucleate cells can act as intermediates in tumor formation will be examined. The transparency of zebrafish skin allows melanocytes and early melanocytic lesions to be easily identified. The accessibility of these lesions will be exploited to determine when the genetic instability and blood vessel formation that facilitate tumor growth first occur. In addition, fluorescently-labeled melanocytes from melanocytic lesions will be isolated for genome-wide analyses. These studies are designed to analyze, in detail, melanocyte number and morphologic changes that occur during melanoma progression and identify the genetic changes that cause or accompany cell number and morphologic shifts. Genes that regulate melanoma onset and other characteristics will be identified and studied. I have developed a means to test, in a high-throughput fashion, candidate melanoma genes. Genes that are present in extra copies and potentially overly functional in human and zebrafish melanomas will be identified. They and other candidates will be tested for effects on melanoma onset, invasiveness and metastasis. These studies may identify diagnostic and prognostic indicators of disease as well as therapeutic targets for cancer treatment.

Daniel Denning, Ph.D.

Massachusetts Institute of Technology

"Identification of ced-3- independent and caspase-independent mechanisms of cell elimination in *C. elegans*"

Programmed cell death (also known as apoptosis) is a genetically regulated mechanism by which animal cells are eliminated in a control manner. Apoptosis occurs during normal development and is also a means of killing and removing damaged, virus-infected, or cancerous cells. Consequently, the dysregulation of programmed cell death is a hallmark of cancer, neurodegeneration, autoimmunity, and many other disorders. The evolutionarily conserved genetic pathway that regulates apoptosis was

identified and characterized in studies of the roundworm *Caenorhabditis elegans*, the development of which involves the deaths of specific cells. Most apoptotic deaths in *C. elegans* require the gene *ced-3*, which encodes a member of the caspase family of proteins. However, some cell deaths can occur in mutants that completely lack *ced-3*. To date, my Postdoctoral research has focused on characterizing *ced-3*-independent cell deaths and identifying the genes that regulate them. To this end, I have demonstrated that a second caspase gene, *csp-1*, contributes to apoptosis in *C. elegans*. Therefore, as in vertebrate cells, multiple caspases promote apoptosis in the worm, and my observation provides a means for studying how these different caspases are regulated. Additionally, I have shown that some *C. elegans* cell deaths occur in the complete absence of caspase activity, resolving a longstanding question whether *ced-3*-independent deaths are in fact caspase-independent. Specifically, I identified two different types of cell elimination that do not require caspases: the first resembles normal apoptotic deaths, whereas the second is strikingly different and involves an extrusion mechanism that expels unwanted cells from the developing worm. Vertebrates also employ caspase-independent mechanisms of cell removal; however, we know very little about the genes that control these mechanisms. I propose to identify the genetic pathways that regulate *csp-1*-mediated killing and the caspase-independent cell elimination processes. I hope to elucidate novel genetic pathways that activate apoptosis or the extrusion of unnecessary cells in *C. elegans*. These studies might facilitate the discovery of similar pathways in vertebrates, contribute to our understanding of diseases like cancer, neurodegeneration and autoimmunity in which apoptosis is disregulated, and identify new targets for therapies to treat these disorders.

Erica Larschan, Ph.D.

Brigham and Women's Hospital

"Mechanisms for targeting histone modifications to regulate gene expression"

DNA is the hereditary material, composed of genes that encode proteins required for every function within a living cell. In order to grow and divide, all cells must tightly regulate which genes are turned on and off at different times. When gene regulation is disrupted, uncontrolled cell growth can occur, causing cancer. If stretched end-to-end, the DNA within each microscopic cell would extend for approximately one yard. Therefore, DNA must be tightly wrapped and compressed to fit within the nucleus of each cell. Our work aims to understand how the packaging of DNA controls when genes are turned on or off. We are using a sophisticated model organism, the fruit fly, to study gene regulation. When packaging of DNA is disrupted in the fruit fly, a blood disorder like leukemia can occur. We hope to understand how disrupting DNA packaging causes this leukemia and other cancers. Because DNA packaging factors are very similar in fruit fly and human cells, our research will be applicable to human cancers, where proteins involved in DNA packaging could be potential targets for anti-cancer drugs. Thus far, our work has provided significant insights about how this process of DNA packaging occurs but also allows important genes to be accessed when necessary. My experiments have identified the specific DNA sequences which are targeted by factors involved in gene packaging. Furthermore, I have identified a key regulator in this process which provides a new target for anti-cancer drugs. Many human cancers including leukemias are caused by misregulation of this type of

packaging. We hope our further studies will yield more insight into how this link can be targeted to inhibit cell proliferation in human leukemias.

Carlos Lopez, Ph.D. (supported by The Harold Whitworth Pierce Charitable Trust)

Harvard Medical School

"Exploring variability in the ErbB signaling network"

The nature of cellular signaling is such that the interactions of chemical entities at the atomic and molecular level results in observable biological responses. Understanding these events, at the interface where molecular interactions transition into life-processes is extremely challenging yet necessary to better treat diseases such as cancer. The most common approach to modeling dynamic chemical signaling in cells involves the tedious task of writing mathematical equations (usually by hand) that describe the reactions in a cellular signaling network. This is reasonable for a few tens of equations but this approach quickly fails for larger models composed of hundreds to thousands of equations, such as those related to cancer signaling due to human limitations to keep track of thousands of equation parameters and how they relate to each other. To alleviate this, I propose the development and implementation of a, so-called, "rules-based" methodology which will allow non mathematics-oriented scientists to approach the modeling problem from a conceptual framework. In rules-based models, the researcher describes the reactions present at a conceptual level as opposed to writing the explicit differential equations needed describe the system. The rules software I will implement and further develop then translates these reactions into the differential equations automatically, saving much time, as well as allowing modeling of far larger and complex systems. The system of choice for this modeling effort will be the epidermal growth factor receptor (EGFR) signaling network. Damage in this network highly correlates with uncontrolled tumor growth in several cancer phenotypes including lung and breast cancers. I expect that the outcome of this project will have significant impact in both, understanding how such chemical signaling networks fail and become deleterious as well as developing tools which will be useful to a broad base of biological researchers. The work I will develop will therefore also have a significant outreach component by coupling my work directly with ongoing efforts in our lab to disseminate scientific data using electronic wiki-based world-wide-web approaches.

Michele Markstein, Ph.D.

Harvard Medical School

"Exploiting drosophila models of stem cell derived colon cancer in high-throughput genetic and chemical screens"

An emerging theme in cancer biology is that stem cells, or cells with stem-cell properties, drive the unregulated growth and metastasis of human tumors. For example, transplantation studies have shown that in breast, brain, blood, prostate, and colon tumors, only a small fraction of cells those with the stem cell properties of self-renewal and differentiation can propagate tumor formation when transferred to a host animal. These findings indicate that regardless of how well a tumor is reduced, it can be expected to return unless all its cancer stem cells are eliminated. Thus, it is now

becoming clear that to design effective cancer therapeutics it is necessary to specifically target cancer stem cells.

This proposal aims to advance cancer therapeutics by conducting large-scale unbiased screens in the fruit fly *Drosophila melanogaster* to identify genes, microRNAs, and chemicals that can prevent the stepwise progression of stem cell colon cancer, the second deadliest of all human cancers. *Drosophila* is an ideal system for these studies because the stem cell biology of the *Drosophila* gut is highly similar to that in mammals. Moreover, I have already developed and optimized two models of stem cell cancer growth in the *Drosophila* gut. These models take advantage of genetic mutations in two genetic pathways known to also drive human cancers. In addition, I engineered *Drosophila* strains that permit me to readily quantify changes in the number of gut stem cells present in each fly. By being able to monitor the growth of gut stem cells so precisely, I can now screen many of the elements that can affect cancer growth, including genes, microRNAs, and chemicals.

The genetic and chemical screens outlined in this proposal should identify two classes of tumor suppressors: those that specifically target stem cell tumors caused by a particular genetic pathway and those that more broadly target all types of tumors. While both classes of tumor suppressors may be beneficial for humans, identifying pathway specific growth inhibitors will be especially exciting because they offer the best chance of directly targeting cancer stem cells. Moreover, by specifically targeting cancer stem cells, they are less likely to cause deleterious side effects in patients. Thus, the identified pathway specific genes, microRNAs, and chemicals, will be prioritized for validation in mammalian models of stem cell colon cancer.

Catherine Merrick, Ph.D.

Harvard School of Public Health

"Epigenetic control of virulence gene expression in the malarial parasite *P. falciparum*"

The proposed research concerns the most important human malaria parasite, *Plasmodium falciparum*. Malaria is one of the world's most debilitating infectious diseases, killing 2 – 3 million every year and affecting up to 300 million. Most of the deaths occur in young children in Sub-Saharan Africa. The lack of an effective vaccine and the emergence of drug-resistant parasites mean that there is now an urgent need for research leading to new vaccine targets and drug treatment strategies for malaria.

This parasite causes illness in humans via the cyclical infection of red blood cells. It multiplies inside these cells and modifies their surfaces with proteins that bind to the walls of blood vessels. This protects the infected cells from passing through the spleen, which might recognize and destroy them. It also contributes to disease, with severe malaria being particularly associated with the sequestration of infected cells in vessels of the brain and placenta.

To prevent the immune system from recognizing parasite proteins exposed on the surface of infected cells, *P. falciparum* regularly switches amongst different protein variants. It possesses a large family of genes for these proteins, and varies their expression by so-called 'epigenetic switching'. It can thus evade immunity and sustain a

chronic infection for months or years, ensuring its transmission to new hosts. Interfering with the switching process could be a key to more effective immune control of malaria.

This proposal focuses on the protein PfSir2, which has been shown to have a central role in controlling epigenetic switching. Experiments will be carried out to compare rates of switching in parasites with and without PfSir2. Any differences between the surface-expressed proteins in these two lines will also be measured, and the ability of each line to adhere to known blood vessel receptors will be assessed. Secondly, since PfSir2 is an enzyme, drugs that affect its activity could potentially influence switching. This idea will be tested using a known inhibitor of such enzymes and a screen for new, more specific drugs will then be conducted. These studies will lead to a better understanding of the mechanisms underlying epigenetic switching in *P. falciparum* and may inform new drug strategies to combat malaria.

Satoshi Namekawa, Ph.D.

Massachusetts General Hospital

"Characterization of germline epigenetic information in mice"

During mammalian reproduction, the offspring receives different contributions from the father's sperm and mother's egg. Although genetic information encoded by the DNA sequence is exactly the same in both sperm and egg, distinct features unique to the father and the mother are memorized as heritable modifications surrounding the DNA sequence. Since these modifications do not change the underlying DNA sequence, they represent epigenetic features of the parents. During the process of sperm formation, heritable modifications occur to uniquely define the sperm's paternal origin. However, how this epigenetic information is established during sperm formation and transmitted to the offspring is unclear. I have discovered that the sex chromosomes (X and Y) are specifically modified during sperm formation in diverse classes of mammals and named this novel epigenetically-modified structure 'postmeiotic sex chromatin' (PMSC). Importantly, epigenetic differences in the sperm and egg are believed to be responsible for the early development of the embryo. Thus, the goal of my research is to understand how epigenetic information is established during sperm formation and transmitted to the next generation with special attention to PMSC as a model system.

Aim 1: Visualization of epigenetic modifiers of PMSC. I propose to visualize specific modifications of PMSC during sperm formation to trace their fates from the sperm to early embryo. To accomplish this, I will generate transgenic mice carrying fluorescent proteins fused to epigenetic modifiers of PMSC. I plan to characterize the father's epigenetic contribution to the early development of the offspring.

Aim 2: Large - scale characterization of epigenetic modifiers of PMSC

Kimie Ng, M.D., M.P.H.

Dana-Farber Cancer Institute

"Influence of the vitamin D pathway on survival in patients with colorectal cancer"

Nutritional therapies are the most common form of complementary therapy among cancer patients. A consensus panel of the American Cancer Society recommended,

"Properly conducted studies of the effect of nutrition and physical activity on the prognosis of cancer survivors are urgently needed, and should be a high priority for all academic and research funding agencies." A growing body of evidence suggests that vitamin D inhibits growth of colorectal cancer cells and may improve the survival of patients with colorectal cancer. Using data from two large ongoing cohort studies, the Nurses' Health Study and the Health Professionals Follow-Up Study (160,000 participants), as well as a large, completed National Cancer Institute sponsored chemotherapy clinical trial in stage III colon cancer, we will examine the influence of various aspects of the vitamin D pathway on colorectal cancer patient outcomes. We will evaluate the relationship between plasma levels of 25 – hydroxyvitamin D [25(OH)D], the main form of vitamin D in the blood, and survival in colorectal cancer patients, as well as the association between a predicted vitamin D score calculated from the main determinants of vitamin D status and patient survival. Moreover, we propose to investigate the impact of genetic variations in an important protein in the vitamin D pathway, the vitamin D receptor, as well as expression of the protein, on patient survival. Finally, interactions between patient blood levels of vitamin D and molecular factors within the tumors, such as K – ras mutations, will be explored. With the advantages of prospective longitudinal assessment of nutritional and lifestyle factors, paraffin – embedded tumor specimens to examine how vitamin D interacts with molecular alterations in tumors, prospectively collected blood and germline DNA specimens, and comprehensive data on cancer recurrence and mortality provided by these cohorts, we hope to further elucidate the role of the vitamin D pathway in colorectal cancer. Our results could potentially lead to a better understanding of the underlying biology of colorectal cancer, and to the identification of novel targets for therapeutic intervention.

Joo-Seop Park, Ph.D.

Harvard University

"The molecular regulation of nephrogenesis in the mammalian kidney"

The mammalian kidney comprises hundreds of thousands of filtering units called nephrons. Their major functions are to remove waste from the blood and to maintain water/salt balance of the body. Although a nephron has a complex tubular shape associated with tiny blood vessels, it is derived from a small ball-like structure called the renal vesicle. The formation of renal vesicles is the first step of nephrogenesis. Beta-catenin, a key component of the canonical Wnt signaling pathway, is known to play an important role in the formation of renal vesicles. Activation or inactivation of beta-catenin can initiate or abolish this process, respectively. It is known that beta-catenin can turn on certain genes during body formation and in some disease conditions. However, little is known about which genes beta-catenin can turn on or off in developing kidneys. I propose to identify these genes by locating binding sites of beta-catenin complexes through out the genome of nephron progenitor cells. In addition, I will profile changes of gene expression caused by activation of beta-catenin in the same cells. Combining two sets of these data will allow genome-wide identification of genes, whose expression is regulated directly by beta-catenin during the formation of renal vesicles. Identification and characterization of direct targets of beta-catenin will profoundly advance our current understanding of not only the mechanism of nephrogenesis but also general responses of the canonical Wnt signaling pathway,

which is important in many biological processes including development and cancer. Currently, two major treatments of chronic renal failure are kidney transplant and dialysis. The adoption of cell-based strategies, which has great potential to improve the outcome of the wide spectrum of kidney diseases, requires a sound understanding of the molecular mechanisms of kidney development and repair. The results from this proposal will lead to new insights that will educate our approach to better treatments of various kidney diseases.

Joshua Roffman, M.D.

Massachusetts General Hospital

"Altered one-carbon metabolism in schizophrenia: molecular and neuroimaging correlates of folate response"

Schizophrenia is a devastating neuropsychiatric disorder affecting 1% of the population. Although certain schizophrenia symptoms can be managed with antipsychotic medications, there remain no good treatment options for the most debilitating aspects of the disorder-cognitive impairment and negative symptoms. These symptoms substantially disrupt social and occupational function, and inflict a terrible burden on affected patients, their family members, and society at large.

Up to 80% of an individual's risk for schizophrenia relates to genetic factors, although the effects of individual genes have been difficult to measure. We are using a variety of new, cross-disciplinary tools to magnify the signals of risk genes, by examining their effects on the levels of molecules, cells, and regional brain function in schizophrenia patients. Our work has focused on a promising candidate gene, MTHFR, that plays an important role in how the body processes folate. Folate is a B vitamin that comes from dietary sources, and deficiencies in folate have been tied to a variety of neuropsychiatric illnesses including schizophrenia. The MTHFR gene breaks down dietary folate into methyl groups, which influence the activity of genes that regulate brain chemistry. We are particularly interested in how the MTHFR gene influences the expression of a protein called COMT, which affects dopamine signaling in a part of the brain that has been consistently implicated in schizophrenia, the dorsolateral prefrontal cortex (DLPFC).

We have shown that a common genetic variant in MTHFR increases the severity of negative symptoms and cognitive impairment in the disorder, and that these effects are worsened in patients with low folate intake. Using functional MRI scans, we have seen that the MTHFR gene also affects activity in the DLPFC during memory tasks in schizophrenia patients. The proposed research will determine whether MTHFR's effect on methyl groups and COMT expression drive its subsequent effects on DLPFC activity and cognitive impairment in schizophrenia. We will also determine whether treatment with folic acid helps to reverse the effects of MTHFR on COMT and DLPFC activity. These studies may provide vital insights into the genetic basis of schizophrenia, and will apply these insights directly to a promising new treatment for cognitive impairment.

Dan Stoleru, M.D., Ph.D.

Harvard Medical School

"Gene replacement with automatic and fully-regulated insulin release in type-1 diabetes"

Type-1 diabetes results from widespread autoimmune destruction of the insulin-producing beta-cells in pancreas. The absence of beta-cells leads to profound imbalances in glucose metabolism, and the life-threatening pathology defining diabetes.

Rapid and proficient regulation of insulin secretion is critical for maintaining a normal level of blood sugar, and beta-cells singularly possess mechanisms for producing and controlling its release. However, the simple replacement of beta-cells, either by transplants or, prospectively, by stem cell technologies, may not represent viable therapies, because of the fundamental problem of autoimmunity that would destroy the new cells just like the old.

To circumvent this obstacle, I propose that other cells in the body be therapeutically transformed into surrogate insulin-producing cells that correctly respond to changes in blood sugar and yet survive the attacks of the immune system. It has been previously shown that mice genetically manipulated to express insulin from endocrine K-cells in the intestine are protected from the effects of diabetes. This was explained by the natural resemblance between these intestinal cells and beta cells: K-cells secrete their hormones in an insulin-like pattern, immediately responding to food intake, and inhibiting secretion upon decreases in glucose concentration, as well. This suggested the notion that K-cells could provide the tight regulatory competence needed for glucose homeostasis, and could serve as beta-cell surrogates.

My goal is, therefore, to develop a therapeutic scheme that will persuade adult K-cells (and them only) to produce insulin in sustainable fashion. I am generating insulin gene-containing viral constructs that will allow insulin to be produced exclusively in K-cells. In addition, I am devising delivery techniques for targeting the therapeutic virus to the recently characterized intestinal stem cells (i.e., progenitors of K-cells). This will allow the insulin gene to be integrated in their genome and transmitted to all daughter cells for the entire life of the organism, while remaining inactive in all but mature K-cells. Several critical safety features will be provided by the extremely rapid turnover of intestinal cells: first, non-stem cells promiscuously infected by the vector are shed within days; second, the immune system may attack the novel insulin-secreting K-cells, but others will replace them fast and continuously. The strategy should provide a properly regulated supply of insulin while avoiding graft rejections and recurring autoimmunity, and lead to safe and effective clinical applications.

Eduardo Torres, Ph.D.

Massachusetts Institute of Technology

"Isolation and characterization of mutants that tolerate aneuploidy in yeast"

The genome of every organism is composed of a set number of chromosomes that remains constant through life. Humans have 22 pairs of autosomes (non-sex chromosomes) and a pair of sex chromosomes, XX for females or XY for males. Sophisticated mechanisms have evolved to supervise and maintain a constant number of chromosomes during cell division. Despite these mechanisms, mistakes occur where cells either lose or gain a copy of a chromosome. Cells that acquire an abnormal number of chromosomes are referred to as aneuploids. Aneuploidy is usually incompatible with life and is the major cause of spontaneous abortions. However, in humans, individuals with an extra copy of chromosomes 13, 18 or 21 can live and have either Edward's, Patau's or Down syndrome, respectively. In addition, almost all human cancer cells are aneuploid. Therefore, it is of great importance to study the effects of aneuploidy in cells. To that end, we have chosen to study aneuploidy in budding yeast. Yeast has 16 chromosomes and can be engineered to become aneuploid by gaining an extra chromosome. We created several yeast strains, each having an extra copy of a given chromosome and characterized them. Surprisingly, we found a common set of characteristics in these cells independent of the identity of the extra chromosome, suggesting that cells have a common response to aneuploidy. Among these characteristics are slower growth, increased cellular volume, and sensitivity to drugs that target cellular processes regulating protein production. The main conclusion of our first set of studies, which were published last year in *Science*, is that aneuploidy in cells leads to deleterious effects. My proposal now focuses on the search for key genes that help cells tolerate aneuploidy. For that purpose, our approach takes advantage of the latest technologies to systematically look for gene mutations and deletions that help yeast cells cope with aneuploidy. We predict that these genes will help shed light onto the nature of mutations observed in human tumors, which are also aneuploid and have misregulated cellular growth. More importantly, these studies have the potential for discovering genes that might serve as novel targets for chemotherapeutics.

2007 Grant Recipients

Briana Burton, Ph.D.

Harvard Medical School

"Mechanism of DNA Transport across Cell Division Membranes"

Hak Soo Choi, Ph.D.

Beth Israel Deaconess Medical Center

"PSMA-Targeted NIR Fluorescent Quantum Dots for Prostate Cancer Surgery"

Joern Coers, Ph.D.

Harvard Medical School

"A Mammalian RNAi Screen to Identify Host Resistance Factors to Bacterial Infections"

Rutao Cui, M.D., Ph.D.

Dana-Farber Cancer Institute

"The Suntan Response: the Transactivation of POMC/MSH and its Mimicking by Small-Molecular Compounds"

Markus Feuerer, M.D.

Joslin Diabetes Center

"Regulatory T Cells, Adipose Tissue and Insulin Resistance"

Wilhelm Haas, Ph.D.

Harvard Medical School

"Combining Chemical Biology and Proteomics to Decipher the Ubiquitin-Proteasome System"

Yujin Hoshida, M.D., Ph.D.

Massachusetts Institute of Technology

"Prognostic Prediction of Hepatocellular Carcinoma Using Fixed Tissue-based Gene Expression Profiling"

Jennifer Hughes, Ph.D.

Whitehead Institute for Biomedical Research

"Insights into Male Infertility from Sequencing the Rhesus Macaque Y Chromosome"

Mary Keebler, M.D.

Massachusetts General Hospital

"Identification of Common DNA Sequence Variants Related to Blood Lipid Traits"

In-Jung Kim, Ph.D.

Harvard University

"A Novel Strategy to Map and Manipulate Neuronal Connectivity in Visual System"

Fernando Monje-Casas, Ph.D.

Massachusetts Institute of Technology

"Asymmetric Localization of MEN Components in *Saccharomyces cerevisiae*"

Jaime Murphy, M.D.

Boston University Medical Center

"Circulating Mesenchymal Precursors with Fibrogenic Potential in Asthma"

Nicolas Preitner, Ph.D.

Harvard Medical School

"Axon Guidance at the Spinal Cord Midline: RNA-based Regulatory Mechanisms"

Miguel Rivera, M.D.

Massachusetts General Hospital

"Characterization of WTX, a Novel Tumor Suppressor Frequently Inactivated in Wilms Tumor"

Satoshi Yoshida, Ph.D.

Dana-Farber Cancer Institute

"Regulation of Rho1GTPase in Budding Yeast"

2006 Grant Recipients

QueeLim Ch'ng, Ph.D.

Massachusetts General Hospital

"Genome-Wide Analysis of Dense Core Vesicle Secretion in *C. elegans*"

Chris D. Ellson, Ph.D.

Massachusetts Institute of Technology

"Understanding Signaling in Primary Neutrophil Apoptosis – A Systems Biology Approach"

David J. Freedman, Ph.D.

Harvard Medical School

"Neural Mechanisms of Visual Category Learning"

Javier E. Irazoqui, Ph.D.

Massachusetts General Hospital

"Signaling Pathways Controlling Innate Immunity in *Caenorhabditis Elegans*"

Patricia Jensen, Ph.D.

Harvard Medical School

"Genetic Sublineages of the Mammalian Serotonergic System"

Serena Mascieri, M.D.

Dana-Farber Cancer Institute

Prevalence of Germline E-Cadherin Mutations in Women with Lobular Breast Cancer

Maitreyi Mazumdar, M.D., M.P.H.

Children's Hospital Boston

"Health Insurance and Utilization of Services by Children with Epilepsy"

Avital Rodal, Ph.D.

Massachusetts Institute of Technology

"Regulation of the Synaptic Actin Cytoskeleton by Nwk"

Susanne Schlisio, Ph.D.

Dana-Farber Cancer Institute

"Neuronal Apoptosis by the Prolyl Hydroxylase EglN3: Hypoxia Sensing and Cancer"

David M. Smith, Ph.D.

Harvard Medical School

"Analysis and Inhibition of Proteasomal Regulation by ATPase Complexes"

Judith Stegmüller, Ph.D.

Harvard Medical School

"Control of Axonal Growth by the Cdh1-APC-SnoN Signaling Pathway"

Tatsuro Takahashi, Ph.D.

Harvard Medical School

"Establishment of Sister Chromatid Cohesion in Vertebrates"

Steven A. Vokes, Ph.D.

Harvard University

"Genome-Scale Identification of the Shh Regulatory Network in the Limb Bud"

Qin Yang, M.D, Ph.D

Beth Israel Deaconess Medical Center

"Maternal Elevation of Serum Retinol Biding Protein (RBP4) Causes Insulin Resistance in Offspring"

Davide Zoccolan, Ph.D.

Massachusetts Institute of Technology

"The Rat as a Novel Model for Understanding Visual Object Recognition"

2005 Grant Recipients

Robert Ajemian, Ph.D.

Massachusetts Institute of Technology

"Dynamic Motor Learning in Alzheimer's Disease"

Anna Delprato, Ph.D.

University of Massachusetts Medical School

"Biochemical and Structural Analysis of the Rab/GEF Interaction"

Jinbo Fan, Ph.D.

Massachusetts Institute of Technology

"Linkage Disequilibrium Mapping on Candidate Genes Across Chromosome 6q16-22 Bipolar Disorder Locus"

Minkyu Kim, Ph.D.

Harvard Medical School

"Termination of Transcription by RNA Polymerase II"

Junhao Mao, Ph.D.

Harvard University

"The Roles of the Hedgehog Pathway in Adult Muscle Stem Cells and Rhabdomyosarcoma"

Emi Nagoshi, Ph.D.

Brandeis University

"Proteomic Analysis of Chromatin Binding Proteins Involved in Circadian Rhythms"

Melanie D. Ohi, Ph.D.

Harvard Medical School

"Structural Analysis of the Fission Yeast Spliceosome"

John S. Pezaris, Ph.D.

Harvard Medical School

"A Visual Prosthesis Based on Thalamic Stimulation"

Niels Ringstad, Ph.D.

Massachusetts Institute of Technology

"Molecular Genetics of Peptidergic and Aminergic Signaling in the C. elegans Nervous System"

Gerhard Schratt, Ph.D.

Children's Hospital Boston

"Investigating the Molecular Mechanism of BDNF-Regulated Local Dendritic Translation in Mammalian Neurons"

Tianzhi Shu, Ph.D.

Harvard Medical School

"Regulation of Neurogenesis by Microtubule-Associated Proteins (MAPs)"

Joseph Wade, Ph.D.

Harvard Medical School

"Regulation of Ribosomal Protein Gene Transcription in Budding Yeast"

Andrew Wilkins, Ph.D.

Beth Israel Deaconess Medical Center

"The Role of RhoBTB2 in Tumorigenesis"

Bin Zheng, Ph.D.

Beth Israel Deaconess Medical Center

"Regulation of GLUT4 Translocation by AMPK Signaling Transduction Pathways"

2004 Grant Recipients

Douglas Allan, Ph.D.

Children's Hospital Boston

"Regulation of Exocytosis at the Drosophila Neuromuscular Junction: Molecular Distinctions Governing Differential Secretion of Neurotransmitters, Neuropeptides, and Postsynaptic Retrogradely-Secreted Molecules"

Michael A. Brehm, Ph.D.

University of Massachusetts Medical School

"Sequential Viral Infections and Transplantation"

Daniel A. Butts, Ph.D.

Harvard University

"The Role of Visual Adaptation in Information Processing in Mammals"

Amy B. Hall, Ph.D.

Harvard Medical School

"The Role of Vav Proteins in Macrophage Migration, Polarity and Phagocytosis"

Tina Huang, Ph.D.

Tufts University School of Medicine

"The Relationship of Fish and Essential Fatty Acids to Dementia and Alzheimer's Disease"

Grzegorz Ira, Ph.D.

Brandeis University

"DNA Double Strand Break Repair in Yeast"

Sigall Kassutto, M.D.

Massachusetts General Hospital

"Predictors of Time to Virologic Suppression and Disease Progression after Initiation of Antiretroviral Therapy in Acute HIV-1 Infection"

Norman J. Kennedy, Ph.D.

University of Massachusetts Medical School

"JIP Scaffolding Proteins in Development and Disease"

Maurits F. Kleijnen, Ph.D.

Harvard Medical School

"Proteasome/Ubiquitin Function in Membrane Fusion"

Dana Borden Lacy, Ph.D.

Harvard Medical School

"Structural Studies of Anthrax Intoxication"

Juliette C. Madan, M.D.

New England Medical Center

"Indomethacin Use for Closure of Patent Ductus Arteriosus in VLBW Infants: Association with Necrotizing Enterocolitis and Spontaneous Intestinal Perforation"

Kenkichi Masutomi, M.D., Ph.D.

Dana-Farber Cancer Institute

"Functional role of Telomerase in the DNA Damage Response in Normal Human Cells"

M. Golam Mohi, Ph.D.

Beth Israel Deaconess Medical Center

"Role of Shp2 and its Binding Protein Gab2 in Leukemogenesis"

Adrian Salic, Ph.D.

Harvard Medical School

"Novel Regulators of the Kinetochore-Microtubule Interaction"

Haihong Shen, Ph.D.

University of Massachusetts Medical School

"Alternative splicing mechanism of spinal muscular atrophy (SMA)"

Efstathios Stratikos, Ph.D.

Harvard Medical School

"Structural Basis for the Antigenic Peptide Trimming Properties of the Newly Discovered ER Aminopeptidases ERAP1 and ERAP2"

Robert Wheeler, Ph.D.

Whitehead Institute for Biomedical Research

"Fungal Recognition by the Innate Immune System"

Jianxin You, Ph.D.

Harvard Medical School

"Treatment of Latent Viral Infections by Disrupting the Virus-Host Interaction"

2003 Grant Recipients

Rajeshwar Awatramani, Ph.D.

Harvard Medical School

"Conditional genetic manipulations at molecular intersection points: a novel, high resolution study of cell fate and circuit formation in the mouse hindbrain"

Nabeel Bardeesy, Ph.D.

Dana-Farber Cancer Institute

"Preclinical mouse model of pancreatic cancer"

Kendra K. Bence, Ph.D.

Beth Israel Deaconess Medical Center

"Tissue-specific deletion of PTP1B: role in resistance to diet-induced obesity"

Heike A. Bischoff, M.D., M.P.H.

Brigham and Women's Hospital

"Effects of Vitamin D&K on symptomatic Osteoarthritis of the Hip and Knee"

Steven Branda, Ph.D.

Harvard Medical School

"Cell-cell signaling in *B. subtilis* biofilm development"

Edda Fiebiger, Ph.D.

Harvard Medical School

"New approaches to study mechanisms that regulate MHC class II-dependent immune responses in vitro and in vivo"

Richard E. Frye, M.D., Ph.D.

Children's Hospital Boston

"Developmental phonological dyslexia: Neural mechanisms"

Peter J. Horn, Ph.D.

University of Massachusetts Medical School

"Heterochromatin structural organization"

Long Ma, Ph.D.

Massachusetts Institute of Technology

"Identification and characterization of C. elegans genes responsible for the promotion of apoptosis by chagocytic cells"

Emi Nishimura, M.D., Ph.D.

Dana-Farber Cancer Institute

"Melanocyte stem cells: Mechanism(s) for lineage renewal and relevance to melanoma"

Carl D. Novina, Ph.D., M.D.

Massachusetts Institute of Technology

"Genetic approaches to mammalian RNAi"

Ka-Ming Pang, Ph.D.

University of Massachusetts Medical School

"Regulation of asymmetric division in C. elegans"

Suzanne Paradis, Ph.D.

Children's Hospital Boston

"A role for ephrin/EphB Signaling in Synapse Formation and Maturation"

James Shorter, Ph.D.

Whitehead Institute for Biomedical Research

"Deconstructing how molecular chaperones intervene in prion conformational conversion and neurotoxicity"

Kim T. Simons, Ph.D.

Harvard Medical School

"Structural characterization of the yeast kinetochore"

Change Tan, Ph.D.

Harvard Medical School

"Cytokinesis and ring canal formation"

Guiliang Tang, Ph.D.

University of Massachusetts Medical School

"Investigation of miRNAs and their targets in plants and animals"

Christine Williams, Ph.D.

Massachusetts General Hospital

"The role of the Mi2b/NuRD chromatin remodeling complex in lymphocyte development and lymphomagenesis"