

Charles A. King Trust Postdoctoral Fellowship Program

Basic Science Research

2011 Grant Recipients

Daniel Bendor, Ph.D.

Massachusetts Institute of Technology

"Cortical-hippocampal Dynamics Governing Memory Consolidation"

The goal of my research is to understand how two brain areas (auditory cortex and hippocampus) are involved in storing memories. Previous work has suggested that the hippocampus plays a critical role in memory consolidation, temporarily storing a new memory and then transferring this memory to cortex during sleep. The proposed research uses electrophysiological, behavioral, and optogenetic methods to study the hippocampus and auditory cortex of rodents during memory consolidation of an auditory-spatial association behavioral task. A phenomenon central to this proposal is hippocampal replay, a process by which neurons in the hippocampus activated in a specific sequential pattern during a behavior get reactivated in the same way while the animal is sleeping (after the behavior). Hippocampal replay provides a neural correlate of the memory, and allows the experimenter to track when and where the memory is being transferred in the brain (e.g. by observing a coordinated replay event between two brain areas).

In Aim 1 of this research proposal, I will study how sensory input can modulate the content of hippocampal replay events and affect memory consolidation. Rats will be trained on an auditory-spatial association task and I will play task-related sounds during sleep to see if replay content is biased toward the spatial location associated with the sound. I will then determine if learning is enhanced for auditory-spatial associations strengthened during sleep (due to a replay content bias). In Aim 2, I will study auditory cortical responses during hippocampal replay, and examine the contribution of supra- and infragranular cortical layers to memory consolidation. In Aim 3, I will use optogenetics to inactivate auditory cortex during hippocampal replay events, and determine if the transfer of memories from hippocampus to cortex requires cortical activity during replay events.

My proposed research plan represents an initial step towards understanding how memories are stored in the brain through cortical and hippocampal networks. Understanding how information is stored and transferred between brain areas can be advantageous in developing new treatments for memory-related disorders such as Alzheimer's disease, dementia, and amnesia.

Brenda Bloodgood, Ph.D.

Harvard Medical School

"Differential Regulation of Subsets of Inhibitory Synapses by the Transcription Factor Npas4"

Neurons receive thousands of excitatory and inhibitory (E/I) inputs throughout their dendrites, soma, and axon initial segment. Proper circuit function requires appropriately matched E/I synaptic activity -- a daunting task considering the heterogeneity of inhibitory neurons and constantly changing excitation. The importance of this balancing act is underscored by the myriad neurological disorders believed to stem from an E/I imbalance, including autism, schizophrenia, and epilepsy. I am interested in identifying signaling pathways that regulate subsets of inhibitory synapses in response to changing excitation.

The activity-dependent transcription factor Npas4 is induced by excitatory activity and regulates inhibitory synapses received by the active neuron, making it unique in its capacity to transcriptionally regulate E/I balance. I am studying Npas4 to address the following:

1. Does Npas4 regulate all or subsets of inhibitory synapses?

2. Through which target genes does Npas4 regulate inhibition?

In the hippocampus, we are using a combination of electrophysiology and viral circuit-tracing techniques to locate the axons and somas of presynaptic interneurons regulated by Npas4. In acute slices, we simultaneously compared evoked inhibitory currents between neighboring wild type and Npas4-knockout pyramidal neurons in response to layer-specific stimulation of axons. Inhibitory currents originating from the soma and basal dendrites decrease while those from the apical dendrites increase in Npas4-knockout neurons suggesting Npas4 regulates at least two types of inhibitory neurons. In organotypic slices, we can infect a single pyramidal neuron with a modified rabies virus that replicates and infects monosynaptically connected presynaptic neurons. We are comparing connectivity between control and Npas4-knockdown neurons to gain insight into Npas4-regulated interneurons.

Npas4 exerts its influence by regulating the expression of genes. To identify targets, we used next-generation sequencing to survey the genome for Npas4 binding and activity-regulated transcription. In organotypic slices, we knocked down 52 of ~200 target genes and recorded corresponding miniature inhibitory postsynaptic currents; we are currently validating hits. Most Npas4 targets are neuropeptides or involved in neuropeptide biosynthesis and secretion. We speculate neuropeptides regulate subsets of interneurons.

These studies are fundamental to our understanding of circuit function and may suggest targeted interventions for neurological disorders associated with E/I imbalance.

Christoph Bock, Ph.D.

Harvard University

"A Model-based Approach to Stem Cell Differentiation and Human Development"

Pluripotent stem cells possess the ability to differentiate into any cell of the human body. If we could effectively utilize this ability it would become feasible to study human disease directly in human cells, and to engineer tissues and organs for transplantation therapy. However, current protocols for stem cell differentiation are cumbersome and inefficient, and protocol development relies heavily on trial-and-error.

The proposed project explores the hypothesis that a quantitative model of human embryonic development could enable rational design of directed differentiation protocols. I will test this hypothesis in a proof-of-concept study that investigates and improves the differentiation of human pluripotent cells into motor neurons and cardiomyocytes. To that end, I have established collaborations with leaders in the fields of neural and cardiac development, and I have developed a genomic scorecard for predictive characterization of human pluripotent cells and their differentiating progeny. This scorecard will be used to obtain time series of neural and cardiac differentiation starting from human pluripotent cells. Based on this unique dataset I will computationally infer the epigenetic and transcriptional mechanisms that control neural and cardiac development in human. These predicted mechanisms will be validated experimentally in a targeted gain-of-function screen.

Finally, I will evaluate bioinformatic algorithms for rational design of improved neural and cardiac differentiation protocols, and I will work with the collaborators to experimentally confirm the efficiency and utility of these protocols. In summary, the proposed project exploits the power of a novel genomic tool to derive a quantitative model of human embryonic development, and it applies this model to computationally design neural and cardiac differentiation protocols. This approach readily generalizes to other lineages and could overcome a critical bottleneck of stem cell research, fostering the use of pluripotent stem cells for drug discovery and regenerative medicine.

Stephanie Dougan, Ph.D.

Whitehead Institute for Biomedical Research

"Transnuclear Mice Derived From NKT Cells With Different TCR Vbetas"

NKT cells recognize lipid antigens presented on CD1d, and have been implicated in a range of diseases. NKT cells produce a variety of cytokines and have both pro and anti-inflammatory properties. NKT cells use a semi-invariant T cell receptor consisting of TCR Valpha14;Jalpha18 paired with a limited set of TCR Vbetas. Subtle differences in TCR Vbeta usage affect both the spectrum of ligands seen and the TCR affinity for those ligands. Functionally distinct subsets of NKT cells have been described, but the contribution of TCR Vbeta usage to NKT cell differentiation has not been explored. We propose to use somatic cell nuclear transfer to clone mice from NKT cells with different TCR Vbetas. The resulting panel of transnuclear mice will serve as a source of genetically identical NKT cells. These transnuclear NKT (TN NKT) cells will be adoptively transferred into Jalpha18^{-/-} mice, which lack invariant NKT cells, and the homing properties of each TN NKT line will be assessed. TN NKT cells will be cocultured with CD1d-expressing primary cells (hepatocytes, intestinal epithelial cells, B cells and dendritic cells) and with plate-bound CD1d loaded with host or pathogen-derived lipids to determine the spectrum of lipids seen by each TN NKT line and the profile of cytokines produced in response to those lipids. Finally, TN NKT cells will be adoptively transferred into Jalpha18^{-/-} hosts and assessed for their ability to cause hepatic damage upon activation with alpha-galactosylceramide or upon infection with adenovirus expressing the hepatitis B viral genome. The transnuclear NKT cell mouse panel will allow, for the first time, a clear distinction between the genetic contribution of the TCR versus the environmental contribution of the local tissues to the appearance of NKT cell functional subsets. Furthermore, TN NKT mice will be an invaluable resource for the NKT cell community as a source of primary NKT cells.

Specific Aims:

- 1) Generate transnuclear mouse lines from NKT cells with different TCR Vbetas.
- 2) Analyze the tissue homing patterns and cytokine profiles of each NKT TN line.
- 3) Assess the contribution of each NKT TN line to a mouse model of autoimmune hepatitis.

Nadine Gogolla, Ph.D.

Harvard University

"Multisensory Integration Deficits in Autism: Molecular Mechanisms of Postnatal Development"

Most of our daily sensory experiences are dominated by inputs from more than one sense. We use these different senses to build a uniform concept of our environment and to improve our perception. To bind the information from different senses, the brain has devoted specialized neuronal circuits to multisensory integration. These circuits are shaped postnatally in an experience-dependent manner. Interestingly, recent research has demonstrated that multisensory integration is deficient in complex neurodevelopmental disorders such as autism spectrum disorders (ASD).

I propose to test the hypothesis that abnormalities of multisensory function in ASD arise from disruption of normal postnatal plasticity. Furthermore, I will test whether restoration of proper critical period plasticity can rescue functional and behavioral impairments in animal models of ASD.

Towards this aim, I recently established a mouse model of cortical multisensory integration. I found that mice with impaired excitatory-inhibitory balance or myelin signaling, as well as an inbred mouse model of ASD, lack proper multisensory integration. These results have started to reveal molecular mechanisms regulating the development of multisensory integration in the healthy and diseased brain and raise further important questions:

(1) How is multisensory integration achieved at a cellular level across development and how do inhibition and myelin signaling contribute to the experience-dependent shaping of these circuits? (2) Do abnormalities of multisensory circuitry in ASD arise from disruption of postnatal, experience-dependent synaptic plasticity and can we (3) functionally and (4) behaviorally rescue multisensory deficits in animal models of ASD through the manipulation of critical period plasticity?

To address these questions, I will record single neuronal responses from the multisensory cortex of anaesthetized control and ASD mice at different ages and use genetic models to reveal which aspects of multisensory integration depend on proper inhibition and/or myelin signaling and whether these mechanisms are disrupted in ASD circuits.

I will then try to restore proper multisensory integration in ASD mice by manipulation of inhibition during postnatal development. Finally, I will address through behavioral testing whether restored multisensory integration in ASD mice also improves behaviorally the autistic phenotype.

Edel Hyland, Ph.D.

Harvard University

"Targeting Antibiotic Resistance by Defining the Molecular Basis for Segregation Dependent Plasmid Incompatibility in Bacteria"

Infection by microorganisms remains one of the most serious threats to human survival in the 21st century. This threat persists due in part to the evolution and spread of mechanisms in bacteria that confer resistance to antibiotics. The majority of resistance genes are harbored on mobile genetic elements known as resistance, or R, plasmids. I believe that directly attacking the plasmids represents an attractive alternative to conventional therapies for suppressing antibiotic resistance. R-plasmids are stably maintained within bacteria over hundreds of generations because they encode stability loci. These loci ensure that both daughters of a dividing bacterium receive at least one copy of the plasmid.

Mutations that break this segregation machinery lead to the loss of antibiotic resistance. Before targeting the segregation machinery it is imperative to know how rapidly it can evolve because this will make it possible to screen for compounds that target the least evolutionarily flexible regions of the target proteins.

To understand the evolutionary pressures on plasmid segregation, I will study the phenomenon of plasmid incompatibility - the inability of two different plasmids that use the same segregation machinery to both be stably maintained in a bacterial lineage. Plasmids escape from incompatibility when the proteins responsible for segregation acquire enough changes so that the machinery of one plasmid no longer interacts with that of the other plasmid.

I aim to define this region of evolvability for a group of stability loci that are homologues to that found on the R-plasmid from multidrug resistant *Staphylococcus aureus* (MRSA). These loci encode three components named ParC, a centromere like DNA sequence, ParM, an ATPase actin like filament, and ParR, a DNA binding protein. My data thus far suggests that sequence similarity specifically between the ParR proteins on two plasmids, is predictive of partitioning incompatibility. This proposal aims to define precisely the region of ParR that governs incompatibility and to use this knowledge to experimentally evolve two compatible partitioning systems. These insights will assist us in identifying optimal targets for anti-plasmid therapies.

Yoh Isogai, Ph.D.

Harvard University

"Molecular Dissection of Innate Defensive Behaviors in Rodents"

Sensory experiences play a fundamental role in sculpting normal brain function and behaviors, and may, in some circumstances, increase the risks for mental disorders. For example, stressful and life-threatening experiences are strongly associated with the emergence of mood disorders such as anxiety, depression and posttraumatic stress disorders. The molecular mechanisms underlying these long-term changes in brain physiology remain elusive. While recent evidence suggests that the establishment of adapted states involves chromatin regulation, precise causal links between chromatin states and mood disorders have not yet been established.

The vomeronasal system of rodents offers an excellent experimental system to dissect stress-induced behaviors. Importantly, the odors of life-threatening predators, mammalian predators and snakes, are known to generate distinct but partially overlapping innate responses with short and long-term consequences such as avoidance, hypervigilance, and modulation of feeding and foraging behaviors.

We recently identified a specific repertoire of vomeronasal receptors that differentially detect various classes of predators such as mammalian predators, snake, and birds of prey, and demonstrated that the vomeronasal organ (VNO) is required for avoidance behavior and feeding inhibition upon predator stress. Our results therefore open new avenues of research on the specific neural pathways controlled by different predator inputs and on the mechanisms by which predator stress generates long-term effects.

Specific Aim 1: What are the targets of predator inputs in the brain?

I will first identify specific brain areas activated by distinct classes of predators (mammals, snake and birds). Subsequently, I will molecularly characterize the activated neurons using RNA-seq. These experiments will reveal if distinct predator information is processed in either "generalist" or "specialist" circuits, and will yield important insights into how predator cues may generate diverse behavioral and endocrine responses.

Specific Aim 2: What are the molecular mechanisms underlying the long-term effects of chronic predator stress?

I will use predator odors to chronically stress animals and identify alterations in gene expression and chromatin composition (DNA methylation, histone modification) associated with long-term changes in physiology and behavior. This comprehensive dataset will allow a detailed analysis revealing potential molecular strategies during long-term adaptation to chronic predator stress.

Shan Liao, Ph.D.

Massachusetts General Hospital

"Nitric Oxide Regulates Lymphatic Vessel Function in Health and Disease"

Chronic disruption of lymphatic function leads to lymphedema, which creates painful and disfiguring swelling. In addition, chronic infections are common in sites of lymphedema, carrying a heavy burden on the patient and requiring frequent and expensive medical treatment. While in the US about 3 million patients develop lymphedema as a result of cancer therapy, the major cause of lymphedema world-wide is lymphatic filariasis, which is caused by mosquito-borne parasitic infections of the lymphatic system. Globally, it is estimated that there are 120 million filarial infections, with over 1.3 billion people at risk.

During both inflammation and lymphedema, an impaired immune response is frequently observed. However, it is not clear which step of the immune response is impaired: i) antigen presenting cell (APC) activation, ii) antigen and APC entry into initial lymphatic vessels, iii) antigen and APC transport through collecting lymphatic vessels, iv) the capacity of APCs to present antigen or v) the induction of regulatory T cells. Here, I hypothesize that major defect is in the transport of antigen and APCs to the draining lymph node and that reduction in lymphatic function is mediated by myeloid-derived suppressor cell produced nitric oxide (NO).

I have developed a mouse model that allows intravital imaging of autonomous lymphatic contractions. Using my novel model, I have determined that NO regulates lymphatic contraction under physiological conditions and that

lymphatic contraction was significantly attenuated during edema induced by inflammation. In this study, I will determine the roles of different nitric oxide synthases in regulating lymphatic contraction under inflammatory conditions (Aim 1). Second, I will characterize which cell types produce NO during inflammation and determine their effect on lymphatic function through functional blockade studies. Finally, I will measure each step in the T-cell response to ovalbumin and a self-antigen myelin oligodendrocyte glycoprotein after inducing inflammation (Aim 3).

The lymphatic contraction model established in this study will provide an invaluable tool to study lymphatic function. It will also improve our understanding of the mechanism of impaired immune response during inflammation and edema. Using this model, strategies to improve immune protection during lymphedema will be developed.

Christine Merlin, Ph.D.

University of Massachusetts Medical School

"Clock Gene Targeting in Monarch Butterflies Using Zinc-Finger Nucleases"

Biological timing is critical to many life processes, at both physiological and behavioral levels. In the last decade, much has been learned about the molecular clock and its behavioral outputs in animals using genetically tractable species, such as *Drosophila* and the mouse. Recently, the migratory monarch butterfly (*Danaus plexippus*) has emerged as a novel system to study animal clock and navigational mechanisms because it possesses a circadian clock necessary for its flight oriented behavior during the fall migration, whose molecular mechanism incorporates features of both *Drosophila* and vertebrate clocks. In addition to a *Drosophila*-like cryptochrome, monarch uses a vertebrate-like protein (designated CRY2). Our knowledge of monarch CRY2 function is limited to cell culture studies in which it functions as a transcriptional repressor of the core clock mechanism. In addition, its spatial and temporal expression patterns in the brain also suggest that CRY2 could be marking the neuronal network involved in time-compensated flight orientation. Yet, defining the roles of CRY2 for clockwork and navigational mechanisms *in vivo* has been challenging because direct genomic manipulation at a specific locus is not feasible. To access the monarch genome, which is being sequenced and annotated, we are pioneering a gene targeting approach using Zinc-Finger Nucleases (ZFNs). Our initial goal and the bulk of this proposal aim at knocking out *cry2* and knocking a membrane GFP tag into the *cry2* locus in individual butterfly lines. Preliminary data validate the use of ZFNs to introduce genomic lesions and mutations *in vivo*; surviving animals after embryo microinjection of mRNAs encoding the ZFNs are mosaic for *cry2* mutations. When germline transformants are recovered, knockout and knock-in lines will be established. The effects of the knockout will be monitored for the disruption of the molecular clockwork, and circadian and orientation behaviors using standard methods. The axonal projections of clock neurons expressing membrane-tagged GFP knocked into the *cry2* locus will be assessed by confocal microscopy for circuit mapping. This work will pioneer a novel and powerful strategy for genetically manipulating monarch butterflies, which could be extended broadly to "non-model" insects for which genome accessibility is still lacking.

Yunsun Nam, Ph.D.

Harvard Medical School

"Structural and Mechanistic Studies of let-7 MicroRNA Regulation by Lin28"

MicroRNAs (miRNAs) are small RNA molecules that regulate specific target gene expression by recognizing mRNAs through sequence complementarity. Recent years' progress revealed major steps in miRNA maturation. However, many mechanistic questions remain, including substrate recognition by the RNaseIII molecules and potential modes of intervention. The *let-7* miRNA family controls a variety of cell fate determination genes to influence pluripotency, development, and tumorigenesis. The first example of post-transcriptional regulation of miRNAs was discovered when *Lin28* was found to inhibit *let-7* maturation. The overarching goals of this study is to understand how microRNAs are regulated at the post-transcriptional level, using the *Lin28:let-7* as a model

system.

To study Lin28 activity, I am using largely structural and biochemical approaches. I have established the groundwork to investigate Lin28:pre-let-7 interactions in biochemical studies, including quantitative binding assays and NMR spectroscopy. Guided by the cumulative understanding of the complex, I recently determined high-resolution crystal structures of Lin28 in complex with pre-let-7. I now have a compelling hypothesis for the specificity of Lin28, guided by the structures, and will use biochemical assays and NMR to validate it. The proposed work will also build upon the Lin28:pre-let-7 complex to decipher how a key downstream factor, terminal uridyl transferase (TUTase) is recruited. In order to map the interaction surface, I can use binding and enzymatic assays as well as NMR spectroscopy. Combining these data with the structural information will already provide considerable insight, but I will also attempt to determine a co-crystal structure of the ternary complex. Finally, I will conduct a high-throughput search for inhibitors targeting the Lin28:pre-let-7 complex, using a fluorescence polarization assay.

By understanding how Lin28 specifically binds its target, recruits a downstream effector, and can be blocked, are all important questions to better understand let-7 regulation with many biological implications. In addition, mechanistic details from Lin28 may be applicable to a rapidly growing list of other RNA-binding factors that appear to regulate other miRNAs. Finally, investigating how Lin28 inhibits central events in miRNA processing will also provide a fresh perspective on mechanisms involved in miRNA maturation itself.

Edward Owusu-Ansah, Ph.D.

Harvard Medical School

"Cytoprotective Factors Activated in Response to Mitochondrial Perturbation in Drosophila"

Mitochondrial injury usually elicits a robust compensatory response where signals emanating from damaged mitochondria activate signaling cascades to re-establish metabolic homeostasis. Studies in mammalian systems have uncovered a network of kinases, nuclear factors, and other molecules that contribute to this adaptive cytoprotective response; but a model system easily amenable to genetic studies has been lacking. I have established a paradigm in *Drosophila* that will allow such studies. Constitutive expression of RNA interference constructs to complex I proteins in *Drosophila* somatic muscles activate several signaling pathways that are required for cytoprotection and cell maintenance when mitochondria are injured. The extended larval phase associated with this phenotype is highly amenable to genetic screens. This *in vivo* system will facilitate the identification of novel regulatory factors that are preferentially required for the induction and sustenance of cytoprotective responses associated with mitochondrial dysfunction. Specifically, I will:

Aim 1. Establish the contribution of ferritin and hsp60 to adaptive mitochondrial stress signaling: Both hsp60 and ferritin are strongly induced in muscles with perturbed mitochondria. Using genetic, cell biological and biochemical analyses, I will examine the consequence of either overexpressing or knocking down the expression of these genes in muscles with perturbed mitochondria.

Aim 2. Characterize the mechanism by which the JAK-STAT pathway confers tolerance to mitochondrial stress: A genetic screen for factors that preferentially confer tolerance to mitochondrial perturbation identified multiple components of the JAK-STAT pathway. I will use various genetic and cell biological assays to assess the impact of this pathway on cytoprotection

Aim 3. Identify additional cytoprotective genes via expression profile analysis and functional validation: Using microarray and shotgun proteomic analyses, I will identify additional genes induced in NDUFS1-disrupted muscles; and establish their functional significance using genetic, cell biological and biochemical assays

In summary, I have established a system in *Drosophila* that allows mitochondrial adaptive stress responses to be studied and plan to identify the molecular framework associated with this phenomenon. Given the high degree

of conservation between the Drosophila and human genomes, I anticipate that cytoprotective factors identified by this study will provide leads for improving/restoring mitochondrial function in humans.

Bernhard Payer, Ph.D.

Massachusetts General Hospital

"Identification of X-reactivation Mechanisms in Mouse Embryos and Induced Pluripotent Stem (iPS) Cells"

X-chromosome inactivation is a classic epigenetic phenomenon, by which female mammals (XX) avoid imbalances in gene dosage with males (XY). It involves multiple epigenetic processes such as DNA methylation, histone modifications, and expression of long noncoding RNAs. While X-inactivation is tightly maintained in differentiated cells, it is reversible both in vivo during embryonic development and in vitro during the generation of induced pluripotent stem (iPS) cells. As a hallmark of the pluripotent cell state, X-chromosome reactivation is a particularly timely research topic. The goal of the proposed projects is to functionally address the interplay and hierarchy of various genetic and epigenetic regulators of X-reactivation during reprogramming towards pluripotency.

In my proposed projects, I am addressing the question of how X-reactivation is achieved from two different angles. In Aim 1, I use a hypothesis-driven functional approach to ask, if the noncoding RNA Tsix is involved in X-reactivation. Tsix is a repressor of Xist, an X-linked long noncoding RNA, which is the master regulator of X-inactivation. While Tsix's function during X-inactivation has been extensively studied, its role during X-reactivation is unknown. To answer this question I am looking at X-reactivation in Tsix-mutant mouse embryos and iPS cells. As both Tsix and Xist are regulated by pluripotency factors, these experiments could advance our understanding of how pluripotency and X-reactivation are mechanistically linked.

In Aim 2, I propose to investigate X-reactivation with an unbiased screening approach. By single-cell expression profiling in mouse blastocysts during X-reactivation, I have previously identified a number of potential "X-reactivator" candidate genes. Using lentiviral RNAi knockdown during reprogramming of fibroblasts into iPS-cells, I am planning to functionally validate the top X-reactivator candidates. While some of them might be necessary specifically for X-reactivation, others might be crucial for iPS-cell formation in general. Therefore this project has relevance both for the epigenetics and stem cell field and could potentially uncover previously unknown pathways required for epigenetic reprogramming and pluripotency.

Lorena Riol Blanco, Ph.D.

Harvard Medical School

"Characterization of the Fundamental Role of the Nervous System in Psoriatic Skin Inflammation"

Experimental data from various animal models of psoriasis, as well as the clinical efficacy of newer systemic therapeutics strongly implicate both innate and adaptive immune response in the pathogenesis of psoriasis. However, to designate psoriasis purely an autoimmune disease is too simplistic. The activation of the immune system alone fails to clarify various clinical and histopathological psoriatic features. In exciting preliminary experiments we have observed the fundamental role by which peripheral nerves determine the magnitude of skin inflammation response in two psoriasis-like models. Therefore, we hypothesize that peripheral nerves control the initiation and determine the magnitude of the immune response during psoriasis. The central objective is to characterize the role of peripheral nerves in the initiation and modulation of the inflammatory response and determine how the interactive behavior between peripheral nerves and immune cells may influence the immunological outcome in psoriasis. To accomplish this objective, first we will perform the degeneration of sensory and sympathetic nerves in order to characterize the neuro-modulation of the skin inflammation. Second, we will introduce a multiphoton intravital microscopy (MP-IVM) technique to study the interaction dynamics between peripheral nerves and immune cells during skin inflammation in two psoriasis-like models. The demonstration of the crucial role of the nervous system on the cutaneous inflammatory response

will lead to new therapeutic targets to treat psoriasis.

Guramrit Singh, Ph.D.

University of Massachusetts Medical School

"Investigating the Molecular Basis of Function of a DEAD-box Protein DDX6 in Translation Control"

DEAD-box proteins, abundant RNA-dependent ATPases, are classically viewed as RNA helicases due to their homology with DNA helicases. However, emerging evidence suggests an anti-dogmatic possibility that DEAD-box proteins may instead function as stable mRNP components with their nucleotide binding states dictating different conformations and activities. For example, the exon junction complex (EJC) protein eIF4AIII stably clamps onto spliced mRNAs in its ATP-bound state, providing a binding platform for other proteins. ATP hydrolysis and subsequent release of ADP and inorganic phosphate from the protein causes it to dissociate from mRNA. Numerous other DEAD-box proteins also constitute stable RNP components. Given that no DEAD-box protein has been shown to be a processive helicase, it is possible that this recently discovered ATP-modulated RNA clamp activity might prove the rule for this protein family rather than the exception. My goal is to test this hypothesis by investigating other DEAD-box proteins that stably associate with Neuronal Granules (NGs), which are cytoplasmic translation repression complexes important for neuronal functions. To begin with, I propose to study a NG-localizing DEAD-box protein DDX6, a well-known translational regulator whose molecular mode of function is poorly understood. To test if DDX6 functions as an ATP-dependent RNA clamp, I plan to use a combination of biochemical, molecular biological and genomic approaches to study how and where on mRNAs this protein is recruited. My first specific aim is to identify proteins that selectively interact with DDX6's "closed" or "open" states, and modulate its activities to impact translation. To this end, proteins from rat cortical neurons that are purified with DDX6 in its closed state (RNA- and ATP-bound) and open state (free of RNA and ATP) will be identified using affinity chromatography and mass spectrometry. In my second aim, I will identify genome-wide RNA binding sites of DDX6 within its mRNA targets. This will be achieved by tandem immunoprecipitation of RNA-protein complexes containing DDX6 and one of its closed state interaction partners from rat cortical neuron extracts. High-throughput sequencing of purified footprints will identify RNA binding sites of the DDX6-nucleated complexes.

2010 Grant Recipients

Mark Andermann, Ph.D.

Harvard Medical School

"Cellular Imaging of Behavioral and Cholinergic Influences on Mouse Visual Cortex"

The long-term goal of my research is to understand how local cortical circuits mediate context-dependent changes in sensitivity to external stimuli. Previous electrophysiological studies have shown that considerable variability in task-modulation of neural responses exists in unidentified, nearby neurons within a cortical circuit. Cortical slice studies have postulated that behavioral modulation may vary across different classes of cells and synapses due to distinct actions of the neurotransmitter acetylcholine, implicating different roles for these cell classes in selective processing of task-relevant visual and intracortical input.

In Aim 1 of this proposal, I will dissect the contribution of identified cell classes within primary visual cortex to shaping neural and behavioral sensitivity to visual stimuli during increased task engagement. To achieve this goal, I will employ a mouse model system I developed for chronic, three-dimensional two-photon calcium imaging of neurons of identified cell type (excitatory, inhibitory) during visual behavior. In Aim 2, I will examine the role of acetylcholine in vivo in driving these changes in sensitivity. Specifically, I will record cellular responses to selective, naturalistic stimulation of basal forebrain cholinergic neurons, by combining the above imaging and anatomical methods with optogenetic tools during passive viewing of visual stimuli.

The proposed aims represent first steps in a research program geared to revealing how and why neighboring visual cortical neurons demonstrate diversity in task-modulated activity. The experiments lay the groundwork for three promising future directions: 1) extension of the current paradigm to investigate behavioral/cholinergic influences on different efferent populations of pyramidal neurons, 2) causal modulation of visual perceptual performance using selective cholinergic stimulation, and 3) in vivo cellular imaging of opposing cholinergic influences on corticocortical vs. thalamocortical synaptic input to visual cortex. These basic studies will help elucidate the mechanisms underlying cholinergic dysregulation of local cortical circuits. More generally, the chronic cellular assay of cognitive function developed in the proposed studies can be used to test the immediate and long-term effects of pharmaceutical and optogenetic therapies in wild-type mice and in mouse models of neurologic disease involving cholinergic dysfunction, such as Alzheimer's disease, schizophrenia, and aging.

Sudipta Basu, Ph.D.

Brigham and Women's Hospital

"Nanotechnology Based Platform for Cisplatin Delivery in Cancer"

Cancer is the second leading cause of mortality in the United States, with an expected 1,479,350 new cases and 562,340 deaths in 2009. Traditional strategy for treatment has primarily been based on using highly cytotoxic chemotherapies alone or in combination with radiation therapy. However, this reductionist approach often fails due to development of drug resistance and toxic side effects. There is clearly a need for a paradigm shift to develop novel therapeutic strategies that are more efficacious than existing options but have lesser side effects. We envision that harnessing a nanotechnology-based strategy for selective targeting tumors can offer clear advantages in addressing some of these issues.

Cisplatin [cis-dichlorodiammineplatinum(II)] has emerged as an important class of antitumor agents, and is widely used for the treatment of many malignancies. But its use is however dose-limited mainly because of nephrotoxicity or toxicity to the kidney.

Our hypothesis in this proposal is that harnessing a nanotechnology based-platform to deliver cisplatin will spatiotemporally target different tumors and hence will increase the efficacy of cisplatin and reduce its toxic side effects.

Specifically we will, 1. Chemically synthesize cisplatin analogues that self-assembled into nanostructures. 2. Engineer cisplatin nanoparticle using self-assembly of cholesterol-cisplatin, phosphatidylcholine and DSPE-PEG-cisplatin. 3. Study the effect of cisplatin nanoparticle in vitro and in vivo. There is clearly an urgent need to develop novel approaches that are more effective and safer than existing strategies. We are proposing an interdisciplinary effort integrating synthetic chemistry, nanotechnology, pharmacology and translational medicine to address one of the major challenges in cancer chemotherapy. Accomplishing the specific aims outlined in this proposal, we anticipate that this project will lead to the development of a novel nanomedicine of the platinate family, which (based on our preliminary data) will demonstrate similar efficacy to cisplatin but reduce the adverse effects, thereby increasing therapeutic index.

Kevin Corbett, Ph.D.

Harvard Medical School

"Structure and Function of the Fungal Monoplin Complex"

In eukaryotes, mitosis and meiosis are related processes by which duplicated chromosomes are accurately segregated to daughter cells or gametes. In both mitosis and meiosis, the accuracy of chromosome segregation depends on the proper geometry of attachment between kinetochores, specialized protein structures assembled onto each chromosome, and microtubules of the spindle. In *S. cerevisiae*, the monoplin complex acts in meiosis I to align two separate kinetochores of duplicated (sister) chromatids, mediating the reductional

division necessary for meiosis. In *S. pombe*, an orthologous complex assures that the multiple sites of microtubule attachment within a single kinetochore are aligned, such that a kinetochore is not simultaneously attached to microtubules extending from both poles of the spindle. The monopolin complex also acts throughout fungi to assist chromatin silencing and suppress harmful recombination within the highly repetitive ribosomal DNA (rDNA) array.

The multiple roles of the monopolin complex in these different contexts can be largely rationalized with a model of the complex as a molecular cross-linker, joining microtubule-binding elements at kinetochores and rDNA repeats in the nucleolus. Indeed, I have shown that this model is likely accurate: the structural core of *S. cerevisiae* monopolin, Csm1/Lrs4, forms a distinctive V-shaped complex, suggestive of a cross-linking function. I have also characterized interactions between Csm1/Lrs4 and their binding partners at both kinetochores and rDNA repeats, and shown that these interactions are important for monopolin function in both of these contexts.

This proposal outlines experiments that will directly test, then expand upon, the model of Csm1/Lrs4 as a molecular cross-linker. I will first determine whether the complex is indeed capable of cross-linking kinetochores or rDNA repeats, using a combination of structural and biochemical methods. I will also examine the roles of the two additional *S. cerevisiae* monopolin complex subunits, Mam1 and the Hrr25 kinase, using both structural methods and in vivo functional assays. Finally, as a simple cross-linking model cannot easily explain monopolin's role in rDNA silencing, I will further outline the complex interactions of Csm1/Lrs4 with the network of proteins involved in rDNA silencing and recombination control.

Adam Douglass, Ph.D.

Harvard University

"Neuromodulatory Control of Reward Learning in Larval Zebrafish"

Central to our understanding of operant reward learning is the notion of "reward centers", areas in the brain that signal when a reward has been attained and reinforce the actions that preceded the reward. In the mammalian brain, primary reward centers are all thought to be neuromodulatory in nature, as they release neurotransmitters such as dopamine (DA), serotonin (5-HT), and noradrenaline (NA). Our understanding of the mechanisms by which these cells enable operant conditioning is still lacking. In particular, it is unclear how disparate reward signals, encoded by different neurotransmitter systems, are integrated to reinforce operant behaviors. It is also unclear how stimuli are differentially interpreted by modulatory neurons as rewarding or otherwise. To address both of these issues, I propose to develop the larval zebrafish as a model system for studying the neuromodulatory control of reward learning.

Due to its small size and optical transparency, the whole brain of a larval fish can be studied with relative ease, and without invasive, surgical manipulations. My project will exploit these properties in the following ways: First, I will use calcium-evoked bioluminescence to characterize neuromodulatory responses to food reward in larval fish. If fish larvae use DA, 5-HT, or NA cells to encode reward, as do adult fish and mammals, then activity in these neurons should change upon delivery of a food reward. I will use an aequorin-based bioluminescence assay to monitor this in freely behaving fish. Second, I will determine whether direct, channelrhodopsin-2 (ChR2)-mediated stimulation of neuromodulatory cells in the larval zebrafish constitutes a reward. If modulatory neurons are true reward centers, then it will be possible to reinforce operant behaviors (i.e. tail movement in a specific direction) via direct photoactivation of these cells. Third, I will map the functional afferents of neuromodulatory cells using a synaptic tracing technique based on rabies virus. In addition to providing a detailed understanding of the architecture of the underlying neural circuits, this will form the basis for further exploration of reward coding, and in particular will help us to understand how stimuli are classified as rewarding or not.

Ryuya Fukunaga, Ph.D.

University of Massachusetts Medical School

"Understanding the Discrete Functions of Loquacious Isoforms in Small Silencing RNA Pathways"

My long term goal is to understand how small silencing RNAs such as small interfering RNAs (siRNAs) and microRNAs (miRNAs) are made, how they are loaded into distinct functional complexes, and how these complexes repress gene expression. I propose to use *Drosophila*, a model organism with well established genetics, genomics, and biochemistry, to study the RNA silencing pathways. *Drosophila* produces at least four distinct small silencing RNAs (miRNAs, endo-siRNAs, exo-siRNAs, and piRNAs), which are classified according to the mechanism of their production. As specific aims in this proposal, I will examine the functions of each of the isoforms of the double-stranded RNA-binding protein, Loquacious (Loqs), which assists Dicer-1 and Dicer-2 in the production of all small silencing RNAs except piRNAs. There are four known Loquacious isoforms (Loqs-PA, PB, PC, and PD) produced by alternative splicing. My working hypothesis is that each Loqs isoform has a specific function in a small silencing RNA biogenesis pathway. To test and elaborate my hypothesis, I will use quantitative biochemistry, enzymology, and fly genetics. I will recapitulate the biogenesis of each small silencing RNA; microRNAs, exo-siRNAs, and endo-siRNAs in vitro using recombinant proteins and/or cell and fly tissue lysates. Then I will quantitatively examine how each factor contributes to RNA biogenesis. I will also examine the characteristics of substrate precursor RNAs that determine which pathway they enter. To analyze Loqs functions in vivo, I will construct transgenic flies that express only specific subsets of Loqs isoforms. For each transgenic strain, I will determine which small RNA pathway is affected using high throughput sequencing. I will identify the protein-binding partners and RNA substrates of each Loqs isoform. A final aim of this proposal is to elucidate the biogenesis mechanism of the recently discovered endo-siRNAs. I will seek to identify novel proteins required for endo-siRNA production and to define the RNA intermediates in endo-siRNA biogenesis. My goal is to bring a quantitative molecular understanding to the biogenesis of these small RNAs.

Mario Halic, Ph.D.

Harvard Medical School

"Argonaute Surveillance and priRNAs Nucleate RNAi and Heterochromatin Formation"

In nearly all eukaryotes, small RNAs associated with the RNA interference (RNAi) pathway contribute to regulation of cell differentiation, gene expression, development, and cancer. In fission yeast, the RNAi machinery is required for the assembly of silent heterochromatic DNA domains. The assembly of fission yeast pericentromeric heterochromatin and the generation of small interfering RNAs (siRNAs) from noncoding centromeric transcripts are mutually dependent processes. How this interdependent positive feedback loop is first triggered is a fundamental unanswered question.

My results suggest that a transcriptome surveillance mechanism based on the random association of small RNAs with Argonautes triggers RNAi-mediated heterochromatin formation within DNA repeats. Two Ago1-dependent pathways mediate the generation of small RNAs from centromeric repeat sequences. First, small RNA profiles in heterochromatin mutants demonstrated that the amplification of a subclass of siRNAs occurred independently of H3K9 methylation and involved RDRC, Dicer and Argonaute activity on specific noncoding RNAs. Second, my work has uncovered a distinct class of small RNAs, called primal small RNAs (priRNAs), which are generated independently of Dicer or RDRC. priRNAs appear to be degradation products of abundant genome-wide transcripts. My data suggest that priRNAs trigger low levels of H3K9 methylation and promote RDRC/Dicer-mediated siRNA amplification.

Although these results suggest that priRNAs are general RNA degradation products, it remains unclear how priRNAs are generated. In this proposal, I will use genetic, biochemical and computational tools to determine whether the exosome, dhp1, or another nuclease is involved in priRNA generation. Furthermore, I will test our model that bidirectional transcription might be sufficient to trigger RNAi. I will determine whether high

expression of antisense transcript is sufficient to trigger RNAi dependent silencing. In addition, my preliminary results suggest that siRNAs undergo processing at their 3' ends, which involves the addition of untemplated nucleotides by the Cid12 and Cid14 nucleotidyltransferases as well as trimming. I will use small RNA sequencing to determine how the activity of different nucleotidyltransferases affects the fate of small RNAs.

Ran Kafri, Ph.D.

Harvard Medical School

"Signal Transduction of DNA Double Strand Breaks"

A central question in signal transduction is how information from multiple different channels is combined and processed to bring about a single cell decision. Signal transduction responding to DNA double-strand-breaks lies at the junction of three different channels of communication involving pathways from (i) double strand break detection, (ii) cell cycle control and (iii) double strand break repair. In this three-way junction, signals reporting detection DNA damage are conveyed to the cell cycle machinery to bring about a cell cycle arrest. In the reverse direction, information regarding a cells' stage in cell cycle is thought to influence the choice between two alternative pathways of repair, homologous recombination (HR) or non-homologous end joining (NHEJ). How these different signals are communicated remains an open question. To confront this question we have devised a methodology of single cell analysis combining complementary information from both live cell time lapse microscopy and systematic, high-throughput, Immunofluorescence (IF) staining. Specifically, we have constructed a stable cell line simultaneously reporting the dynamics of 53BP1, a mediator of double strand break detection and Rad52, a repair proteinsd of the HR pathway. Using a fluorecence microscope equipped with automated stage and perfect focus, we will examine and record dynamics of thousands of cells, capturing both mean behavior and cell to cell variation. Large scale IF will be used to establish correlations between these observed dynamics to levels, localization or phosphorylation states of regulators of cell cycle and DNA damage. Our data will be constructed into distributions reporting the population level dynamics of the measured proteins. These data will be interpreted using a mathematical approach developed in our lab, which has recently been implemented, revealing growth regulation in mammalian cell cycle. The central principle of this technique is that, in a dynamic process, deconvolution of distributions from close time points reveal regulatory relationships with accuracy. Following this approach, we will be able to identify the means by which information is channeled from the very early events of double strand detection (appearance of 53BP1 foci) to mechanism of cell cycle arrest and the decision which repair pathway to activate.

Maria Lehtinen, Ph.D.

Children's Hospital Boston

"Regulation of Adult Neurogenesis by the Cerebrospinal Fluid (CSF) Proteome"

Maintenance of the human cerebral cortex is essential for normal cognitive function, and is disrupted in a wide range of human neurological processes, including aging and age-associated neurodegenerative disease. In designing new therapies for the aging brain, insight can be gained from what is already known about the early development of the mammalian cerebral cortex. The developing embryonic cortex is continuously in contact with an enclosed space filled with embryonic cerebrospinal fluid (CSF). The CSF has historically been viewed as a fluid cushion for the CNS and a passive sink for CNS debris and biomarkers. However, our preliminary data have elucidated this fluid niche as a distributed source of signals for stem cells in the developing rat and mouse brain. We have found that embryonic CSF stimulates proliferation and subsequent maintenance of neural stem cells in vitro, in part through the signaling capacity of the insulin-like growth factor IGF2. In this proposal, I will employ a multi-tiered experimental approach to explore an exciting, new role for CSF in the adult rat brain: That CSF constitutes a dynamic stem cell niche which instructs and regulates neural stem cells in the adult and aging brain. My specific aims are the following: (1) analyze CSF constituents in embryonic, young adult, and aging rats by mass spectrometry, (2) test the ability of adult CSF to promote a neural stem cell niche in vitro in cortical explants and in neurospheres, and (3) in vivo by intra-ventricular infusion of CSF, IGF2, and other candidate

factors into young adult and aged adult rats. The proposed experiments should elucidate a role for CSF as a tractable fluid niche for stem cells in the adult brain. Since the CSF is a surgically accessible medium to remove or supplement in even in humans, the proposed experiments will open avenues for novel stem cell therapies throughout life.

Kunal Rai, Ph.D.

Dana-Farber Cancer Institute

"Investigating the Mechanism of Action of Epigenetic Modifications and Factors during Metastatic Progression of Melanoma"

Metastatic melanoma is one of the most aggressive kinds of cancer whose incidence rate worldwide is currently highest. Although, recent work has identified genes responsible for melanoma initiation and metastasis, the contribution of epigenetic events to different stages of melanoma progression are poorly understood. Therefore, the goal of this proposal is to identify the epigenetic changes that occur during metastasis and understand how they help in metastatic spread of melanoma. This proposal builds on recent work done in the Chin lab where novel genes helping in the metastatic spread of melanoma have been identified through combination of cross-species oncogenomics (using mouse models and human patient samples) and genetic screens in human melanoma cell lines. Eighteen pro-metastatic genes were identified after two rounds of screening for invasive potential through boyden chamber assay. Of these, four genes (RNF2, UCHL5, ASF1B and HMGB1) are known to be involved in epigenetic regulation of the genome. I propose to study the mechanism of metastasis regulation by RNF2. RNF2 has established role in adding ubiquitin marks at histone H2A lysine 119. In first aim, I will verify the metastatic roles of RNF2 using mouse models. RNF2 dependent biological process(es) with roles in metastasis will be identified. In the second aim, I will determine the molecular mechanism of RNF2 action. A genome-wide profiling of selective multiple histone and DNA modification marks and nucleosome positioning will be performed in cell lines derived from primary melanoma of mouse models with differential metastatic capabilities (HGF-Met versus HRAS driven). Genome-wide occupancy of RNF2 in metastatic cells will be determined and locations of chromatin marks be determined in RNF2-deficient cells to identify its direct gene targets. Finally, it will be assessed as to which protein complex of RNF2 helps in its metastatic roles to gain further insight into molecular mechanism. Occupancy of RNF2/chromatin marks will be correlated to gene expression profiles to assess the causative roles of these chromatin modifiers/modifications in gene expression changes. The final goal is to identify cellular systems critical for metastasis and their regulation by RNF2 and chromatin modifications

Manas Santra, Ph.D.

University of Massachusetts Medical School

"Characterization of FBXO31 Tumor Suppressor Function and Identification of Therapeutic Targets for FBXO31-Deficient Cancers"

Eukaryotic cells are constantly at risk of damage to their DNA from both external and internal sources. If left unrepaired, DNA damage can lead to detrimental biological consequences, including mutations, malignant transformation and cell death. Consequently, eukaryotes have evolved a number of mechanisms to monitor the integrity of their genome and, if necessary, repair damaged DNA. For example, genome integrity is monitored by a series of DNA damage-inducible "checkpoint" systems that can transiently delay cell cycle progression, thereby providing time for the cell to repair the damaged DNA. A variety of observations have led to the view that in general cancer cells have a reduced capacity, relative to normal cells, to repair damaged DNA. The loss of DNA repair/checkpoint function is thought to be responsible for the genomic instability that is frequently observed in cancer. Thus, defects in DNA repair/checkpoint pathways may represent a general vulnerability of cancer cells that may be exploited for therapeutic benefit. We have recently identified the F-box protein FBXO31 as factor that is required to induce G1 arrest following DNA damage. Interestingly, FBXO31 is a candidate tumor suppressor encoded in 16q24.3, a chromosomal region in which there is loss of heterozygosity in breast, ovarian,

hepatocellular and prostate cancers. However, whether loss of FBXO31 function confers susceptibility to cancer is not known. In this application, I propose to characterize the role of FBXO31 in tumor prevention using a mouse model. Specifically, I will use FBXO31 knockout mice to determine whether loss of FBXO31 enhances spontaneous, carcinogen-induced and irradiation-induced tumorigenesis. Furthermore, I will perform a genome-wide RNA interference-based screen to identify genes that are preferentially required for viability or proliferation of cells in which FBXO31 function has been lost. Candidates from the primary screen will be validated by confirming synthetic lethal interactions in directed experiments with single shRNAs and by ruling out off-target effects. The results of this screen may identify selective therapeutic targets for cancers in which FBXO31 function has been lost.

Asaf Spiegel, Ph.D.

Whitehead Institute for Biomedical Research

"Breast Cancer Metastasis--The Role of Systemic Activation of Immune Cells by the Carcinoma Cells"

The most damaging change during cancer progression is the switch from a locally growing tumor to a metastatic killer. This switch involves numerous alterations that allow tumor cells to complete the complex series of events needed for metastasis. Tumors are highly complex tissues composed not only of neoplastic cells, but also of stromal cell compartments. The latter contain a variety of mesenchymal cells as well as cells associated with the immune system. While it is apparent that these cells are not just passive bystanders, the mechanism underlying their active role in tumor growth and metastasis development is not fully understood. We hypothesize that metastatic progression depends not only on interactions between the tumor and its stromal microenvironment but also involves systemic effects initiated by the malignant cells. More specifically, I propose to determine the role of recruitment of hematopoietic cells from the bone marrow on tumor growth and metastasis. Preliminary results suggest that perturbation of hematopoiesis in the bone marrow and consequent mobilization of immune cells facilitate tumor growth and metastasis. I aim to decipher the mechanism governing these processes and characterize the signals used by the cancer cells to activate and recruit the immune cells. Furthermore, I plan to identify the hematopoietic cells that facilitate tumor development, and determine which heterotypic signals that are secreted by the immune cells affect the growth, invasion and metastasis of the malignant cells. Studying tumor-associated immune cells both in a physiological mouse model as well as at the molecular level will provide a comprehensive view on the role of systemic activation in malignant tumor progression. Understanding the mechanisms that drive metastasis formation may lead to new approaches to detect and prevent metastasis at its earliest inception. Leukocytes and/or molecules involved in their activation and recruitment may stand out as new cancer bio-markers and therapeutic targets, as a complement to conventional therapy based on targeting only the malignant cells.

Tim van Opijnen, Ph.D.

Tufts University School of Medicine

"Functional Organization of the Streptococcus Pneumoniae Two Component System Regulatory Network"

The worldwide increase of antibiotic resistance among virtually all bacterial pathogens and a rise of multi-drug resistance in several of the major pathogenic species is a serious problem. To effectively battle established and emerging infectious diseases we are in need of new and preferably genome-wide approaches that will lead to the successful development of new antimicrobials. In this proposal I describe experiments to reconstruct the two component system (TCS) regulatory network for the important bacterial pathogen Streptococcus pneumoniae. TCSs serve as the decision-making circuits within bacteria and enable them to sense, respond and adapt to the environment by triggering changes in specific transcriptional programs. To obtain a genome-wide picture of the transcriptional programs and molecular pathways under control of each TCS, the network will be reconstructed predominantly from two types of data: Conditional genetic interactions between each TCS and the rest of the genome, determined using our recently developed Tn-seq method; And temporal and conditional

gene expression profiles, obtained by performing genome-wide microarrays. Subsequent data integration into a unified network will fulfill the two main aims of the proposal: Aim 1: Identify the transcriptional programs - both individual genes and complete pathways - that are triggered under specific circumstances by each TCS. Aim 2: Determine the architecture of the TCS regulatory network, which will both reveal where the network gets its robustness from, and identify the hubs. Hubs are the most important "information carriers" in a network, which makes them at the same time the most vulnerable nodes for a network breakdown. The reconstruction of the TCS regulatory network will serve three long-term goals: 1) It may lead to new approaches and the identification of new targets to tackle infectious diseases, e.g. hubs would make ideal antimicrobial targets; 2) It will aid in the engineering of sensitive and controllable gene-circuits that can be used for the development of new vaccines and industrial purposes; 3) It will serve as a first step in the creation of predictive (clinical) models for infectious diseases such as *S. pneumoniae*.

Nadine Vastenhouw, Ph.D.

Harvard University

"The Role of Chromatin in Pluripotency and Cell Fate Specification"

Changes in chromatin are thought to underlie many biological processes, from development to cancer. I propose to study the *in vivo* roles of chromatin modifications in pluripotency and cell fate specification, using zebrafish as a model system. During early embryogenesis, cells transition from a period of transcriptional repression to genome activation. At this time, embryonic cells are pluripotent until they receive cell fate specification signals.

We recently found that during genome activation many developmental regulatory genes become bivalently marked by histone H3K4me3 (an "active" mark) and H3K27me3 (a "repressive" mark), similar to results obtained in embryonic stem cells. In addition, we discovered that many genes are not transcribed but monovalently marked by H3K4me3, suggesting that H3K27me3 is not absolutely required for the repression of transcription from H3K4me3-marked promoters. These results have started to reveal the chromatin landscape during vertebrate embryogenesis but raise two major questions: What is the relationship between these histone modifications and subsequent cell fate specification? What is the role of these histone modifications in the establishment of pluripotency?

Specific Aim1: How do pluripotent chromatin marks change during cell fate specification? The relationship between the chromatin signature for pluripotency and subsequent cell fate specification is unclear. I will analyze the chromatin profile of Nodal target genes during the transition from pluripotency to fate specification in the presence and absence of Nodal signaling. These experiments will reveal whether, when and how pluripotent chromatin signatures change during cell fate specification.

Specific Aim 2. What are the roles of H3K4me3 and H3K27me3 in Zygotic Genome Activation and Pluripotency? Specific histone modifications have been associated with transcriptional output and pluripotency, but it remains unclear what regulatory role these modifications play during embryogenesis. I hypothesize that the appearance of these histone marks poises zygotic genes for activation (H3K4me3), while ensuring the proper regulation of lineage commitment (H3K27me3). By disrupting histone modification marks *in vivo*, I will determine the functional relationship between the appearance of H3K4me3 and H3K27me3, the activation of the genome and embryonic pluripotency

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.

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 Sapiaha, 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, C20:4n-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 dysregulated, 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.

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

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.

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"

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*"

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"

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

Anna Delprato, Ph.D.

University of Massachusetts Medical School

“Biochemical and Structural Analysis of the Rab/GEF Interaction”

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”

Grzegorz Ira, Ph.D.

Brandeis University

“DNA Double Strand Break Repair in Yeast”

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”

Kenichi 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”

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”

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 phagocytic 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”