

Charles A. King Trust

Postdoctoral Fellowship Program

Basic Science

2018 Grant Recipients

Monther Abu-Remaileh, Ph.D.

Postdoctoral Fellow

Whitehead Institute for Biomedical Research

Mentor: David M. Sabatini, M.D. Ph.D.

“The Biochemical and Molecular Pathogenesis of Batten Disease”

Neuronal ceroid lipofuscinoses (NCLs) are the most prevalent childhood neurodegenerative disease group with an incidence of 1 in 12,500 live births in the United States. These diseases are characterized by accumulation of lipoprotein aggregates (lipofuscin) in the lysosome and a progressive loss of vision, motor skills and cognitive abilities leading to early death. Juvenile neuronal ceroid lipofuscinoses (known as Batten disease) is the most common form of NCL and is caused by mutations in *CLN3* gene, which encodes a lysosomal transmembrane protein. *CLN3* has been implicated in many cellular processes but its exact molecular function remains elusive. Pinpointing the molecular function of *CLN3* will aid in deciphering how its loss drives the cellular changes that lead to the neurodegenerative symptoms typical of NCLs. Through our recently developed lysosomal metabolomics approach, we identified a group of phospholipid-derived metabolites (glycerophosphodiester, GPDs) to massively accumulate in the lysosome upon loss of *CLN3*. In this proposal, we hypothesize that *CLN3* is a lysosome transporter for GPDs, a function that is essential for maintaining neuronal cell function and survival. To test our hypothesis we will:

Aim 1: Determine whether *CLN3* is a transporter of GPDs in vitro and in vivo.

Aim 2: Determine how *CLN3* dysfunction alters phospholipid metabolism in the lysosome.

Aim 3: Determine the effects of GPDs accumulation in the lysosome on neuronal homeostasis in Batten disease models.

We believe that our work will establish a direct link between phospholipid metabolism and neurodegeneration and identify novel nodes that can be targeted to improve the management of Batten and other neurodegenerative diseases. In the long term, this work will set up a new paradigm for studying lysosomal storage diseases by focusing on the effects of gene mutations on lysosome-specific metabolism, which will help to better understand the molecular basis of these debilitating disorders.

Hume Akahori Stroud, Ph.D.

Research Fellow

Harvard Medical School

Mentor: Michael Greenberg, Ph.D.

“Understanding the Developmental Programs that Control Transcription in Inhibitory Neurons”

Dysfunction of inhibitory neurons underlies diverse neurodevelopmental and psychiatric disorders. To understand how such dysfunction can occur, it is important to understand how inhibitory neurons mature in the normal brain. Neurons undergo dynamic changes in morphology and function during brain development, and this process is associated with changes in expression of specific genes. However, the gene programs underlying this process are poorly understood. By analyzing gene expression profiles in specific inhibitory neuron subtypes over a developmental time-course, I have identified many genes with specific neuronal functions that become highly expressed during the early postnatal period, and found that this process occurs in a neuron subtype-specific manner. The goal of this proposal is to uncover the molecular mechanisms controlling these developmental changes in gene expression. My preliminary results suggest that increases in gene expression are mediated in part by the activation of neuronal subtype-specific enhancers. These enhancers are significantly enriched for sequence motifs recognized by AP1 transcription factors, which are induced by growth factors and neuronal activity. Furthermore, by performing whole-genome DNA methylation analyses in specific neuron subtypes, I have found that DNA methylation patterns are dynamic at these enhancers such that methylation is actively removed during neuronal maturation. Based on these preliminary findings, I hypothesize that the maturation of inhibitory neurons is mediated by a combined action of extrinsic factors in a neuron's environment (e.g. neurotrophic factors or neuronal activity) and intrinsic factors (e.g. DNA demethylases) within the neuron that together lead to the licensing of enhancer sequences that control inhibitory neuron gene expression in a neuronal subtype specific manner. In this application, I propose two specific aims that will test this hypothesis: 1) Identification and functional testing of regulatory elements that control developmental gene programs in maturing neurons and 2) exploring the role of DNA methylation in controlling enhancers.

Mehmet Burak, M.D.

Post-Doctoral Research Fellow

Harvard T.H. Chan School of Public Health

Mentor: Gökhan Hotamisligil, M.D., Ph.D.

“The Role of Circulating aP2 in the Pathogenesis of Allergen Induced and Obesity Related Asthma and New Therapeutic Strategies Against Asthma Using anti-aP2 Agents”

Asthma is a chronic inflammatory airway disease with increasing prevalence and limited treatment options. The pathogenesis of asthma is not fully understood, importantly, it has a major immunometabolic component, as lipid mediators are directly promoting airway inflammation. Interestingly, accumulating evidence implicates obesity as an important risk factor for asthma, where these lipid mediators are upregulated. There is a great need for novel treatments for obesity-related asthma, which is less-responsive to conventional therapies.

The fatty acid binding protein aP2 is a fundamental immunometabolic regulator, which is increased in obese humans and mice. aP2 deficiency improves the dysregulated metabolic outcomes of obesity, diabetes and atherosclerosis, which share similar lipid derangements and immunometabolic

underpinnings. Moreover, we reported that aP2 secreted from adipose tissue acts as an endocrine hormone, supporting inter-organ communication.

We detected elevated aP2 levels in bronchial epithelium and broncho-alveolar lavage fluid (BALF) in mice models of asthma and higher serum aP2 levels in overweight/obese asthmatic humans. Notably, aP2 expression in lung tissue and aP2 levels in serum and BALF were highly responsive to Th2 cytokines IL-4 and IL-13, important asthma regulators. Strikingly, aP2^{-/-} mice were markedly protected against allergen-induced asthma. We propose to study the role of aP2 in allergen-induced and obesity-related asthma and evaluate its potential as a therapeutic target in the following aims: 1) Determine the mechanistic role of aP2 in the pathogenesis of airway inflammation and hyperresponsiveness (AHR) in allergen-treated mice and in genetic models of obesity, 2) Examine the therapeutic activity of a neutralizing monoclonal anti-aP2 antibody and a small molecule inhibitor of aP2 in mouse models of asthma, and, 3) Determine the levels of aP2 in serum and BALF from asthmatic vs non-asthmatic overweight and obese humans. With this line of investigation, we hope to generate critical insights into asthma pathogenesis and generate novel translational opportunities.

Yongzhi Chen, Ph.D.

Postdoctoral Fellow

University of Massachusetts Medical School

Mentor: Katherine A. Fitzgerald Ph.D

“Regulation of Immune Gene expression and Inflammatory Disease by the Cellular Nucleic Acid Binding Protein (CNBP)”

An inducible program of inflammatory gene expression is a hallmark of antimicrobial defenses. Germline-encoded receptors recognize microbial products and activate signaling pathways that lead to the expression of hundreds of inflammatory response genes. This proposal expands on these studies by defining a new regulator of immune gene expression; the CCHC-type zinc finger protein cytosolic nucleic acid binding protein (CNBP). We have generated mice lacking CNBP and found that CNBP-deficient macrophages fail to induce transcription of the IL-12/IL23 family. Cnbp resides in the cytosol of macrophages and translocates to the nucleus in response to multiple microbial ligands and pathogens. Cnbp regulates IL12 via c-Rel, an NFB/Rel family member known to control IL12 gene transcription. c-Rel nuclear translocation and DNA binding activity require Cnbp. Furthermore, Cnbp itself a DNA binding protein bound the IL12 promoter. CNBP-deficient mice were more susceptible to acute toxoplasmosis associated with reduced production of IL12, as well as a reduced Th1 cell IFN response essential to control parasite replication. Collectively, these findings identify Cnbp as a new signaling molecule downstream of multiple Pattern Recognition Receptors, that acts as a key regulator of IL12 gene transcription and Th1 immunity. This proposal will test the hypothesis that CNBP represents a novel signaling molecule that acts as a transcriptional coactivator to bind the genome and coordinate expression of IL12p40 to regulate IL12 and IL23 in innate cells and direct TH1/TH17 dependent adaptive immunity and inflammation. We will explore these hypotheses using the following specific aims: (1) defining detailed molecular mechanisms of CNBP-dependent control of the IL12/IL23 gene family; (2) defining the cell type specific contributions of CNBP in control of Th1 immunity to *Toxoplasma gondii* and TH17 responses to *Candida Albicans*; and (3) defining the role of CNBP in controlling Inflammatory Bowel Diseases using DSS colitis and related models.

Ben Ewen-Campen, Ph.D.

Postdoctoral Fellow

Harvard Medical School

Mentor: Norbert Perrimon

“Systematic Analysis of Genetic Redundancy in the Wnt Signaling Pathway”

Wnt signaling is a highly conserved pathway for intercellular communication, with numerous critical roles during development and adult life in all multicellular animals. While the core components of this pathway have been characterized in great detail, both the ligands for this pathway and their receptors belong to large families of duplicated genes (paralogs), which retain partially overlapping functions in different contexts, and have therefore proven challenging to study via traditional loss-of-function genetic analysis.

I have developed two new potent and scalable techniques for studying redundant paralogs in *Drosophila*, and I propose to apply these tools to perform the first systematic analysis of redundancy amongst Wnt ligands and their receptors. Specifically, I will perform comprehensive pairwise double knock-outs in multiple tissues using multiplexed CRISPR, and a series of targeted gain-of-function experiments using Cas9-based transcriptional activators. In Aim 1, I will generate and validate reagents for all pairwise double knock-outs and over-expression of the seven Wnt ligands and four Frizzled receptors. In Aim 2, I use these reagents to perform a systematic double knock-out screen in multiple tissues enriched for Wnt family gene expression. In Aim 3, I will adapt the “split-Gal4” technique to characterize, and gain genetic access to, the specific tissue domains that co-express pairs of ligands or receptors. Together, these studies will provide new mechanistic insight into how multiple closely related ligands and receptors for a single signaling pathway can shape pathway output in different cellular contexts. Because aberrant Wnt signaling is implicated in numerous diseases, these results may also have biomedical implications.

Madeline Hayes, Ph.D.

Postdoctoral Fellow

Massachusetts General Hospital

Mentor: David Langenau, Ph.D

“Characterization of the Role for Non-canonical Wnt/Planar Cell Polarity (Wnt/PCP) Signaling in Growth and Self-renewal of Rhabdomyosarcoma”

Relapse is a major clinical problem in rhabdomyosarcoma (RMS), with continued tumor growth maintained by undifferentiated self-renewing tumor-propagating cells (TPCs). My long-term goal is to define therapeutically relevant pathways that maintain TPCs. The objective of this proposal is to characterize the role of the non-canonical Wnt/Planar Cell Polarity (Wnt/PCP) signaling pathway in RMS and uncover novel targets to inhibit relapse in patients. My work has defined a role for Van Gogh-like 2 (Vangl2), a core regulator of Wnt/PCP signaling, in self-renewal of RMS (Hayes et al., in press). VANGL2 knock-down inhibited growth, led to reduced TPC number, and increased differentiation of human RMS. In a zebrafish model, Vangl2 expression enriched for TPCs, revealing novel approaches to dynamically visualize cells in live animals and isolate them to further characterize TPCs on a molecular basis. The rationale for this work is that factors involved in Wnt/PCP signaling are highly expressed in the majority of human RMS and therapeutic strategies based on Wnt/PCP inhibition could potentially benefit many patients. Aim 1 will further assess the role for Wnt/PCP in tumor growth, testing my hypothesis that

Wnt/PCP signaling regulates self-renewal and maintenance of TPCs in RMS. Aim 2 will characterize Vangl2 as a marker of TPCs in zebrafish ERMS, providing unprecedented access to dynamically visualize roles for the Wnt/PCP pathway in self-renewal and cell fate choices in vivo. Aim 3 will define the effector pathways downstream of Vangl2 and Wnt/PCP signaling, further elucidating roles of the small GTPase, RhoA. Given that factors involved in Wnt/PCP signaling are expressed in other tumor types, I expect my work will have significant translational impact by advancing the understanding of processes that affect self-renewal in human cancer. This work will also provide pre-clinical data supporting Wnt/PCP targeting for the suppression of RMS tumor growth in vivo.

Wonsik Lee, Ph.D.

Research Fellow

Harvard Medical School

Mentor: Suzanne Walker, Ph.D.

“Platform for Study of Peptidoglycan and Lipid II Binding Molecules”

Antibiotic resistant bacteria, and methicillin-resistant *Staphylococcus aureus* (MRSA) in particular, account for 0.7 million deaths every year and thus present a significant global economic and health burden. Many important antibiotics used in the clinic target bacterial cell wall synthesis, which is essential for cell survival. Peptidoglycan (PG), a major component of the cell wall, is synthesized from the lipid-linked precursor Lipid II. Our previous studies have shown that Lipid II is an essential and druggable substrate in the PG pathway and established methodologies to isolate and modify *S. aureus* Lipid II. These studies led to the development of a novel concept for Lipid II (or its glycopolymer) immobilization on a solid surface using enzymatic or chemical modification, where the immobilized Lipid II enables affinity-based characterization of Lipid II binding proteins and molecules that may reveal new antibacterial treatment strategies to supplement current approaches. Here, I seek to develop chemical tools enabling affinity-based study for proteins and small molecules that bind Lipid II or nascent peptidoglycan glycopolymer. My research will apply multidisciplinary approaches to develop technologies for discovering new antibiotics and protein targets, to understand the biogenesis of bacterial cell wall, and to exploit this knowledge for designing new treatments. The Walker lab and Harvard Medical School provide an ideal environment for my research with unique resources that will enable me to conduct the proposed research and continue building additional skills. The proposed research integrates chemical biology and bacterial genetic approaches with the aim of providing insight into our understanding of protein networks governing peptidoglycan synthesis, identifying potential targets for development of new therapeutics, and applying this methodology to other important pathogens. The research and career development plan I proposed will provide the basis for me to make significant contributions to the microbial pathogenesis research community.

Yoav Livneh, Ph.D.

Postdoctoral Fellow

Beth Israel Deaconess Medical Center / Harvard Medical School

Mentor: Mark Andermann, Ph.D.

“Convergent and Divergent Coding in Insular Cortex and Amygdala Across Hunger and Thirst”

Physiological need states, such as hunger and thirst, produce powerful motivations to obtain specific goals, such as food or water. Yet it remains unknown how the specificity of goal-seeking is achieved.

How is one goal prioritized over another to satisfy the current physiological need? (e.g., water-seeking but not food-seeking during thirst).

Human neuroimaging studies implicated insular cortex (InsCtx) in these phenomena. However, it remained unknown how InsCtx activity guides action based on current physiological state. We have recently investigated the influence of hunger on InsCtx processing of food-predicting cues. We developed a microprism-based method for cellular-resolution imaging of InsCtx in behaving mice. InsCtx neurons demonstrated food cue-biased responses that were abolished following satiation. Circuit mapping and pathway-specific manipulations uncovered an underlying pathway linking hypothalamic “hunger neurons” (AgRP-expressing) to InsCtx, via paraventricular thalamus and basolateral amygdala (BLA).

This proposal builds on these findings to explore how specificity of need-driven goal-seeking is achieved. We hypothesize that InsCtx receives predicted reward information from BLA and combines this with current physiological state information. Thus, InsCtx produces specific predictions of whether the associated outcome will satisfy current physiological need (“how will this make me feel?”). We will test this hypothesis by contrasting InsCtx and BLA→InsCtx responses to food-and water-predicting cues during hunger and thirst in the following aims:

1. Determine whether InsCtx neurons respond to water-predicting cues during thirst but not after its quenching, and whether this can be rescued by activation of “thirst neurons”.
2. Determine whether different InsCtx neurons respond to distinct need-relevant cues specific to hunger or thirst.
3. Determine whether BLA→InsCtx neuronal responses to reward-predicting cues are not specific to distinct need states (hunger/thirst).

These experiments should provide important insights into decision making during natural and pathological need states that underlie various clinical conditions, from compulsive eating to drug addiction.

Xin Mu, Ph.D.

Postdoctoral Fellow

Boston Children's Hospital

Mentor: Sun Hur, Ph.D.

“Molecular Dissection of MDA5 Filament in Antiviral Immunity”

Effective immune defense against viral infection is dependent upon efficient detection of viral RNA by innate immune receptors. One such receptor is MDA5 that recognizes double-stranded RNAs (dsRNAs) originated from a broad range of viruses and elicits the type I interferon response to restrict viral propagation. While its proper function is critical for host defense against viral infection, mis-regulation of MDA5 causes a variety of auto-immune and auto-inflammatory diseases. More recently, MDA5 was also shown to be important for successful cancer immunotherapies. Despite the importance, deep molecular mechanisms of how MDA5 recognizes viral RNAs and activate signaling are incompletely understood. Previous studies from the Hur lab showed that MDA5 forms filaments upon dsRNA binding and filament formation is required for signal activation. However, key molecular events that occur between filament formation and signal activation remain poorly characterized. The goal of my research

is to dissect these poorly understood molecular events using a combination of structural biology and biochemistry. In particular, my current focus is on the role of ATP binding and hydrolysis by the MDA5 helicase domain, the activity implicated in multiple functions, including MDA5 filament regulation and auto-inflammatory diseases. Using a combination of cryo-electron microscopy, biochemistry and cellular assays, I am currently investigating the structural basis for ATP-mediated MDA5 filament disassembly and the role of ATP in regulating of MDA5 auto-repression. This work builds upon my previous work in the Hur lab, which led to one co-first authored publication in Cell and another first authored manuscript under revision in Nucleic Acid Research. Together with my expertise from Ph.D. in virology, I believe the proposed work in structural biology and biochemistry will help me achieve my goal of becoming an independent scientist who explores new important questions in host-virus interaction using a multi-disciplinary approach.

Enzhi Shen, Ph.D.

Post Doc

University of Massachusetts Medical School

Mentor: Craig C. Mello, Ph.D.

“Dissection of Self/Non-self Nucleic Acid Recognition and Signaling”

A long-standing question in eukaryotic gene regulation is how, and to what extent, are the nuclear events of transcription and splicing coupled to the subsequent cytoplasmic events controlling mRNA translation. Argonaute systems provide potential links between these fundamental steps in gene expression. In *C. elegans*, Argonautes engage both mature cytoplasmic transcripts and nascent nuclear transcripts. For example, the Argonaute CSR-1 binds chromatin and mRNAs of active genes, while WAGO Argonautes target silent genes and mRNAs. This connection is best understood for the WAGO/silencing pathway wherein chromatin factors, including homologs of Polycomb and heterochromatin protein 1 (HP1), are required for WAGO-mediated silencing. Thus, mRNAs produced within silent chromatin are thought to feed the amplification and maintenance of silencing, while mRNAs produced within euchromatin are somehow protected. Recently, I analyzed mRNA targeting by a third Argonaute system in the germline, the piRNA/Piwi Argonaute pathway. Targeting by piRNAs, which are genomically encoded, triggers WAGO-dependent silencing. My analysis revealed that piRNA targeting follows micro-RNA-like “seed” and supplementary base pairing rules. Surprisingly, even expressed mRNAs, although protected from piRNA-induced silencing, are abundantly targeted by piRNAs within both their open reading frames (ORFs) and untranslated regions (UTRs). I hypothesize that the piRNA pathway senses features of mRNAs that reflect their origin within euchromatin or heterochromatin and, in response to these cues, recruits distinct downstream factors that regulate the fates of their target mRNAs. Here, I will explore the downstream factors recruited to mRNAs by piRNA targeting (Aim 1) and the upstream factors that modulate targeting (Aim 2). I will also search for new factors that influence transcriptome surveillance and epigenetics (Aim 3). This study will uncover fundamental mechanisms that link transcription and translation in the germline, and perhaps other tissues, and will further our understanding of epigenetic programming important in both normal development and disease.

Gloria Sheynkman, Ph.D.

Research Fellow

Dana-Farber Cancer Institute

Mentor: Marc Vidal, Ph.D.

“Deciphering the Deregulated Network Driven by Transcription Factor Isoform Switching in Melanoma”

Accumulation of genomic mutations within oncogenes or tumor suppressor genes is widely seen as the primary cause of cancer progression. However, another mechanism of molecular perturbation, likely just as important yet vastly understudied, is the widespread changes in alternative splicing typical of all cancers. Indeed, alternative splicing changes the composition and expression levels of protein isoforms, drastically remodeling the cancer transcriptome and proteome. These changes are particularly impactful in the rewiring of gene regulatory networks (GRNs) through changes in the primary structure of transcription factors (TFs). Despite recent studies correlating isoform expression with cancer phenotypes, how cancer TF isoforms functionally impact cancer progression remains largely unknown. Therefore, new global approaches are needed to dissect the mechanistic implications of dysregulated splicing on GRNs at genome-scale.

We have selected melanoma as a model system to initiate global studies of cancer-specific TF isoforms. In my preliminary studies, I observed that MITF, a central factor in melanocyte differentiation whose locus is frequently amplified in melanomas, undergoes an isoform switching event by which non-melanocyte MITF forms become the dominant isoforms. Here, I will comprehensively discover melanoma-specific TF isoforms using a novel isoform capture technology that I developed. This technology enables enrichment of transcripts from target genes of interest for their subsequent full-length sequencing, creating a deep-coverage census of aberrant splice changes correlated with melanoma. I will perturb the expression of these isoforms in melanoma short term cultures to assess their functional effects on melanoma proliferation and growth. Furthermore, to understand the underlying mechanisms of differential isoform functions, I will employ a high-throughput functional profiling platform I helped develop where I will map changes in protein-protein/protein-DNA interactions between normal and cancer specific isoforms. This work will set a new paradigm for assessing the global rewiring of cancer-specific networks and will likely result in precise therapeutic targets.

Konstantinos Sousounis, Ph.D.

Postdoctoral Fellow

Brigham and Women's Hospital

Mentor: Jessica Whited, Ph.D.

“Elucidation of Molecular and Cellular Events that Drive Ocular Tissue Regeneration in Axolotls as a Basis for Future Therapeutic Approaches”

Incurable eye diseases that cause blindness currently affect eleven million Americans. Loss of vision is due to degeneration of retina cells, a destroyed optic nerve, or a cloudy lens, all associated with the pathophysiology of common eye diseases including age-related macular degeneration, diabetic retinopathy, glaucoma and cataracts. My plan is to induce autologous regeneration, so the human eye can repair itself and restore function. To accomplish that I will study how regeneration of ocular tissues occurs naturally in axolotls. These amphibians have tremendous regenerative abilities and a wealth of available genetic and molecular tools that will enable me to study the mechanism in detail. My long-

term goal is to find how amphibians utilize the eye's pigmented epithelium to regenerate the retina and the lens following injury, and translate this mechanism to innovative medical treatments. I will focus on axolotl lens regeneration; following removal of the whole lens, the iris pigmented epithelium (IPE) will reprogram to regenerate a new lens in just three days. Using this model, I can tease apart the molecular and cellular events involved in regenerating a new ocular tissue. My experimental design utilizes cutting-edge axolotl editing technologies including novel transgenic as well as CRISPR/Cas9-based knock-in and knock-out animals, to unbiasedly investigate: (SA1) a previously identified candidate gene for axolotl lens regeneration, Eyes Absent 2 and its role during IPE cell reprogramming, and (SA2) whether specific IPE cell subpopulations are responsible for lens regeneration. These experiments will provide fundamental insights on the molecular and cellular mechanisms underlying the reprogramming of the pigmented epithelium during regeneration of ocular tissues. Understanding how this process is controlled naturally in amphibians is critical to safely induce autologous regeneration in humans. Translating this mechanism to the clinic will revolutionize regenerative medicine and provide groundbreaking treatments to incurable human eye diseases.

Bing Zhang, Ph.D.

Postdoctoral Fellow

Harvard University

Mentor: Ya-Chieh Hsu, Ph.D.

“Elucidate the Cellular and Molecular Mechanisms for Slow Wound Healing Upon Chemotherapy”

Chemotherapy combined with surgery is the most prevalent approach to treat cancer. However, chemotherapy leads to significantly delayed injury repair in the skin. Slow wound healing hinders surgical recovery and increases the chance of life-threatening infections. These side effects increase the mortality rate and create physical and emotional burdens for cancer patients and caretakers. Nevertheless, the cellular and molecular mechanisms leading to delayed wound healing upon chemotherapy remain poorly understood. This gap in knowledge prevents the development of strategies to enhance injury repair in chemotherapy patients.

Chemotherapeutic drugs ablate fast-dividing cells. Most somatic stem cells rarely divide and are spared during chemotherapy. By contrast, stem cells' fast-dividing progeny, transit-amplifying cells (TACs), are often destroyed. Work published from the Hsu lab, including my own, suggest that TACs of the hair follicle (HF-TACs) play diverse regulatory roles in the skin. My preliminary data further suggest that HF-TACs are indeed ablated by chemotherapeutic agents. Moreover, my preliminary data suggest that HF-TACs are involved in wound repair. Therefore, loss of HF-TACs might be the basis for the slow wound healing observed during chemotherapy.

My objective is to elucidate the cellular and molecular mechanisms by which HF-TACs regulate skin injury repair and to identify genes and/or pathways that can enhance wound healing under chemotherapy. My central hypothesis is that HF-TACs govern changes in diverse skin cell types to promote wound healing through secreted factors. In Aim1, I will focus on delineating the function of Sonic Hedgehog (SHH, a known HF-TAC secreted protein) in wound healing. In Aim2, I will test the function of other secreted proteins expressed by HF-TACs in promoting wound healing during chemotherapy. Results from these aims will be the foundation to new therapies that can promote injury repair in cancer patients that have undergone chemotherapy and surgery, thereby enhancing treatment efficacy.

Ting Zhang, Ph.D.

Postdoctoral Fellow

Brigham and Women's Hospital

Mentor: Matthew Waldor, M.D., Ph.D.

“Colonization, Dissemination, and Transmission of the Enteric Pathogen *Listeria Monocytogenes*”

L. monocytogenes is Gram-positive foodborne facultative intracellular pathogen that can disseminate from the gastrointestinal tract to distal sites and cause systemic infection. Numerous studies have been performed of bacterial and host factors that mediate its internalization and intracellular spread, as well as of the host response to intracellular bacteria. In contrast, there has been minimal analysis of *L. monocytogenes* as an enteric pathogen, and the genetic requirements for *L. monocytogenes* colonization of the GI tract have not been determined. In addition, little is known about how *L. monocytogenes* responds to the harsh environment within the gallbladder, a distal colonization site that we found is the source of fecally shed bacteria, nor about how the gallbladder responds to the presence of the pathogen.

Using a murine model of infection, we determined that a relatively small number of bacteria (generally <100) give rise to the population at each colonization site, despite use of large infectious doses. However, we have found that pretreatment of mice with streptomycin markedly augments these “founding populations”, and thereby enables analysis of bacterial factors required for colonization using transposon insertion libraries (TnSeq).

The long term objective of this proposal is to understand how pathogen and host factors control the proliferation of *L. monocytogenes* within the gastrointestinal tract and gallbladder, in order to better combat pathogenesis and improve the biosafety of *L. monocytogenes*-based vaccines. Toward that end, we propose to:

1. Characterize the genetic requirements for *L. monocytogenes* to overcome host defenses and colonize the gastrointestinal tract.
2. Analyze the interplay between *L. monocytogenes* and the gallbladder.

Using several high throughput approaches, and a selection of bacterial mutants and knock-out mice, we will identify bacterial factors that *L. monocytogenes* relies on to colonize the gastrointestinal tract and gallbladder, and also define the gallbladder transcriptional response to *L. monocytogenes* infection.

Zhibin Zhang, Ph.D.

Postdoctoral Fellow

Boston Children's Hospital

Mentor: Judy Lieberman, M.D., Ph.D.

“The Effect of Gasdermin E Cleavage by Caspase-3 and Granzymes on Inflammation and Tumor Responses to Chemotherapy and Tumor Immunity”

Pyroptosis, the inflammatory death triggered by cytoinvasive pathogens and danger signals was mediated by inflammatory caspase cleavage of gasdermin D (GSDMD). The N-terminus of cleaved gasdermin D forms pores in the membrane to permeabilize and kill the cell and release inflammatory

cytokines. GSDMD belongs to a family of six homologous human gasdermin (GSDM) proteins, most of which exhibit pore-forming activity. However, how other family members are activated is unknown. An exception is GSDME. Two recent studies found that GSDME is cleaved and activated by caspase-3, the key effector caspase protease in apoptosis, suggesting that noninflammatory apoptosis secondarily activates pyroptosis. I found that two granzymes, the death-inducing serine proteases in NK and Cytotoxic T cells, also cleave GSDME to liberate an N-terminal fragment that causes cell death. GSDME are expressed in many normal tissues but its expression is diminished in multiple human cancer cell types because of promoter hypermethylation. Moreover, spontaneous GSDME mutations have been found in many cancers. I hypothesize that loss of GSDME expression or loss-of-function (LOF) mutations protects cancer cells from inflammatory death induced by caspase-3 and granzymes to promote tumor survival and growth. Furthermore, I hypothesize that proinflammatory cytokines and other inflammatory mediators are released in a GSDME-dependent manner during pyroptosis, which activates immune responses to the tumor and enhances the effectiveness of chemotherapy, i.e. that GSDME activation of pyroptosis leads to “immunogenic” tumor cell death. In this proposal, I will determine if GSDME cleavage causes secondary pyroptosis and whether cancer cells inactivate GSDME to avoid caspase-3 or Gzm-induced pyroptosis. I will also use mouse model to determine the roles of GSDME and pyroptosis in cancer cell elimination. Understanding this new inflammatory pathway that some cancer cells evade may provide insights into developing novel cancer therapeutics and understanding drug resistance.

Mark Zimmerman, Ph.D.

Research Fellow

Dana-Farber Cancer Institute

Mentor: A. Thomas Look, M.D.

“MYC-driven Core Regulatory Circuits in Neuroblastoma”

Despite their known importance in cancer, direct targeting of MYC and MYCN as a therapeutic strategy in pediatric tumors such as neuroblastoma has proven extremely challenging. However, we now know that in tumor cells, MYC functions in concert with a small group of highly expressed transcription factors referred to as the core regulatory circuit (CRC) that uniquely establishes cellular identity and disease states. In neuroblastoma, this CRC includes the highly expressed transcription factors HAND2, ISL1, PHOX2B, GATA3 and TBX2, which along with MYC or MYCN, form a feed-forward autoregulatory loop that regulates the entire cellular transcriptome. The goal of this proposal is to understand how the malignant state of neuroblastoma cells is supported by the CRC, and how this process can be exploited for the benefit of patients. My central hypothesis is that disruption of the CRC and the signaling pathways it regulates will result in loss of malignant cell viability and provide novel strategies for targeted therapy. Furthermore, by elucidating the epigenetic mechanisms through which maintenance therapy with retinoic acid regulates the neuroblastoma CRC, I will provide critical insights that will inform the development of future treatments. The specific aims for this project are: 1) dissect the specific mechanisms by which disruption of individual core regulatory circuitry (CRC) component genes inhibit the growth and survival in human neuroblastoma cells.; and 2) determine the epigenetic mechanisms through which retinoid-based therapeutics reprogram the neuroblastoma CRC and lead to differentiation, arrest or apoptosis in human cells and PDX tumors in mice. The information gained from these approaches will be critical to the rational design and use of circuitry-directed therapeutics that will be used to treat malignancy on a disease-specific basis.

2017 Grant Recipients

Halil Aksoylar, Ph.D.

Research Fellow

Beth Israel Deaconess Medical Center / Harvard Medical School

Mentor: Vicki Boussiotis M.D. Ph.D.

“Regulation of Macrophage Activation by Mitochondrial Metabolism”

Macrophages form an innate immune line of defense against invading pathogens. During a microbial infection, macrophages respond to LPS, a component of gram negative bacteria, by upregulating inflammatory cytokine production and antimicrobial activity. While LPS activation is important for host defense during microbial infection, exaggerated inflammatory responses impair tissue functions and must be restrained by negative feedback mechanisms. Indeed, sustained exposure to LPS stimulates entry to an unresponsive state called LPS tolerance, in which macrophages are profoundly refractory to LPS stimulation and fail to induce an inflammatory response. How macrophages coordinate the switch between induction and suppression of inflammatory responses to allow for optimal host defense yet limit excessive and maladaptive inflammation is not known.

Our preliminary studies demonstrate that, upon LPS signaling, AKT mediated glucose utilization impinge on the mitochondria to determine the state of macrophage activation or tolerance. A critical regulator of this process is GPD2, a component of the electron transport chain (ETC) that receives electrons from glycolysis to link glucose utilization to electron transport chain activity. Acute/low dose LPS signaling stimulates GPD2 activity to increase ETC activity, TCA cycling, Ac-CoA availability, and histone acetylation at inflammatory genes. However, sustained/high dose LPS signaling prolongs glucose utilization to saturate electron transport capacity, leading to reduced Ac-CoA availability and histone acetylation at inflammatory genes. In this way, mitochondria monitor the extent of LPS signaling to orchestrate the switch between LPS activation and tolerance.

The goal of this project is to further test the working model proposed above. Specifically, I will determine the roles of Akt, glucose utilization, and GPD2, in regulating LPS activation and tolerance. I will also determine if GPD2 regulates inflammatory response induction and suppression in vivo. These studies will reveal novel modes of regulation that control macrophage function in the context of inflammatory diseases.

Reza Behrouzi, Ph.D.

Postdoctoral Fellow

Harvard Medical School

Mentor: Danesh Moazed, Ph.D.

“Structural Characterization of Silent Chromatin Domains by Single Particle Cryo-EM”

Transcriptionally silent and structurally distinct chromatin domains, known as heterochromatin, play critical roles in gene expression regulation, cellular identity, and chromosome stability in eukaryotic cells. Heterochromatin nucleation at specific genomic loci requires specific DNA sequences and associated proteins, which recruit effector complexes that contain both histone-modifying activity and

modification-binding domains. Following initiation, the effector complexes spread out tens to hundreds of kilobases without sequence specificity, presumably by stepwise modification and binding to nucleosomes.

The structure and functions of heterochromatin are strikingly conserved through evolution. Homologous complexes of human heterochromatin machinery have been identified in other mammals, flies, and unicellular fission yeast. However, despite considerable progress in characterizing heterochromatin systems in various model organisms, models of heterochromatin formation, epigenetic inheritance, and function remain largely speculative, due to the absence of high resolution structural models of heterochromatin.

My postdoctoral research has focused on quantitative studies of heterochromatin as a biomolecular assembly process. I arrived at a new understanding of how yeast silencing complexes organize on chromatin to form heterochromatin domains. Here, I describe a research plan that builds on my previous work to determine the first atomic resolution models of heterochromatin domains by cryo electron microscopy. Preliminary negative stain EM images of single particles and the resulting class averages confirm the success of the proposed strategy in structural characterization of heterochromatin. Ultimately, the findings are expected to provide fundamental insights on how silencing factors change the structure of chromatin and restrict access to DNA.

Caroline Heintz, Dr. sc. nat.

Postdoctoral Fellow

Harvard T.H. Chan School of Public Health

Mentor: William Mair, Ph.D.

“The Physiological Role and Regulation of Splicing Factor 1 in Aging and Longevity”

Aging is driven by a loss of cellular and organismal homeostasis, and is the key risk factor for multiple chronic diseases. Interventions that attenuate or reverse systemic dysfunction seen with age therefore have potential to reduce overall disease risk and prolong healthy years in the elderly. Dietary restriction (DR) is currently the most potent intervention known to increase health- and lifespan and protect against multiple chronic diseases. Loss of protein and transcriptional homeostasis are well-established causes of aging. However, a link to RNA homeostasis and splicing fidelity, key processes in the central dogma of molecular biology, in aging was largely unexplored. Using in vivo splicing reporters in *C. elegans* and RNA-Seq, we recently showed that RNA splicing deregulates with age and DR promotes longevity via increased pre-mRNA splicing efficiency. I identified splicing factor 1 (SFA-1) to be specifically required for DR and target of rapamycin complex 1 (TORC1)-mediated longevity. Further, we identified TOR signaling as a mediator of the effects of *sfa-1* in DR. Our transcriptome data suggest that SFA-1 mediates longevity via regulation of lipid metabolism. Lastly, increased expression of SFA-1 is sufficient to extend lifespan of wildtype nematodes, suggesting that interventions targeting RNA splicing present novel therapeutic avenues for age-onset diseases.

The current proposal aims at specifying the role and mechanism of SFA-1 in aging and longevity. First, I aim to define the downstream physiological effects and targets of *sfa-1* to promote healthspan, lifespan extension and in DR/TOR-mediated longevity. The second aim will delineate the regulation of *sfa-1* in aging and by TORC1 on translational level. I will combine *C. elegans* and mammalian tissue culture approaches to assess the mechanistic link between *sfa-1* and TOR signaling in splicing regulation.

Together, this proposal will lead to increased mechanistic understanding of SFA-1 both on gene and protein level regulating longevity.

Ni Ji, Ph.D.

Postdoctoral Fellow

Massachusetts Institute of Technology

Mentor: Steven Flavell, Ph.D.

“Circuit Mechanisms Underlying Sustained Behavioral States in *C. Elegans*”

The internal state of our mind--whether we are sleepy or alert, motivated or satiated--can have profound influences on our behavior. Despite much effort in research, we still lack circuit-level understanding of how internal brain states are generated and maintained over long time scales. Past studies have revealed a key role of neuromodulators, such as serotonin and dopamine, in impacting brain states. However, only in a few cases have we successfully uncovered the circuit-level mechanisms by which neuromodulation impacts internal and behavioral states.

In this study, I will use *C. elegans* as a model to examine circuit-level mechanisms underlying internal brain states and their control by neuromodulation. Many behaviors of *C. elegans* are modulated by the satiety state of the animal. When exposed to food, *C. elegans* exhibits two long-lasting locomotory states, the roaming state and the dwelling state, during which the animals exhibit sharp contrast in locomotory speed. A recent study by Dr. Flavell found that

serotonin and the pigment dispersal factor (PDF) function antagonistically to extend the durations of roaming and dwelling, respectively. It remains unclear what types of circuit activity underlie the roaming-dwelling states, and how neuromodulatory effects propagate through its immediate target neurons to exert global impact on the circuit.

To address these open questions, I propose to image circuit-wide neural activity in freely moving animals during roaming and dwelling behaviors. I will then use a combined computational and experimental approach to identify the circuit-wide activity patterns that causally impact the roaming-dwelling states. Next, I will measure and analyze circuit activity patterns in mutant animals with disrupted neuromodulation. Finally, I will use data-driven network modeling to decipher the mechanism by which neuromodulation impacts circuit-wide activity states. This study will be one of the first to connect circuit dynamics with network topology, neuromodulation, and emergent behavior.

Lan Jiang, Ph.D.

Research Associate

Boston Children's Hospital

Mentor: Yi Zhang, Ph.D.

“Identifying and Functional Characterizing Imprinting Control Regions of Novel DNA Methylation-Independent Imprinting Genes”

Genomic imprinting is an allelic gene expression phenomenon that is controlled by differential DNA methylation at the imprinting control regions (ICR). Imprinted genes play important roles in a wide range of biology processes and dysfunction of these genes is the cause of several human syndromes. Despite the

role of DNA methylation in controlling imprinted gene expression, recent studies have revealed several genes that exhibit DNA methylation-independent imprinted expression, raising the possibility that novel imprinting mechanism may exist. By comprehensive mapping of allelic-specific DNase I hypersensitive sites (DHSs) and gene expression using physically-separated parental pronuclei, as well as gynogenetic embryos, we identified several dozen paternally-expressed genes that harbor paternal allelic DHSs (Ps-DHSs) that are highly enriched for tri-methylation H3K27 (H3K27me3) but devoid of DNA methylation in maternal allele. Importantly, ectopic removal of the H3K27me3 turns Ps-DHSs into bi-allelic DHSs and induces maternal allele expression in genes that include all the known DNA methylation-independent imprinted genes *Gab1*, *Sfmbt2*, *Slc38a4*, and *Phf17*. These results establish maternal H3K27me3 as a novel DNA methylation-independent genomic imprinting mark.

The discovery of the novel imprinting mechanism and the identification of new non-canonical imprinted genes provide me the opportunity to dissect the novel imprinting mechanism. Toward this goal, I propose to identify and characterize ICRs of this group of non-canonical imprinted genes with the following two aims:

- 1) Define the ICRs of the H3K27me3-controlled imprinted genes through integrated analysis of allelic H3K27me3, allelic DHSs, and allelic gene expression affected by H3K27me3-specific Kdm6b injection;
- 2) Functionally validate putative ICRs by CRISPR-based epigenome editing.

Completion of the proposed study will not only identify ICRs of the newly discovered non-canonical imprinted genes, but also prove that H3K27me3 as a novel imprinting mark. Understanding this novel imprinting mechanism has important implications for understanding imprinting related disorders.

Mei Ng, Ph.D.

Postdoctoral Fellow

Massachusetts General Hospital

Mentor: Rakesh Jain, Ph.D.

“Alleviating Solid Stress to Enhance Immunotherapy in Metastatic Breast Cancer”

Metastatic breast cancer (mBC) is the chief cause of mortality among breast cancer (BC) patients. Our incomplete understanding about many aspects of the microenvironment of BC metastatic lesions currently hinders our treatment of the disease. In this fellowship application, I will explore how mechanical forces in the mBC microenvironment affect its immune evasion and resistance to immunotherapy. In primary BC, the mechanical pressure accumulated within the solid components of the tumor-- termed solid stress-- is elevated, and causes pronounced vascular compression. This vascular compression leads to decreased blood perfusion and increased hypoxia, both of which promote BC immunosuppression. The cause of elevated solid stress in primary BC is desmoplasia, characterized by high levels of cancer-associated fibroblasts and extracellular matrix deposition. Primary BC desmoplasia and solid stress can be reduced using angiotensin II receptor 1 blockers (ARBs). What remains unknown, however, is whether solid stress elevation in the metastatic setting of BC is also associated with desmoplasia, angiotensin II signaling and hypoxia, and what are the consequences of elevated solid stress on the tumor immunology of mBC. I will address these questions by modulating solid stress in a mouse model of triple-negative BC brain metastases through direct mechanical manipulation (Aim 1) and pharmacological inhibition of angiotensin II signaling (Aim 2). In the pharmacological approach, I will use a novel ARB-based therapeutic that becomes selectively activated

in the mBC microenvironment, minimizing systemic side effects. I will characterize the changes in the immune microenvironment in response to solid stress alterations. Importantly, I will test whether alleviation of solid stress can improve the outcome of immune checkpoint inhibitors in mBC. By elucidating the contribution of solid stress to mBC immunosuppression, the proposed work will lead to new paradigms for treating mBC, where anti-desmoplastic agents are rationally applied to enhance the efficacy of promising immunotherapeutics.

Andrey Parkhitko, Ph.D.

Postdoctoral Fellow

Harvard Medical School

Mentor: Norbert Perrimon, Ph.D.

“Studying Methionine Flux and its Role in Aging and Neurodegeneration”

Aging is a risk factor for various human pathologies including neurodegenerative diseases. Using targeted high-throughput metabolite profiling in *Drosophila melanogaster* at different ages, we have demonstrated that methionine metabolism changes during aging. Methionine generates the methyl donor, S-adenosylmethionine (SAM), which is converted via methylation to S-adenosylhomocysteine (SAH). Ahcy13 is the only known enzyme and the rate-limiting step in the conversion of SAH into homocysteine. Elevated homocysteine level in human plasma is a known risk factor for dementia, Alzheimer's disease, and stroke. Understanding how the flux via methionine metabolism is reprogrammed with age will help develop strategies that prevent its age-dependent changes and have a great impact on human health.

We previously showed that SAH accumulates with age and that downregulation of dAhcyL1/dAhcyL2, which can heterotetramerize with Ahcy13 and inhibit its function, extends life- and healthspan. Based on our model, downregulation of dAhcyL1/dAhcyL2 activates Ahcy13 that promotes SAH and homocysteine processing, activates flux via methionine metabolism and phenocopies the effect of methionine restriction (MetR). However, how the flux via methionine metabolism is reprogrammed into alternative pathways during aging is unknown, representing the critical knowledge gap. My central hypothesis is that tissue-specific activation of methionine flux promotes SAH and homocysteine processing and extends *Drosophila* health- and lifespan. An important corollary of this hypothesis is that activation of methionine flux can improve healthspan of *Drosophila* models with age-dependent neurodegeneration. Ultimately, translating findings from the *Drosophila* model to mammalian systems may provide better therapeutic options for patients with age-dependent neurodegenerative diseases.

To address my hypothesis, I propose two Specific Aims:

Aim 1: Test whether downregulation of dAhcyL1/dAhcyL2 prevents muscle and brain age-dependent changes, and improves healthspan of *Drosophila* models with age-dependent neurodegeneration.

Aim 2: Determine how aging reprograms methionine flux and establish *Drosophila* genetic MetR model for characterizing tissue-specific effects of MetR.

Jianbin Ruan, Ph.D.

Postdoctoral Fellow

Boston Children's Hospital

Mentor: Hao Wu, Ph.D.

“The Killer Protein Gasdermin D: Activation Mechanism and a New Potential Therapeutic Target”

While inflammation is an important defense mechanism against pathogens and other types of dangers, it often causes serious human diseases when overactivated. One source of inflammation is elicited from intracellular macromolecular complexes dubbed as inflammasomes, which activates inflammatory caspases such as caspase-1 and caspase-11. Activated caspases cleave pro-IL-1 β and other pro-inflammatory cytokines into the active isoforms thus amplifying the inflammatory response. For a long time, it is known that inflammasomes also induce a form of pyroptotic cell death characterized by cell lysis. However, the specific molecule involved in pyroptosis was not elucidated until recent research indicated that caspase-1 cleaves Gasdermin D (GSDMD), a member of the Gasdermin superfamily, to cause cell death. Our preliminary studies show that the GSDMD N-terminal fragment triggers pyroptosis by forming pores that compromise the integrity of the cell membrane. Therefore, inhibition of pyroptosis by targeting GSDMD may represent a unique therapeutic strategy to limit cell death and overt inflammation. In this application, I propose systematic studies on elucidating the pore assembly mechanism of Gasdermin D, and on identifying small molecule inhibitors against the pore formation. The molecular understanding on how the N-terminal region of Gasdermin forms lytic pores would not only provide new insights on the mechanism for pyroptosis but also afford a new therapeutic strategy for human diseases especially sepsis and other inflammatory diseases.

Brian Russo, Ph.D., M.A.

Research Fellow

Massachusetts General Hospital

Mentor: Marcia Goldberg, MD

“The Type 3 Secretion System Translocon Pore in Bacterial Pathogenesis”

Diarrhea is a leading cause of death and morbidity of children less than 5 years old. Globally, Shigella is the leading cause of diarrhea in children. To cause disease, Shigella invades and spreads among intestinal epithelial cells; both invasion and spread requires activity of a type 3 secretion system (T3SS). T3SSs are required for the virulence of approximately 30 different bacterial pathogens that cause disease in humans. Contact with a mammalian cell activates the T3SS to form the translocon pore in the membrane of the cell. This pore is essential for the T3SS to deliver virulence proteins into the mammalian cytosol. T3SSs, including the translocon pore, are highly conserved and further understanding of how they function will likely identify conserved virulence strategies shared among these human pathogens. At invasion, the T3SS translocon pore interacts with intermediate filaments, structural proteins in mammalian cells. This interaction enables docking of Shigella to cells by unknown mechanisms. Docking activates secretion of virulence proteins through the T3SS. In contrast to invasion, Shigella spread requires T3SS activity, but it occurs without intermediate filaments. The overall goals of this project are to investigate how the translocon pore supports Shigella infection and to test whether the translocon pore can be used as a therapeutic target to block infection. Aim 1: To test whether the conformation of the translocon pore protein IpaC is different in docking competent versus docking incompetent translocon pores. Aim 2: To test whether docking is required to activate secretion of virulence proteins. Aim 3: To test whether the conformation of the T3SS translocon pore can be

manipulated. Due to the conserved and important role of T3SSs among bacterial pathogens, this proposal is likely to identify novel bacterial virulence mechanisms that have the potential to be exploited for the development of new therapeutic interventions.

John Salogiannis, Ph.D.

Postdoctoral Fellow

Boston Children's Hospital

Mentor: Thomas Schwarz, Ph.D.

“Mechanisms of Microtubule-Based Transport via Organelle Contacts”

Eukaryotic cells use microtubule-based transport and molecular motors for the delivery of vesicles and organelles required for cell growth, maturation, and survival. Perturbations in microtubule-based transport are an early hallmark of neurological diseases. My long-term goals are to determine how movements of a diverse set of cargos are spatially and temporally regulated in cells, and how they go awry in disease. The canonical view of how cargos move is that each cargo has an adaptor protein that directly links it to the transport machinery. In contrast to this view, my postdoctoral work, and other recent studies, revealed a new mode of transport called "hitchhiking": some organelles can move by connecting to rapidly moving endosomes that have already recruited the transport machinery. Consequently, hitchhiking cargos bypass the need to recruit their own set of molecular motors, and are tightly coupled to the movement of endosomes. Peroxisomes, lipid droplets, and the endoplasmic reticulum all achieve motility in filamentous fungi by hitchhiking. My preliminary data demonstrates that peroxisomes also hitchhike on endosomes in hippocampal neurons.

The molecular mechanisms dictating organelle hitchhiking are not well understood. My published work identified the novel tether PxdA as a central regulator of endosome-mediated hitchhiking of peroxisomes, but how it achieves this remains unclear. To determine the mechanism of organelle hitchhiking, I will use a combination of live-cell imaging, electron microscopy, biochemistry, and genetics. In aim 1 I will determine how peroxisomes, lipid droplets, and endoplasmic reticulum hitchhike on endosomes in the filamentous fungus, *Aspergillus nidulans*. In aim 2 I will determine if hitchhiking is conserved in mammals. I will follow up on my preliminary observations that peroxisomes hitchhike in neuronal axons and determine if other organelles hitchhike in neurons. These experiments will lead to a mechanistic understanding of hitchhiking, a new paradigm in microtubule-based transport.

Kfir Sharabi, Ph.D.

Research Fellow

Dana-Farber Cancer Institute

Mentor: Pere Puigserver, Ph.D.

“Dissecting Mechanisms that Control Hepatic Glucose Production Using a Newly Identified Anti-Diabetic Drug”

Type 2 diabetes is currently a world-wide epidemic with an urgent medical need for novel anti-diabetic drugs. Suppression of hepatic glucose production ameliorates diabetic symptoms, suggesting that targeting regulatory components within this pathway can potentially be used to treat diabetes. The transcriptional coactivator PGC-1 α is a potent activator of hepatic glucose production during fasting and diabetic states, through induction of genes encoding enzymes in the gluconeogenic pathway. PGC-1 α

gluconeogenic activity is negatively regulated through acetylation catalyzed by the GCN5 acetyl transferase, decreasing its ability to promote hepatic glucose production. Thus, small molecules that induce PGC-1 α acetylation could be potential drugs that might be used to control systemic blood glucose levels. We have designed and developed a high-throughput chemical AlphaLisa screen and used MLPCN and DOS libraries to identify chemical scaffolds that increase PGC-1 α acetylation. As a proof of concept for the potential use of selective targeting of PGC-1 α as treatment for diabetes, I have shown that a single compound from this screen, SR-18292, potently improves whole body insulin sensitivity leading to improved glucose homeostasis in diabetic mouse models. The improved glucose homeostasis is a result of reduced hepatic glucose production, since glucose uptake is not affected.

I will use compound SR-18292 as a tool to gain new insights into how hepatic glucose production is regulated. This is an exciting opportunity since the proteins or enzymes that are targeted by this compound are currently unknown. In aim 1, I will use biochemical and proteomic tools to determine the molecular pathways that are activated in response to SR-18292 treatment and lead to formation of an inactive PGC-1 α transcriptional complex. In aim 2, I will identify the molecular changes, at the proteome, genome and metabolome levels, to dissect the molecular changes whereby SR-18292 confers improved glucose homeostasis.

Clare Smith, Ph.D.

Postdoctoral Fellow

University of Massachusetts Medical School

Mentor: Christopher Sassetti, Ph.D

“System Genetics of Tuberculosis Susceptibility”

The outcomes of *Mycobacterium tuberculosis* (Mtb) infections are extremely diverse. Many infected individuals show no signs of disease. Among those that do become ill, the timing, location, and severity of pathology are also variable. Genetic diversity in the host is a major contributor to this variability, but the responsible alleles and mechanisms are poorly defined and have been difficult to address experimentally. In this project, I approach this inherently complex problem by modeling host diversity using the Collaborative Cross (CC), a genetically diverse panel of mice. The unique structure of this population allows the sequential evaluation of variable traits that reflect distinct aspects of pathogenesis. I have used the CC combined with high-throughput bacterial genetics to dissect the genetic basis of tuberculosis (TB) susceptibility at multiple levels, including the gross disease and underlying physiological levels. By defining genetic interactions between host and pathogen, I have developed a system to understand how natural genetic variation in the host alters the immune stresses experienced by Mtb and ultimately influence outcome of infection.

My initial studies implicated Kit Ligand (KitL) variation as an important determinant of TB disease in mice and humans, and associated this genetic variation with alterations in myeloid cell differentiation that I hypothesize to underlie susceptibility. Here, I aim to causally link KitL to TB susceptibility and elucidate the underlying mechanisms driving susceptibility to disease by 1) Investigating the molecular basis of KitL linked TB susceptibility and 2) Defining the biological basis of KitL association with TB susceptibility.

In the long term, this proposal will serve as a blueprint of the strategies I will use in my future independent career to couple this genetically diverse mouse panel with high throughput bacterial genetics to identify new susceptibility genes and interrogate the underlying biological mechanisms

driving outcome to TB.

Yixing Yuchi, Ph.D.

Postdoctoral Fellow

Joslin Diabetes Center

Mentor: Mary-Elizabeth Patti, M.D.

“Resolution of T2D after Gastric Bypass Surgery: Deciphering the Impact of ESM1”

Roux-en-Y gastric bypass (RYGB), considered the gold standard procedure of bariatric surgery, is increasingly recognized as a potent approach for the treatment of both obesity and type 2 diabetes (T2D), as it results in not only sustained weight loss but also diabetes remission and/or improvement in diabetes control. The primary factor(s) and underlying molecular mechanisms contributing to these effects of RYGB to improve glycemic control remain largely undetermined, but are essential to understand in order to develop novel nonsurgical approaches to treat T2D. We have performed comprehensive analysis of the proteome in human blood samples obtained from our longitudinal clinical trial, which randomized individuals to RYGB or intensive medical management and followed them for 3 years postoperatively. This study revealed rapid and sustained improvement in glycemic control in the surgical group as compared with medical management. Proteomics analysis revealed sustained, and significant >2-fold upregulation of 3 members of the IGFBP superfamily: IGFBP1, IGFBP2 and a novel related protein ESM1. All 3 proteins are inversely correlated with hemoglobin A1c. Moreover, upregulation occurred as early as 3 months, before significant weight loss, suggesting the possibility that these factors represent weight-independent contributors to improved glucose levels. These 3 proteins share high protein sequence similarity, including the ability to interact with cell surface integrin β receptor. Therefore, in this proposal, we delineate our plans to investigate the impact of ESM1 in physiology and metabolism by AAV-mediated overexpression of ESM1 in the liver of mice with diet-induced obesity, and to study the impact of ESM1 on hepatic metabolism, signal transduction and transcriptional regulation. Our translational study thus represents an important first step toward determining whether ESM1 may have novel therapeutic potential for the non-surgical treatment of T2D, and to decipher the mechanisms responsible for improved glycemic control after bariatric surgery.

2016 Grant Recipients

Kristopher Burkewitz, Ph.D.

Harvard School of Public Health

Mentor: William Mair, Ph.D.

“Targeting the ER-Mitochondrial Interface to Combat Age-Related Disease”

The combination of an aging global population and the prevalence of age-onset diseases is generating a public health burden that is becoming insurmountable. Interventions causing real or perceived energy-depletion, e.g. dietary restriction or activating the low-energy sensor AMPK, robustly protect against age-related pathology and disease, but carry clinically unacceptable side-effects precluding use as therapeutic regimes. Using an AMPK-dependent model of longevity in *C. elegans*, we uncoupled the negative side-effects of energy-depletion from the benefits on aging through the conserved AMPK

target and transcriptional coactivator, CRTC-1. We showed that the longevity-specific mechanisms downstream of AMPK/CRTC-1 involve reprogramming of mitochondrial metabolism and dynamics.

New data indicate that the unfolded protein response (UPR) of the ER interacts with this same AMPK/CRTC-1 pathway to promote longevity. Through this link, we subsequently identified the first example of an interaction between ER and mitochondrial behaviors that plays a role in aging. Emerging research has revealed that the ER plays critical roles in regulating mitochondrial dynamics and function through interorganelle contact sites, but how the mediators of the UPR play a role in this regulation is not well described. Through the genetic strengths of *C. elegans* and their amenability to *in vivo* microscopy, I will expand on preliminary data to reveal the mechanism by which mediators of ER homeostasis regulate mitochondrial behavior to promote healthier aging. I will define how ER-mitochondrial communication changes during normal aging. Finally, I will combine synthetic and genetic approaches to directly modulate interorganelle communication between ER and mitochondria to begin defining how these organelle interfaces impact the aging process and how they can be targeted to protect metabolic homeostasis in aging animals. Through these aims, the ultimate goal of this proposal is to understand how evolutionarily conserved energy-sensing pathways coordinate subcellular interorganellar communication with metabolic function to promote healthier aging.

Ashesh Dhawale, B.Sc., Ph.D.

Harvard University

Mentor: Bence Ölveczky, PhD

“Role of the Basal Ganglia in the Learning and Execution of Motor Sequences”

The basal ganglia (BG) play an important role in learning and producing motor skills vital to our everyday lives, such as speaking and writing. Their dysfunction - which can be caused by disorders including Parkinson's or Huntington's disease -- gravely impair the execution of learned skills and the capacity to acquire new ones. Despite the importance of motor skills, little is known about the neural mechanisms underlying their learning and execution and the contributions made by the BG.

Motor sequence learning in the BG is usually studied in simple sequencing tasks. These studies have led to models of the BG as high-level controllers that select and initiate entire movement sequences stored in downstream circuits. However, these studies do not monitor movement kinematics or timing of skilled motor sequences, leaving open the question of whether these low-level motor features are encoded by the BG. I will determine whether the BG encode sequence microstructure using a novel sequence learning task for rodents. I will also utilize a chronic extracellular recording platform I developed to track the activity of single neurons in the BG over several weeks, in combination with high-resolution behavioral tracking as rats are performing skilled sequences. I will analyze these datasets to determine whether neural activity encodes low-level microstructure of motor sequences, and if so, what the encoding scheme is. I will then utilize our long-term recording platform to determine how the motor representations in the BG change over the time-course of skill learning and how acquisition of a novel sequences affects representations of pre-existing motor sequences such as grooming. These experiments will identify the role of BG circuits in motor sequence learning and execution that will inform basic understanding of how the brain generates motor skills and inform clinical practice relating to a variety of movement disorders.

Jeffrey Farrell, Ph.D.

Harvard University

Mentor: Alexander F. Schier, PhD

“Dissection of a Newly Discovered DNA Damage Response Pathway in Early Zebrafish Embryogenesis”

Cells with extensive DNA damage are typically eliminated via apoptosis, but pre- and early-gastrulation embryos of most organisms cannot induce apoptosis. What happens to damaged cells in early embryogenesis? I profiled zebrafish embryos by single-cell RNAseq and discovered a new cellular expression program expressed in 'seven-sleeper' cells, which combines genes associated with apoptosis, cellular stress, p53 activation, and several developmental regulators. I hypothesize this program replaces apoptosis in response to damage in the early embryo. Indeed, my preliminary data shows that this program can be induced by DNA damage. Surprisingly, they do not die, even after apoptosis becomes active. The goal of this project is to dissect this newly discovered program and determine its roles in early development and in responding to DNA damage.

In Aim 1, I will determine the origin of the 'seven-sleeper' cells by testing what forms of DNA damage induce them, whether the 'seven-sleeper' cells have encountered DNA damage in vivo, and what aspects of traditional apoptosis they activate. In Aim 2, I will track 'seven-sleeper' cells' fate using two live reporter lines to quantify how many cells die later in development, whether they alter their cell cycle, and which tissues they contribute to. In Aim 3, I will test mutants for each gene in the 'seven-sleeper' pathway for changes in embryonic viability, cell survival, DNA repair, and changes in the expression in other 'seven-sleeper' genes to reconstruct the regulatory cascade and determine the role of this pathway in development.

Together, these experiments will determine how embryonic cells respond to DNA damage without apoptosis, and may shed light on the question of why embryos inhibit apoptosis. Finally, since many of the genes in this pathway are expressed in the immune system or tumor cells, understanding 'seven-sleeper' cells may have implications in other tissues or disease.

Saiyu Hang, Ph.D.

University of Massachusetts Medical School

Mentor: Jun Huh, Ph.D.

“Regulation of Th17/Treg Balance by Gut Bacterial Modification of Bile Acids”

Gut commensal bacteria play critical roles in shaping the host immune system. Perturbations of this community are linked to multiple autoimmune diseases such as inflammatory bowel disease (IBD) that increases the risk of colorectal cancer, and autoimmune uveitis, a major cause of blindness. Interestingly, both IBD and autoimmune uveitis are driven by T lymphocytes, especially recently identified subset of CD4+ T helper cells that secrete interleukin-17 (Th17). On the other hand, anti-inflammatory T helper cells, known as regulatory T (Treg) cells, act to counter-balance pro-inflammatory responses and control autoimmunity. Recent studies have provided compelling evidence that gut bacteria and their metabolites directly modulate both Th17 and Treg cell differentiation and function.

Our small molecule screens identified several bile acid derivatives in regulating Th17 and Treg cell differentiation. Bile acids are produced by enzymes encoded in the mammalian genome, but are further transformed by bacterial enzymes. In fact, one of bile acid derivatives that we identified as a potent Th17 suppressor can be produced in vitro with the help of human commensal bacteria from precursor bile acids. Furthermore, our preliminary data suggest that this particular bile acid metabolite is present

in mouse fecal samples. Moreover, we also identified another bile acid metabolite as a potent enhancer of Treg differentiation, with minimal effects on Th17 cells.

In sum, we hypothesize that bacterial modification of bile acids represents a previously unrecognized mechanism of immune regulation in the gut, contributing to Th17/Treg balance as well as immune homeostasis. Such a regulatory mechanism may provide therapeutic benefits in autoimmune inflammation, associated cancer and blindness. In this proposal, I aim (1) to elucidate molecular mechanisms by which the identified bile acids enhance Treg differentiation and (2) to identify commensal bacteria and their specific enzymes responsible for necessary modification of bile acids to produce biologically active ones.

Jin-Mi Heo, Ph.D.

Harvard University

Mentor: Jeffrey Wade Harper, Ph.D.

“Determine Pathophysiological Mechanisms How Mutations on OPTN and TBK1 Cause Glaucoma”

Glaucoma is a neurodegenerative eye disease caused by damages to the optic nerve that conveys the retinal signal to brain. While it is the second leading cause of blindness affecting ~2% of people over ages of 40, there is no cure, mostly due to the lack of our knowledge on the disease progression. Interestingly, recent genome-wide association studies revealed that mutations in Optineurin (OPTN), an autophagy receptor, and TBK1 kinase, a known interacting protein of OPTN, are tightly associated with glaucoma progression. In my recent work as well as that of others has uncovered a pathway by which OPTN and TBK1 promotes selective autophagy of protein aggregates, pathogens, and damaged mitochondria. Consistent with their critical roles in autophagy, series of studies showed abnormal autophagy in cells expressing glaucoma-associated mutations in OPTN and TBK1. The goal of this proposed study is, therefore, to systemically characterize the molecular defects associated with mutations in these genes found in glaucoma, which, I believe, will provide inroads into therapeutics.

In this proposal, utilizing various disciplines including a state-of-the-art quantitative proteomics approach, I will systemically examine the molecular defects caused by OPTN mutations and elevated TBK1 levels found in glaucoma. First, I will elucidate how glaucoma-associated OPTN mutants alter flux through selective autophagy systems. Secondly, I will define the pathophysiology of TBK1-associated glaucoma and identify the regulatory mechanisms for autophagy-dependent TBK1 activation. I am confident that I can successfully accomplish my goal with my expertise obtained from my training. I believe that this work will provide a mechanistic understanding of how mutations found in OPTN and TBK1 in glaucoma affect their functionality in clearance of organelles and proteins and will set the stage for curing the glaucoma patients.

Soyon Hong, Ph.D.

Boston Children's Hospital

Mentor: Beth Stevens, Ph.D.

“Early Role of Complement and Microglia in Mediating Synapse Loss in Alzheimer's Disease”

Region-specific synapse loss is an early critical hallmark of Alzheimer's disease (AD); however, mechanisms underlying synaptic vulnerability remain elusive. Recent genome-wide association studies suggest microglia to play a major role in AD pathogenesis, but their biological significance is unknown.

Whereas phagocytic microglia have been implicated in plaque clearance and neuroinflammation in late stages of AD, it is not yet known whether, or how, microglia contribute to synapse loss and dysfunction.

Emerging research in healthy brain is providing insight into fundamental roles of microglia and immune molecules in brain wiring. Recent works suggest microglia eliminate developing synapses partly through complement-dependent mechanism, raising the intriguing hypothesis that dysfunction of such microglia-synapse interactions may lead to synapse loss and dysfunction during disease.

My studies in AD mouse models suggest that C1q, the initiating protein of the classical complement cascade, is upregulated and deposited onto vulnerable synapses early, long before plaques deposit. Moreover, genetic or antibody-mediated inactivation of C1 protects synapses against synaptotoxic A β oligomers. Interestingly, microglia in A β -challenged adult mouse hippocampus were found to engulf synaptic material, suggesting that microglia may act as cellular mediators of synapse loss. I thus propose a model in which C1q and A β oligomers act in a common pathway to drive microglial engulfment of synapses early in AD. I propose to address the following: 1) Do microglia play an early and active role in driving synapse loss in AD? 2) Do A β and C1q interact to eliminate specific synapses? 3) Does inactivation of complement ameliorate synaptic deficits in AD models? This mechanistic proposal highlights a potential role of microglia in very early, pre-plaque stages of AD when synapses are already vulnerable. Furthermore, I propose to test novel mechanisms by which A β and C1q mediate early synapse loss that will elucidate new pathways underlying synapse loss in AD.

Benjamin Kleinstiver, Ph.D.

MGH Cancer Center

Mentor: J. Keith Joung, MD, Ph.D.

“Improving the Efficiency of Precise CRISPR/Cas9 Mediated Genome-Editing”

The nearly exponential reduction in both cost and speed of genome sequencing has led to an enormous wealth of genetic data that can be used to study genetic variation. A major challenge associated with repositories of human genome sequencing data is correlating single nucleotide polymorphisms (SNPs) with functional outcomes such as being causative for disease. Scientists can use genome editing technologies to recapitulate SNPs in cell-lines or organisms to determine whether genetic variation is involved in disease progression and subsequently develop targeted therapies.

One recently described genome editing technology that can be used for such applications is derived from the bacterial clustered regularly-interspaced short palindromic repeats (CRISPR) immune system. CRISPR-associated protein 9 (Cas9) is an endonuclease that can easily be programmed to generate double-strand breaks (DSBs) in DNA at specified sites in human cells. While this platform has been transformative in its ability to knockout genes by disrupting coding frames via non-homologous end-joining (NHEJ) DNA repair, precise changes necessary to model SNPs proceed through the homology-directed repair (HDR) pathway and are generally much less efficient.

Therefore, the purpose of this proposal is to investigate methods that increase the efficiency of the precise HDR-mediated repair. To do so, I will: 1) determine whether the inherent properties of the Cas9 nuclease can be engineered or exploited to improve HDR, 2) optimize a protein tethering strategy to enable recruitment of pro-HDR factors to the sites Cas9-induced DSBs, and 3) utilize genome-wide libraries of molecules to identify previously unknown endogenous factors that may bias DNA repair to HDR outcomes instead of NHEJ. The implications of this proposal are of broad interest to both the academic and therapeutic communities, as methods to increase the efficiency of precise DNA repair will enable scientists to more rapidly generate disease models and to develop targeted disease therapies.

Marieke Kuijjer, Ph.D., M.Sc.

Dana-Farber Cancer Institute

Mentor: John Quackenbush, Ph.D.

“Modeling the Effects of Gene Regulation on Cancer Survival”

Cancer is a complex, heterogeneous disease that results from the intricate interplay of diverse cellular and molecular factors, including genetic, epigenetic, and proteomic factors. Data from large-scale projects, such as The Cancer Genome Atlas (TCGA), have shown that each individual tumor is characterized by a unique set of genetic mutations and a distinctive pattern of gene expression. A natural extension of this is the hypothesis that each individual tumor should be defined by a distinctive gene regulatory network that mediates the link between genotype and phenotype. We recently developed two computational methods, one that integrates protein-protein interactions, DNA binding, and gene expression to model phenotype-specific gene regulatory networks (PANDA), and a method that allows us to extract robust gene regulatory networks for individual patients (LIONESS). In this application, we propose to reconstruct single-sample cancer gene regulatory networks by applying these methods on a large publicly available cancer dataset from TCGA, which includes data for >2,000 individual cancer patients and 30 different cancer types. For each individual cancer type, we will analyze these single-sample networks to identify features predictive of patient survival and to identify biological pathways that are aberrantly regulated in poor survivors. We will also perform a pan-cancer analysis to identify pathways associated with poor survival across different cancer types. We have already started analyzing single-sample glioblastoma networks, and found that genes in the PD1 signaling pathway are repressed in patients that have relatively good survival profiles. We are looking forward to expanding our methodology, and to analyzing all TCGA cancers. We believe our approach will identify potential targets for treatment of each cancer type, will help find pathways that influence cancer survival, and will demonstrate the potential of precision network medicine.

Xing Liu, Ph.D.

Boston Children's Hospital

Mentor: Judy Lieberman, M.D., Ph.D.

“Mechanism of Global mRNA Decay During Apoptosis”

Apoptosis is an orchestrated program of cell death. Defective or inefficient apoptosis is the acquired hallmark of cancer cells. Understanding underlying mechanisms of apoptosis should improve our ability to design effective immune and cytotoxic therapies for cancer. Our laboratory recently uncovered global mRNA decay as an overlooked key event of apoptosis. mRNA decay arrests new protein synthesis and promotes cell apoptosis. During apoptotic decay, mRNA decay intermediates are 3'-uridylylated near the stop codon and further digested by the 3'-5' exoribonuclease DIS3L2, which specifically recognizes 3'-uracils. However, the trigger and complete molecular mechanism responsible for mRNA decay during apoptosis are unknown. This proposal aims to identify the trigger of mRNA decay and further elucidate the downstream pathways. Using a cell-free system, I found that mitochondrial material released during mitochondrial outer membrane permeabilization (MOMP) specifically triggers the decay of mRNAs, but not noncoding RNAs. Thus, I hypothesize that a component(s) of the mitochondrial intermembrane space released during apoptosis triggers mRNA decay. My preliminary data identify the exoribonuclease PNPT1, located in the mitochondrial intermembrane space, as the trigger of mRNA decay in apoptosis. PNPT1 is released at the onset of apoptosis and its knockdown inhibits apoptotic mRNA decay. I plan to use biochemical and genetic methods, including deep sequencing of RNA decay intermediates, to

confirm the mitochondrial trigger and to define the pathway of apoptotic mRNA decay. Knowledge of the trigger and enzymes responsible for apoptotic mRNA decay could be exploited to enhance cancer cell killing and potentially sensitize resistant cancer cells for eradication.

Gillian Matthews, Ph.D.

McGovern Institute for Brain Research at MIT

Mentor: Kay Tye, Ph.D.

“Dissecting the Neural Circuitry of Loneliness”

The formation and maintenance of social bonds is vital for a social species to develop and thrive. Situations of social isolation, exclusion, or disconnection can lead to loneliness, which is a highly aversive emotional state in humans and severely detrimental to physical and mental health. However, we have a poor understanding of the neural circuitry which underlies our fundamental need for social connection. I have gathered exciting preliminary data implicating an understudied population of dopamine neurons in the dorsal raphe nucleus (DRN) in representing the subjective experience of social isolation. These neurons are sensitive to acute social isolation, and manipulations of their activity in vivo can induce or suppress a 'loneliness-like' state, in a manner predicted by social rank. With the experiments proposed here, I seek to unravel this dopaminergic circuit, and identify the essential components of this system which underlie our innate need for social contact.

Specific Aims:

Aim 1. Map the input-output connectivity of DRN dopamine neurons and determine which components are modulated by acute social isolation.

Aim 2. Establish the functional role of divergent DRN dopamine projections.

Aim 3. Determine the causal nature of the correlation between DRN dopamine function and social hierarchy.

Dopamine is heavily implicated in the control of social behavior and the pathogenesis of numerous neuropsychiatric disorders and, consequently, is a popular target for symptomatic treatment. However, current treatments fall short in their efficacy and specificity, in part, owing to indiscriminate targeting of a system which exhibits considerable functional heterogeneity. The broader objectives of this project are to transform our understanding of how social behavior is regulated by a heterogeneous dopamine system. This will not only shed light on the neural representation of loneliness, but also has strong potential to reveal new targets for the treatment of social impairments.

Upasna Sharma, Ph.D.

University of Massachusetts Medical School

Mentor: Oliver J. Rando, M.D., Ph.D.

“Epigenetic Inheritance of Paternal Environmental Conditions”

Several studies show that parental nutrient availability can affect metabolic phenotypes in the offspring. However, the mechanisms by which parental conditions influence offspring remain poorly understood. Previously we found that mice fed on low protein diet sire offspring with altered lipid and cholesterol metabolism. My recent work showed that small RNA in sperm, specifically tRNA-derived fragments (tRFs), play roles in the inheritance of paternal dietary information. Protein restriction in mice affects small RNA levels in mature sperm, with decreased let-7 levels and increased amounts of 5' fragments of glycine transfer RNAs. These studies also revealed surprising dynamic aspects of small RNA biogenesis

during sperm maturation; tRFs are scarce in testicular sperm but increase in abundance as sperm mature in the epididymis. To further examine the mechanism of this RNA-mediated epigenetic inheritance, this project aims to address two broad questions 1) how are sperm small RNAs generated in response to low protein diet 2) what is the effect of sperm RNA on early embryonic development? To address the first question, here I propose to first definitively identifying the tissue of origin of tRFs in sperm (Aim1). I will use three distinct methods for generating "tracer" RNAs specifically in a tissue of interest to uncover the tissue of origin for tRFs. Secondly, my data shows that the small RNA payload of sperm changes during epididymal maturation. This has important implications for assisted reproduction in humans, which in some cases utilizes relatively immature sperm from testicular biopsies, for example, to fertilize oocytes. It is therefore of biomedical interest to investigate the function of sperm small RNAs in embryonic development. Here I propose to generate embryos using a variety of combinations of sperm and exogenous RNAs and characterize regulatory consequences in early development, and later phenotypic consequences in offspring carried to term (Aim 2).

Elenoe Smith, Ph.D.

Boston Children's Hospital

Mentor: Stuart Orkin, M.D.

“Modeling and Disrupting Non-Coding Regulatory DNA Sequences to Inform Sickle Cell Disease Therapy”

Elevated fetal hemoglobin (HbF, $\alpha_2\beta_2$) levels in red blood cells are predictive of milder disease in sickle cell anemia patients. Strategies to increase β -globin expression have been ineffective, primarily because mechanisms of β -globin gene silencing are poorly understood. BCL11A is a major negative regulator of β -globin expression. We defined an erythroid specific enhancer of BCL11A whose activity can be disrupted without affecting BCL11A's essential functions in brain, immune, and blood stem cells. This proposal seeks to define the minimal DNA element(s) required for human BCL11A erythroid enhancer activity. Interpretation of the functional consequences of modification or deletion of human BCL11A enhancer sequence requires an in vivo model. I will "humanize" the murine BCL11A locus by swapping low HbF or high HbF associated human enhancer variants into the orthologous region in mouse embryonic stem cells (mESCs). Enhancer activity will be assessed by BCL11A and globin gene expression in mice derived from the "humanized" mESCs. This new model will further develop BCL11A as a therapeutic target. Deletional mapping of genomic DNA from patients with elevated HbF suggests β -globin expression can also be affected by cis-regulatory sequences within the β -globin gene cluster. To identify novel repressors of HbF, I will interrogate the entire β -globin gene cluster using CRISPR/Cas9 genome editing technology in a human erythroid progenitor cell line. Application of this high throughput mutagenesis will allow rapid examination of non-coding DNA elements necessary for proper regulation of the human globin gene locus.

Collectively, these studies will contribute to a fuller understanding of β -globin gene regulation and provide in vivo models for molecular characterization of hemoglobin switching. Both approaches aim to identify critical DNA sequences that are required for maximal β -globin gene silencing. Defining the minimal sequences to target for HbF reactivation are crucial for the advancement of gene therapy as a treatment option.

Pei-Chi Wei, Ph.D.

Boston Children's Hospital

Mentor: Frederick W. Alt, Ph.D.

“Roles of Recurrent DNA Break Cluster Genes in Neural Development and Disease”

We have employed unbiased, high-throughput, genome-wide, translocation sequencing (HTGTS) to discover 27 recurrent DNA double strand breaks (DSBs) clusters ("RDCs") in mouse neural stem and progenitor cell (NSPC) genomes. Most RDCs occur in long genes ("RDC genes") that have large numbers of exons separated by long introns. Moreover, most RDC genes are involved in synapse function and/or neural cell adhesion, with majority also implicated in mental disorders. As RDC-genes lie in single topological-associated domains, the frequency of joining between two separate DSBs within an individual RDC-gene should be high. Therefore, we hypothesize that joining of frequent DSBs within long introns of RDC-genes may lead to deletion of exons or other rearrangements that promote expression of altered products. Such "hard-wired" RDC-gene changes might be transmitted to daughter cells, including mature neurons, and contribute both to genomic mosaicism of the brain and also to neural diseases. To test this hypothesis, our first aim proposes to adapt HTGTS to map junctions between separate endogenous DSBs within given RDC-genes. Furthermore, we will employ RNA-Seq to examine NSPCs and mature neurons for the expression of implicated new transcripts. Many RDC genes also are implicated in human cancers, with several located near genes implicated in medulloblastoma (MB), a deadly pediatric cancer. We have generated a robust mouse model for MB that exhibits characteristic translocations and amplifications. In our second aim, we propose to perform whole genome sequencing to identify the recurrent breakpoints of these MB genomic alterations. We will use this information to tailor HTGTS studies of NSPCs and cerebellar granule cell progenitors (likely MB precursor cells), to both identify DSBs that lead to recurrent MB genomic alterations and also to elucidate mechanisms that cause them. Our proposed aims should shed light on the roles of DSBs in brain diversification and brain diseases including cancer.

Moran Yassour, Ph.D.

Broad Institute of MIT and Harvard

Mentor: Ramnik Xavier, M.D, Ph.D

“Decoding the Origin and Development of the Newborn Gut Microbiome”

The human gastrointestinal tract harbors one of the highest densities of microorganisms on Earth. There is clear evidence that early events in microbial colonization bear a profound effect on immune education in the gut, thereby impacting disease susceptibility. Starting from birth, microbes from the mother and the surrounding environment colonize the infant intestine and microbial composition differs markedly between different delivery modes. For example, children born by C-section have a decreased microbial diversity and increased susceptibility to autoimmune diseases such as asthma and allergies. While healthy neonatal colonization of intestinal microbiota is critically important to host physiology, neither the microbial drivers nor the environmental sources of newborn microbiota have been fully elucidated. I propose to decipher the source of the newborn microbiome and how it develops with respect to a child's delivery mode. To this end, I will establish an unprecedented prospective birth cohort of 120 families analyzing infants born vaginally and by elective or non-elective C-section. I hypothesize that non-elective C-section may result in an intestinal microbiota that is distinct from vaginal or elective C-section, as the child may have been exposed to the microbiota of the birth canal. Infant stool (as a proxy for their gut) will be collected along with potential bacterial "source" samples, including the birth canal, maternal stool, parental skin, hospital bassinet, and breast milk. By performing metagenomic sequencing of these samples, I will characterize initial bacterial colonization in the context of various delivery modes. I will construct a temporal map of the newborn gut microbes (by analyzing several time points post-delivery) along with identifying their potential source. This study will provide a baseline for newborn gut microbiome development. A clear understanding of the microbial trajectory in relation to birth mode, will provide the foundation for future infant microbiome disease-focused studies.

2015 Grant Recipients

Luke Chao, Ph.D.

Harvard Medical School

Mentor: Stephen C. Harrison, Ph.D.

“Capturing Conformational Change in Flavivirus Membrane Fusion”

Flaviviruses (West Nile, Dengue) are pathogens with a global human health burden. The envelope protein (E), as the membrane fusogen mediating cell entry, represents a prime under-explored target for antiviral treatment. Viruses distill fundamental principles of membrane fusion providing general insight into cellular membrane processes. The E-protein undergoes a series of conformational rearrangements to mediate membrane fusion. Understanding the relationship between E-protein structural changes and membrane remodeling requires that we study the reaction mechanism and visualize the macromolecular complex in multiple intermediate states. To date, I have adapted a single-particle TIRF method to study flavivirus hemifusion with target membranes, and through mutagenesis and kinetic simulations developed a model for the sequential conformational rearrangements that the E protein undergoes on the virion surface (Chao et al., eLife 2014).

The research proposed here will determine new structures by electron cryo-microscopy (cryo-EM) to reveal the regulatory mechanisms that control flavivirus membrane fusion. I will determine a sub-nanometer cryo-EM reconstruction of a full-length post-fusion West Nile virus (WNV) E to visualize essential trans-membrane and membrane-proximal interactions important for late stages of membrane fusion. I will determine by cryo-EM a structure of an intermediate form of the virion immediately following low-pH triggered dimer dissociation and subunit extension. I will characterize the extension of the envelope protein due to pH and temperature and the importance of interactions between E and M seen in the fully compact, pre-fusion state. I will use fluorescent conjugates of small-molecule inhibitors to determine the relationship between stoichiometry of inhibitor binding and observed delay in hemifusion. Finally, I will develop new approaches to study the late-stage transitions to test models derived from the cryo-EM structures using new probes for membrane pore opening.

Yoandris Del Toro Duany, Ph.D.

Boston Children's Hospital

Mentor: Sun Hur, Ph.D.

“Interplays Between ADAR1 And MDA5 In The Pathogenesis Of Aicardi-Goutière Syndrome”

MDA5 is a conserved viral double-stranded RNA (dsRNA) sensor in vertebrates. While an appropriate function of MDA5 is important for effective immune defense against viral infection, my recent study showed that its dysregulated activity via gain-of-function (GOF) mutations can lead to a severe inflammatory disease, Aicardi-Goutières Syndrome (AGS) (Rice* and del Toro Duany* et al, Nat Genetics, 2014, *co-first authors). The goal of this proposal is to further elucidate the molecular mechanisms by which these mutations cause chronic inflammation and AGS. In particular, I will focus on defining the potential relationship between MDA5 and a dsRNA-modifying enzyme, ADAR1, of which loss-of-function (LOF) mutation has been also shown to cause AGS in the absence of the mutations in MDA5. I hypothesize that MDA5 and ADAR1 represent two balancing arms of the immune-tolerance relationship.

That is, cellular dsRNAs, such as inverted Alu elements, are normally prevented from stimulating MDA5 through modification of adenosine (A) to inosine (I) by ADAR1, whereas LOF mutations in ADAR1 or GOF mutations in MDA5 may allow stimulation of MDA5 by these endogenous dsRNAs.

To test this hypothesis and further expand our understanding of the AGS pathogenesis, I here propose a multi-disciplinary research project involving a combination of biochemistry, biophysics and cell biology. I will first determine the impact of A-to-I modifications on the MDA5:dsRNA interaction using biochemical and biophysical assays that we have developed in our laboratory (Aim 1). I will next identify the endogenous dsRNA stimulating MDA5 using cells derived from AGS patients (Aim 2). This project builds upon my previous work on the molecular mechanisms of MDA5 and the strong preliminary data. I believe that the proposed research would provide novel insights into the pathogenesis of AGS, and also help us define a new paradigm of the complex interplays between the pathogen sensing and cellular RNA modification.

James Dewar, Ph.D.

Harvard Medical School

Mentor: Johannes Walter, Ph.D

“The Mechanism Of Replication Termination In Vertebrates”

DNA replication terminates when replication forks converge, but the mechanism is unknown. Furthermore, it is unclear how replisome components are removed from DNA, particularly the CMG replicative helicase, which forms a closed ring around the DNA. I have developed a powerful new system to study replication termination in vertebrates. A plasmid containing a lac repressor (LacR) array is replicated in *Xenopus* egg extracts, causing two forks to stall on the outer edges of the array. When IPTG is added, LacR dissociates, forks resume synthesis, and termination occurs synchronously in a narrow zone.

Using this system I elucidated a model for vertebrate termination in which replisomes converge and freely pass each other, allowing leading strands to be rapidly ligated to downstream lagging strands. The CMG helicases, which normally translocate along single-stranded DNA, are unloaded after the DNA is fully ligated, implying that they pass over and encircle dsDNA. CMG unloading occurs during decatenation of the daughter molecules by Topoisomerase II, suggesting these processes may be linked.

In Aim 1, I will determine the trigger for CMG unloading. I will test whether CMG unloading is initiated once it encircles dsDNA, or whether fork convergence or decatenation is the signal for CMG unloading.

Recent work shows that CMG ubiquitylation is required for its unloading, and implicates the ATP-driven 'unfoldase' p97 in this process. In Aim2 I will determine when CMG is ubiquitylated, and investigate how p97 recognizes CMG. In parallel, I will identify new termination proteins by mass spectrometry and investigate their involvement in CMG unloading.

Together, these experiments will coalesce into a detailed molecular model of CMG unloading, which includes p97 and CMG ubiquitylation, as well as novel termination proteins.

Long term objectives

Future work will build upon this study and address other questions about termination, such as how topological stress is dealt with between converging replication forks. It will also be important to test whether the anticancer properties of Topoll and p97 inhibitors can be attributed to inhibition of termination.

Xianchi Dong, Ph.D.

Boston Children's Hospital

Mentor: Timothy A. Springer, Ph.D.

“Molecular Basis for Integrin AlphaVbeta6 Mediated TGF-beta Activation and Structure-based Drug Design”

Integrins are cell surface receptors that link to the cytoskeleton, bind extracellular ligands, and transduce intracellular and extracellular signals. In vivo, alphaVbeta6 and alphaVbeta8 integrins specifically activate latent TGF-beta (pro-TGF-beta). TGF-beta regulates tissue development, wound healing, immune responses, and cancer progression. Furthermore, alphaVbeta6 is an emerging target for fibrosis and cancer therapies.

How alphaVbeta6 and alphaVbeta8 specifically activate TGF-beta is unclear. Our recent crystal structure of pro-TGF-beta3 peptide-bound alphaVbeta6 headpiece provides insights into how alphaVbeta6 achieves ligand specificity. Together with mutational studies, affinity measurements, and evolutionary analysis, we identified three surface exposed loops (specificity-determining loops) in alphaVbeta6 critical for ligand-specificity. We also demonstrated that an amphipathic helix following the integrin-binding RGD contributes to high-affinity binding.

The project goals are to 1) determine molecularly how alphaVbeta6 binds and activates pro-TGF-beta, and 2) rationally design a de novo integrin alphaVbeta6-binding protein (alphaVbeta6-BP) based on the crystal structure we determined and probe its therapeutic usage.

In Aim 1, we will measure affinity, kinetics, and thermodynamics to further probe alphaVbeta6/proTGF-beta binding. To test our hypothesis that other regions or conformational changes in alphaVbeta6 and pro-TGF-beta1 are important for their interaction, we solved a preliminary 3.6 Å crystal structure of the proTGF-beta1/alphaVbeta6 head complex. We will mutate residues revealed from this structure and assay their importance for binding and activation of pro-TGF-beta by alphaVbeta6.

In Aim 2, in collaboration with David Baker's lab, we design, totally de novo, an alphaVbeta6-BP that binds similarly to pro-TGF-beta. We will optimize the alphaVbeta6-BP structure to obtain higher affinity and specificity for alphaVbeta6. Lastly, we will test the therapeutic usage of the binder as a TGF-beta-activation inhibitor, a cancer cell label, and for a nanocage-based drug deliver system.

Our pro-TGF-beta1/alphaVbeta6 head complex structure represents the first reported co-crystal structure for an integrin head bound to physiological, macromolecular ligand. Since the ligand-binding pocket of integrins are structurally similar, determining how pro-TGF-beta and alphaVbeta6 interact will not only further our understanding of TGF-beta activation, but also provides insight into integrin-ligand interactions in general. Our work will have fundamental implications for the development of therapeutics for diseases associated with TGF-beta and alphaVbeta6.

Thomas Guettler, Ph.D., M.Sc.

Harvard Medical School

Mentor: Tom Rapoport, Ph.D.

“Capturing Co-Translational Protein Translocation in Action”

The goal of this project is to understand the mechanism by which proteins are transported across membranes. Specifically, we aim to elucidate how polypeptides are moved through the "translocon", an evolutionarily conserved protein-conducting channel in the endoplasmic reticulum (ER) of eukaryotes and the plasma membrane in bacteria and archaea. Although the players involved in the process are known and the structure of the idle channel has been determined, the mechanism of translocation is still only poorly understood, largely because there is no structural information about the translocon in action. Visualizing the active translocon with a translocating polypeptide chain inside the channel has been the major (and elusive) goal in the field for more than a decade. We will employ a newly developed system that allows us to trap intermediates of co-translational protein translocation at defined stages in intact *Escherichia coli* cells. We established a novel purification strategy to prepare ribosome-nascent chain (RNC)-translocon complexes for structural studies. These will be used for structure determination by state-of-the-art single particle cryo-electron microscopy. First, we will determine a high-resolution structure of a complex of the non-translating ribosome bound to the translocon (Aim 1). This structure will serve as a reference for actively translocating complexes. Second, we will determine a high-resolution structure of the RNC-translocon complex with a short nascent secretory polypeptide chain representing an early stage of translocation (Aim 2). These studies will clarify how the signal sequence of a substrate is recognized by the translocon. Aim 3 focuses on how signal sequences are cleaved during translocation. Newly developed in vitro and in vivo assays will be used to elucidate when the signal sequence is cleaved, and cryo-EM experiments (Aim 4) will clarify how the channel changes after signal sequence removal. This project will provide structural snapshots of co-translational translocation of a polypeptide at different stages. It will reveal how the translocon accommodates a nascent polypeptide, how it is activated by a translating ribosome, and how signal sequences are recognized and processed.

Monika Kowalczyk, M.D., D.Phil.

Broad Institute of MIT and Harvard

Mentor: Aviv Regev, PhD

“Deconstructing Different Modes Of Heterogeneity In Human Leukemia”

Over the last several decades, there has been a revolution in our understanding of cancer growth, and leukemia in particular. It became increasingly clear that leukemia is not just one disease, as any single leukemia doesn't have just one genome, but instead, comprises of multiple genomes that belong to distinct subclones. These subclones may evolve in parallel over the lifetime of cancer and contribute to intratumoral genetic heterogeneity. However, even within single genetic subclone, not all cells are phenotypically and functionally equal, which adds another dimension to the intratumor heterogeneity. For example, cells vary in regard to their state of differentiation, capacity for self-renewal, and long-term clonal maintenance. It is, therefore, compelling to hypothesize that careful characterization of intratumor heterogeneity and understanding its mechanisms and sources may hold the key to improvement in cancer diagnosis, prognosis, monitoring of each patient, and ultimately for more effective therapies and control of cancer. Here, I will catalogue genetic and transcriptomic cell-to-cell variability within normal and malignant hematopoiesis to determine their functionality and shed light on

a number of fundamental issues: the origin of leukemia (cell of origin), identification of pre-leukemic clone, distinguishing driver from passenger mutations, coexistence of normal and malignant hematopoiesis within a patient, as well as, understanding and preventing therapy resistance. By delineating individual cell states (RNA-Seq) with superimposed mutation makeup, I will be able to tease apart the unique aspects of leukemia biology that until now has been obscured by population-based studies. With these distinctive insights, I will recreate genetic diversity of primary leukemia in vitro by generating a set of cell lines recapitulating genetic diversity found in individual leukemic cells and test them individually for leukemia initiation ability.

Ultimately, studying individual cancer cells may bring new implications for the cancer stem cell model, and may help to determine the extent to which it accounts for therapy resistance and disease progression. In the clinic, these approaches will have important applications in early detection and non-invasive monitoring.

Hsiang-Ying Lee, Ph.D.

Whitehead Institute for Biomedical Research

Mentor: Harvey Lodish, Ph.D.

"Dissecting Molecular Mechanisms Underlying Erythroid Progenitor BFU-E Self-renewal"

Many acute and chronic anemias, including hemolysis, sepsis, and genetic bone marrow failure diseases such as Diamond-Blackfan Anemia (DBA), are not treatable with erythropoietin (Epo), because the colony-forming unit erythroid progenitors (CFU-Es) that respond to Epo are either too few in number or are not sensitive enough to Epo to maintain sufficient red blood cell production. One attractive approach is to devise strategies to promote self-renewal of the upstream burst-forming unit erythroid progenitors (BFU-Es). My proposed study focuses on understanding the molecular mechanism that regulates a key decision made by BFU-E progenitors -- self renewal or differentiating into the later CFU-E progenitors -- which has major implications for both basic biology and the development of drugs to treat rare forms of anemia.

We previously showed that glucocorticoids (GCs) specifically target and stimulate self-renewal of BFU-E progenitors. My recent study demonstrated that PPAR α ; synergizes with glucocorticoid receptor (GR) to promote BFU-E self-renewal, and over time greatly increase production of mature red blood cells in mouse and human erythroid cultures. Indeed, PPAR α agonists alleviate anemic symptoms in acute and chronic anemia mouse models.

My ChIP-Seq data shows that both GR and PPAR α predominantly occupied distal intergenic and intronic chromatin sites. Importantly, 67.9% of GR peaks and 44.3% of PPAR α peaks co-localize. I analyzed the DNA sequences underlying these presumed enhancer regions, and uncovered enriched DNA binding motifs for 10 transcription factors, most of which are important for self-renewal of stem cells. I hypothesize that GR and PPAR α co-localized chromatin sites are critical regulatory elements to establish the BFU-E specific gene expression program, and are likely to be "super-enhancers", which are clusters of transcriptional regulatory elements that are highly occupied by critical transcription factors and coregulators and are important for cell identity. In my proposed study, I will use rigorous experimental and bioinformatic approaches to determine the key regulators and the enhancer functions of GR and PPAR α mediated BFU-E self-renewal. By understanding how these regulatory elements modulate BFU-E function, small molecules or gene editing approaches could be developed as new therapeutic strategies for Epo-resistant anemias such as DBA.

Oren Levy, Ph.D.

Brigham and Women's Hospital

Mentor: Jeffrey M. Karp, PhD

"A Metastasis-Targeted Cell-Based Delivery Platform For Prostate Cancer Therapy"

Prostate cancer (PCa) is the second leading cause of cancer-related deaths in American men. PCa lethality is mostly derived by the continuous development of disseminated metastasis, commonly found in the bone marrow. Despite significant progress in PCa research, there still exists a major need to efficiently target anti-cancer drugs to sites of PCa mets, while sparing host toxicity. Systemic administration of therapeutics may encounter multiple challenges, including severe adverse effects, uncontrolled drug levels, early degradation and rapid clearance from the body. While drug encapsulation in nano/micro delivery systems may reduce host toxicity and protect the drug from early degradation, effective targeting of such delivery systems to specific sites remains challenging. In this proposal, we aim to create a metastasis-guided cell-based drug delivery platform by transfecting mesenchymal stem cells (MSCs) with mRNA encoding metastasis-targeting ligands to specifically direct systemically infused MSCs to PCa mets. To be used for drug delivery, mRNA-engineered MSCs will then be loaded with microparticles encapsulating toxic payloads. We predict that this mRNA-guided MSC-based delivery vehicle will display active targeting upon systemic administration and facilitate the delivery of therapeutic agents to sites of PCa mets, resulting in significant anti-tumor effect, while minimizing host toxicity and adverse effects. To develop this targeted platform, we propose 3 specific aims. The first aim is to evaluate homing properties of mRNA-engineered MSCs in-vitro in a bone mets-simulated environment. The second aim is to assess homing of systemically infused mRNA-guided MSCs to PCa tumors in a mouse model simulating human PCa bone metastatic lesions. Finally, the third aim is to evaluate the therapeutic efficacy of systemically infused mRNA-targeted MSCs loaded with anti-cancer drug microparticles using the "humanized" PCa bone mets mouse model. Overall, pursuing this proposal may eventually lead to development of a systemic cell-based metastatic PCa therapy that may help millions of PCa patients.

Falong Lu, Ph.D.

Boston Children's Hospital

Mentor: Yi Zhang, Ph.D.

"Dissecting Mechanisms Regulating Totipotency in Mouse"

In mammals, pluripotent stem cells can give rise to cells of embryonic lineage. Guided by the mechanism underlying pluripotency, pluripotent stem cells have been successfully induced through manipulating the transcriptional and epigenetic networks of various differentiated cell types. For therapeutic use of stem cells in regenerative medicine, it is important to obtain stem cells in a lineage-unrestricted state. The most unrestricted state is the totipotent state in which cells can contribute to both embryonic and extra-embryonic lineages. However, the factors that confer totipotency are poorly understood due to the limited availability of totipotent cells (eg. 1-cell and 2-cell mouse embryos). Recently, a rare and transient subpopulation of ESCs called 2-cell-embryo (2C)-like cells that express a group of 2-cell embryo-specific genes are identified. Amazingly, the 2C-like cells can contribute to both embryonic and extraembryonic tissues when injected into pre-implantation embryos, indicating that they are totipotent. When analyzing the role of Ten-eleven translocation (Tet) family 5-methyl-cytosine dioxygenases in ESCs, I found that triple KO of Tet genes results in a 6-fold increase in 2C-like cell population. Besides Tet proteins, only three factors (Kap1, Lsd1 and G9a) are known to regulate the totipotent 2C-like state.

Since the CRISPR-Cas9 (clustered regularly interspaced short palindromic repeat) system enables genome-wide functional screen in mammalian cells, I propose to systematically identify regulators of totipotent 2C-like state by performing genome-wide knock-out screen and to understand how the identified factors involve in the establishment and exit of the totipotent state in pre-implantation embryos.

Genome-scale CRISPR-Cas9 Knock-out screening will be carried out in cell line with tdTomato reporter driven by a 2C-specific promoter to identify genes responsible for increased or decreased tdTomato-positive cell population. Using this unbiased systematic approach, we anticipate to identify both positive and negative regulators of the 2C-like state. Factors identified in the screen will be further evaluated through depletion or forced expression in 1-cell zygotes and functionally evaluated by analyzing their effect on the 2-cell embryo-specific gene expression and embryo developmental potential. Completion of the proposed study will reveal insights into the establishment, maintenance and exit of totipotency, which will ultimately facilitate regenerative medicine.

Yael Mandelblat-Cerf, Ph.D.

Beth Israel Deaconess Medical Center

Mentor: Bradford Lowell, Ph.D., M.D.

"In Vivo Study Of Homeostatic And Non-homeostatic Control Of Feeding By Hypothalamic Neurons"

Consuming sufficient amount of food to maintain energy balance is fundamental for survival. However, environmental factors contribute to the motivation to eat and can override homeostatic signals to stimulate eating in sated states, or inhibit eating in states of hunger. These factors are believed to underlie the increasing vulnerability to obesity and anorexia nervosa in the developed world. Deeper understanding of the basic neural microcircuits controlling food homeostasis, motivation, and their interaction will provide critical clues for understanding and treating eating disorders.

Agouti-related peptide (AgRP)-expressing neurons in the arcuate nucleus of the hypothalamus are influenced by peripheral homeostatic signals, to drive or block feeding. These neurons were shown to be necessary and sufficient to drive feeding and weight gain: Their activation elicits voracious feeding even in sated states and their inhibition blocks hunger even after fasting. Recent studies indicated that AgRP neurons receive potent excitatory and inhibitory neuronal inputs, which can also regulate feeding. This raises the possibility that AgRP-neurons may be influenced also by non-homeostatic signals that arrive from the central nervous system, such as those concerning the availability of food and the motivation to pursue it, to control feeding behavior. To elucidate this question, direct monitoring of AgRP-neurons in awake behaving animals (in vivo) is necessary.

In my study I investigate AgRP neuronal activity across homeostatic states (fasted to well-fed) and in response to the circadian rhythm, homeostatic hormonal signals (ghrelin), and non-homeostatic sensory signals known to induce feeding. Subsequently, I will examine which upstream inputs to AgRP neurons contribute to the response of AgRP neurons to these signals.

My preliminary data indicates that AgRP neurons demonstrate a slow increase in activity throughout the light-cycle in accordance with the gradual decrease in energy balance. Surprisingly, they also show a fast decrease in activity at the onset of food availability, which cannot be explained by slower changes in hormonal signals of energy balance.

Together, my experiments will not only provide a new avenue for insights into hypothalamic control of feeding, but they should help overcome previous barriers in developing molecular therapies for obesity and other eating disorders.

Shankar Mukherji, Ph.D.

Harvard University

Mentor: Erin O'Shea, Ph.D.

"The role of mRNA localization in establishing and maintaining organelle identity"

One of the hallmarks of the eukaryotic cell is its organization into spatial compartments known as organelles. Organelles provide the cell with optimal subcellular environments in which to carry out fundamental processes critical to life, from gene transcription in the nucleus to ATP generation in the mitochondria. To maintain organelle identity, cells express organelle-specific receptors that selectively bind proteins from the cytoplasm and pass them to protein conducting channels for transport to the organelle interior, but how receptors find the correct organelle has remained unclear. I have established at single molecule resolution that the mRNAs encoding organelle receptor proteins localize to the sites of biogenesis for a variety of organelles, including the mitochondria, peroxisomes and endoplasmic reticulum. Based on this finding, I hypothesized that mRNA localization and spatially restricted translation of organelle receptor genes directs receptor proteins to the correct target organelle. In this proposal, I will test this hypothesis 3 ways. In Aim 1, I will measure the spatiotemporal dynamics of organelle receptor gene expression by placing the genes under inducible control and visualizing the mRNA and protein levels in space and time following addition of inducer. In Aim 2, I will identify the complexes responsible for mRNA traffic to target organelles by fusing the receptor mRNAs to a biotinylatable RNA sequence, affinity purifying the receptor mRNAs on streptavidin beads, and performing mass spectrometry on the purified material to identify proteins that co-purify with the mRNAs. In Aim 3, I will measure the phenotypic consequences of mislocalizing receptor mRNAs with a synthetic RNA binding protein I have developed that can bind RNAs and localize them to alternative organelles in a chemically-inducible fashion. Following receptor mRNA mislocalization I will measure the resulting protein distribution and effects on organelle physiology. Successful completion of these aims would establish mRNA localization and spatial control of translation as a major determinant of organelle identity in the eukaryotic cell, giving us unique insight into possible molecular origins of a wide range of developmental and metabolic disorders called organelle biogenesis disorders, highlighted by mitochondrial biogenesis defects, peroxisome biogenesis disorders and lysosomal storage disease.

Adam Norris, Ph.D.

Harvard University

Mentor: John Calarco, Ph.D.

"Combinatorial control of alternative splicing in determining neuronal specification and animal behavior"

Alternative splicing is an important means of regulating gene expression and of increasing the diversity of the transcriptome and proteome. This diversity has been demonstrated to be important for a number of complex organs including the nervous system, which exhibits high levels of alternative splicing. Indeed, dysregulation of neuronal alternative splicing or neuronal splicing factors has been implicated in a number of neurological diseases. Until my recent studies, however, little was known about alternative splicing within the nervous system between different classes of neurons, or about the factors responsible for regulating such alternative splicing. I demonstrated that alternative splicing between functional classes of neurons is widespread in the *C. elegans* nervous system, and showed that complex

neuron-specific alternative splicing patterns can be generated by partially-overlapping RNA-binding proteins working combinatorially.

These findings, along with a wide range of tools available in *C. elegans*, have set the stage for answering many outstanding questions in the field of post-transcriptional regulation, such as: How widespread is RNA-binding protein control of important neuronal and behavioral phenotypes? How extensive is combinatorial control of alternative splicing in the nervous system? What are the relationships among neuronal fate, splicing factors and the splicing events they control? How do RNA-binding proteins refine the identity of neuronal subtypes generated by the activity of transcription factors? The experiments outlined in this proposal aim to answer these questions and to expand our understanding of the role of post-transcriptional regulation in shaping neuronal fate, function and behavior.

AIM 1: How does neuronal fate shape alternative splicing networks?

1a: What are the factors linking cell fate to alternative splicing in the mechanosensory neurons?

1b: What is the mechanosensory-neuron alternative splicing network?

1c: How tightly linked is the relationship between neuronal fate and alternative splicing?

AIM 2: CRISPR/Cas9-mediated double-mutant analysis of neuronal RNA binding proteins

2a: Generating a library of single and double RNA-binding protein mutants

2b: Assaying defects of behavior and neuronal function in RNA-binding protein double mutants

2c: Focused analysis of RNA-binding protein synthetic interactions

Karl Schmitz, Ph.D.

Massachusetts Institute of Technology

Mentor: Robert Sauer, Ph.D.

“Function And Dysregulation Of Mycobacterial Clp Proteases”

Infection by *Mycobacterium tuberculosis* (Mtb) is a major cause of global mortality. Efforts to prevent and treat tuberculosis are challenged by the emergence of Mtb strains resistant to some or all conventional antibiotics. Consequently, there is an urgent clinical need for novel therapeutics that kill resistant Mtb, and a parallel need to identify and characterize new antibiotic targets.

The Clp-family of self-compartmentalized proteases is one promising class of targets. These enzymes consist of an unfoldase (ClpC1 or ClpX) and a peptidase (ClpP1P2) that together carry out regulated degradation of protein substrates. Clp protease activity is essential for Mtb viability, and inhibition or dysregulation of these enzymes can cause cell death. In recent work, I have shown that these enzymes are subject to complex regulation, and that Clp protease activity is tightly coupled to substrate availability. However, key information about how these proteases assemble and how they recognize substrates remains unknown. A comprehensive biochemical understanding of Clp protease function and the role of these enzymes in the cell will help to identify and characterize compounds that perturb their activity.

This project will address a number of major unanswered questions surrounding Mtb Clp protease structure and function. Aim 1 explores how the two unfoldases, ClpC1 and ClpX, interact with ClpP1P2. Using a set of functional and biophysical assays, I will determine if ClpC1 and ClpX compete for binding or interact simultaneously with ClpP1P2. Aim 2 will employ an assay for Clp protease activity in vitro to carry out high throughput screening for novel compounds that inhibit or dysregulate function. Aim 3 will

investigate how ClpC1 and ClpX recognize substrates for degradation, and will use a novel counter-selection system to build a profile of N- and C-terminal sequences that direct proteins for degradation by Mtb Clp proteases. By clarifying how these proteases function, this work will lead to a better understanding of their essential role in the cell and may lead to new therapeutics to combat tuberculosis.

2014 Grant Recipients

Ozan Aygun, Ph.D.

Massachusetts Institute of Technology

Mentor: Alice Ting, Ph.D.

“Unveiling the Functional Complexity of Heterochromatin by Spatially-Resolved Live-Cell Proteomic Mapping”

Eukaryotic chromosomes are organized into distinct subdomains that are critical for genome function. Heterochromatin is a substantial nuclear structure, which constitutes highly compacted DNA predominantly from centromeres and perinuclear domains. Constitutive heterochromatin is essential for the three-dimensional organization and integrity of the genome, as well as epigenetic silencing of large chromosomal domains containing repetitive DNA sequences. Despite the spatially distinct organization of heterochromatin in the nucleus, its exact protein composition remains as a fundamental unresolved research problem.

Here we propose a novel technology, which combines concepts in microscopy, mass-spectrometry (MS), and enzyme engineering to provide spatially- and temporally-resolved, unbiased proteomic maps of distinct chromosomal domains in living cells. By using this innovative strategy, we will delineate the proteomic complexity of heterochromatin in intact, living human cells, at nanometer scale resolution. Importantly, we will take the advantage of the temporal resolution of our approach to identify the factors that are specifically recruited to heterochromatin during DNA replication. These analyses will lead to identification, functional and mechanistic characterization of the key missing factors that regulate heterochromatin stability and dynamics. Given the impact of heterochromatin on genome stability and its remarkable redistribution during aging, we expect to significantly advance our fundamental understanding of the molecular mechanisms that influence human longevity.

The proposed interdisciplinary project represents a unique and powerful combination of chemical synthesis, enzyme engineering and chromosome biology, and it will introduce technological innovations that will benefit the entire chromatin research field. The outstanding broad long-term goal of this study is to improve the resolution of this novel technology to characterize the proteome of any single-copy genomic locus in human cells. The development and applications of this unique strategy will help us to tackle complex biological questions pertaining chromosome biology, which have been impossible to address by traditional approaches.

Luke Berchowitz, Ph.D.

David H. Koch Institute for Integrative Cancer Research at MIT

Mentor: Angelika Amon, Ph.D.

“Translational Control in Germ Cell Development: Mechanisms and Signaling”

Production of haploid gametes from diploid progenitor cells is mediated by a specialized cell division called meiosis in which two divisions, meiosis I and II, follow a single S phase. Errors in meiosis lead to aneuploid and polyploid gametes. In comparison to somatic cell production, gamete production relies heavily on translational control i.e. the activation and repression of previously transcribed RNA to

govern gene expression. Using budding yeast, I have identified a translational control pathway that is essential for establishing the meiotic chromosome segregation pattern. This pathway regulates the message-specific expression of gene clusters. Few examples of pathways governing the translational control of gene clusters have been identified. Despite the prevalence of translational control, the mechanisms by which translational control pathways act is poorly understood especially when compared to analogous knowledge of transcription control.

I have shown that the RNA-binding protein Rim4 and the kinase Ime2 regulate the timing and order of the meiotic divisions by controlling translation. Rim4 binds and inhibits translation of its targets during meiosis I. At the onset of meiosis II, Ime2 kinase activity rises and triggers a decrease in Rim4 protein levels thereby alleviating translational repression.

Moving forward, this proposal will address the following scientific aims: 1) Discover the mechanism by which Rim4 represses translation. Using genetic, biochemical, and cell biological approaches, I will determine co-factors of Rim4-mediated repression. I will determine how phosphorylation affects RNA-binding, degradation, and/or localization. Rim4 harbors low complexity domains which are important for intracellular compartmentalization. I will determine the function of these domains. 2) Determine the RNA-structure recognized by Rim4. I will use the PAR-CLIP method to purify Rim4 crosslinked to its target RNAs. Bioinformatic analysis of RNA sequences purified in this manner will lead to discovery of motifs recognized by Rim4. 3) Understand the signals that turn translational control on and off during meiosis. I will use genetic and cell biological approaches to determine how the translational control machinery is activated in meiosis and how Ime2 kinase is held inactive until meiosis II. Completion of these aims will give broadly applicable insight into gamete production and translational control.

Alison Burkart, Ph.D.

Joslin Diabetes Center

Mentor: Mary-Elizabeth Patti, M.D.

“Dissecting Mechanisms by which Insulin Resistance Impairs Stem Cell Function and Metabolism in Human iPSC Cells”

Type 2 diabetes (T2D) has reached epidemic rates worldwide. Human and animal studies have defined genetic and environmental contributions to this complex metabolic disease and identified insulin resistance as a predictor of T2D. However, the molecular basis of insulin resistance remains unknown. We propose to utilize a novel tool to approach this critical problem: induced pluripotent stem cells (iPSC) derived from patients with genetically determined insulin resistance. These cells will be analyzed in vitro to dissect the mechanisms by which insulin resistance alters cellular metabolism and proliferation.

We have successfully generated iPSC by reprogramming fibroblasts from 5 healthy controls and 4 patients with severe insulin resistance due to insulin receptor mutations and confirmed pluripotency of these iPSC. We demonstrate that insulin resistance alters critical functions of iPSC, potently reducing proliferative capacity and inducing pathway-specific defects in insulin signaling. Furthermore, preliminary experiments aimed at identifying molecular mechanisms driving these functional defects revealed that insulin-resistant iPSC have increased energetic stress, as indicated by increased ADP/ATP ratios and robust activation of the energetic sensor AMP-activated protein kinase (AMPK). Mitochondrial size, number, and functional capacity are also dysregulated in insulin-resistant iPSC.

Based on these exciting data, we will test the hypothesis that insulin resistance directly impairs cellular metabolic homeostasis, contributing to reduced proliferative capacity of critical stem cell populations.

To identify the cellular mechanisms responsible for defective energy metabolism (aim 1), we will perform detailed quantitative analyses of key metabolic pathways (e.g., glycolysis, TCA cycle, and oxidative phosphorylation) and cellular proliferation and identify regulatory signaling and transcriptional pathways contributing to these defects. To determine whether acute insulin resistance similarly impairs cellular metabolism (aim 2), we will use 2 approaches in control iPSC: a) treatment with a pharmacological antagonist of the insulin receptor and b) genome-editing technology (CRISPR) to introduce insulin receptor gene mutations. Together, these studies will address a fundamental question about the mechanisms by which insulin resistance dysregulates cellular metabolism, contributing to diabetes risk. More broadly, these data will illuminate the role of insulin resistance as an important modifier of stem cell function and development.

Brian Conlon, Ph.D.

Northeastern University

Mentor: Kim Lewis, Ph.D.

“Determining the Mechanism of Persister Formation and Resuscitation in Staphylococcus Aureus”

Bacterial infections caused by *Staphylococcus aureus* are the most common cause of complicated infection in U.S. hospitals. Even when appropriate antibiotic treatment is administered, patients often fail to fully clear the infection. Persister cells are phenotypic variants of regular cells which are dormant and tolerant to antibiotics. We recently established that *S. aureus* persisters can be killed by dysregulation of ClpP protease by ADEP4, facilitating eradication of a *S. aureus* mouse biofilm infection, highly recalcitrant to other antibiotics. This emphasizes the importance of persisters in deep-seated infection. Unlike other bacteria in stationary phase, the entire population of *S. aureus* behave as persisters and are tolerant to antibiotics. Under normal growth conditions, upon entry to stationary phase, ClpP mediates regulated degradation of various proteins, which, interestingly, mediates persister formation. A *clpP* mutant forms 100-fold less persisters. Antibiotic targets such as gyrase and the ribosome are among the proteins degraded at the onset of stationary phase. We will determine the impact of antibiotic target degradation by ClpP on persister formation. ClpP activity is regulated by its associated ATPases. We will determine the contribution of each ATPase to the persister phenotype. Furthermore, we will identify the mechanism of persister resuscitation. Understanding the precise mechanism of persister formation and resuscitation will facilitate the development of treatments to target and kill these cells and eradicate infection.

Aim 1: Identify the mechanism of ClpP mediated persister formation. Antibiotic target levels in a wild-type and a *clpP* mutant isolate will be monitored under a variety of growth conditions and correlated to persister levels. Antibiotic target levels will be artificially controlled in the wild-type and *clpP* mutant using RNA interference. The impact of this on persister formation will be measured.

Aim 2: Determine the role of Clp ATPases in persister formation. Persister formation in mutants of ClpX, ClpC, ClpB and ClpL will be examined under a variety of growth conditions.

Aim 3: Determine the mechanism of persister resuscitation. Using proteomics and metabolomics, we will analyze the changes within the cell as it transforms from an antibiotic tolerant state to an antibiotic susceptible state and will identify resuscitation factors.

Stavroula Hatzios, Ph.D.

Brigham and Women's Hospital

Mentor: Matthew Waldor, M.D., Ph.D.

“Chemoproteomic Studies of Serine Hydrolases active during Vibrio Cholerae Infection”

The chemoproteomic technique of activity-based protein profiling (ABPP) enables the direct evaluation of enzyme activity within complex proteomes. Small-molecule probes that are chemically tuned to react with a particular enzyme class allow selective detection, enrichment, and identification of active enzymes by mass spectrometry. ABPP can provide a more accurate assessment of enzymes that function under a particular set of conditions than either transcriptomic or non-activity based proteomic analyses, since post-transcriptional and post-translational regulation can lead to an imperfect correlation between gene/protein expression and enzyme activity. In preliminary work, I used ABPP to identify four secreted serine hydrolases from the cholera pathogen *Vibrio cholerae* that are consistently active in the infant rabbit model of infection. One of these four predicted proteases, VC0157, is also active in biofilm culture supernatants, but not under typical growth conditions, and appears to undergo significant post-translational processing to generate active enzyme. In the proposed research, I will investigate the hypothesis that these enzymes contribute to *V. cholerae* pathogenicity in infected rabbits, determine how VC0157 activity is regulated in vitro, and identify host-secreted serine hydrolases that modulate *V. cholerae* infection. I will generate a panel of mutants to test whether individual or collective loss of the *V. cholerae* proteases affects bacterial virulence in infected rabbits and to determine their in vivo proteolytic targets using ABPP and mass spectrometry. I will also characterize the molecular and environmental factors that govern the conditional activation of VC0157 in vitro. Finally, I will use ABPP to identify host enzymes that are specifically activated by *V. cholerae* in infected rabbits and human enterocyte-like cells. My work will define the enzymatic crosstalk between this global enteric pathogen and the host, with the long-term goal of identifying potential therapeutic approaches for cholera. In addition, these studies will provide a roadmap for future applications of ABPP to elucidate enzymes that facilitate infection by other pathogens.

Katherine McJunkin, Ph.D.

University of Massachusetts Medical School

Mentor: Victor Ambros, Ph.D.

“Molecular Mechanisms of microRNA and Argonaute Turnover”

MicroRNAs are small 22-23 nucleotide RNAs which bind to Argonaute proteins to negatively regulate protein-coding genes through sequence-specific complementarity. The expression of microRNAs is frequently dysregulated in many human diseases, including cancer. The biogenesis of microRNAs is extensively studied, and comparatively little is understood about their turnover. Untemplated nucleotide additions at the 3' end of microRNAs are correlated with destabilization, but their functional role remains unclear. The objective of this work is to discover the regulators and effectors of microRNA and Argonaute turnover. In addition, we will examine the role of 3' nucleotide additions in microRNA turnover.

We will use three approaches in *C. elegans* to identify regulators and effectors of microRNA turnover. First, microRNA biogenesis will be acutely inactivated in a temperature-sensitive mutant, and proteomic analysis will identify proteins that are preferentially bound to microRNAs over the course of their decay. In a second approach, we will transiently tag Argonaute at its endogenous locus; tagged proteins will be immunoprecipitated over the course of their decay to identify binding partners which are enriched during this process. Third, we will conduct a forward genetic screen for suppressors of a hypomorphic

microRNA phenotype, with the rationale that a subset of these suppressors will increase microRNA levels and may impact microRNA turnover. We will use the systems established herein to assess the impact of candidate proteins on rates of microRNA decay, Argonaute decay, and 3' untemplated nucleotide additions. By examining all three processes, we aim to elucidate if and how their mechanisms are functionally coupled.

Because microRNA levels are likely to be regulated by the balance of the rates of biogenesis and turnover, understanding the determinants and mechanisms of turnover will add to our overall understanding of microRNA regulation and normal development. Because microRNA dysregulation contributes to human disease, these studies may also open novel therapeutic avenues through the targeted inhibition or modulation of microRNA turnover.

Gulcin Pekkurnaz, Ph.D.

Boston Children's Hospital

Mentor: Thomas L. Schwarz, Ph.D.

"O-GlcNAcylation: A Glucose Sensing Pathway for Mitochondria and Energy Homeostasis"

The central nervous system plays an important role in regulating feeding behavior and metabolism; disruption of this regulation leads to obesity, metabolic syndrome, and Alzheimer's disease. This proposal addresses critical questions about how nutrient availability is sensed in the nervous system and what molecules are involved in the regulation of metabolic homeostasis. It builds upon our recent findings that the cytosolic glycosylating enzyme O-GlcNAc Transferase (OGT) diminishes mitochondrial motility based on glucose availability via a direct interaction with, and GlcNAc modification of, a key protein in the motor adaptor complex. I now seek to put this finding into a physiological context by determining the mechanisms by which extracellular metabolic cues regulate mitochondrial function via OGT, and how it contributes to bioenergetics, particularly in hypothalamic neuroendocrine circuit. I will accomplish these goals by investigating the following Specific Aims: Aim 1 concerns how mitochondria sense nutrient availability and how their subsequent change in localization regulates cellular bioenergetics and metabolic homeostasis in neurons. Aim2 investigates how the glucose-sensitivity of mitochondria affects whole-body energy balance by specifically studying this pathway in the arcuate nucleus of the hypothalamus. The anticipated findings should facilitate our understanding of the biochemical pathways that underlie diabetes, other endocrine and metabolic diseases, nutritional disorders, and obesity.

Hyun Cheol Roh, Ph.D.

Beth Israel Deaconess Medical Center

Mentor: Evan Rosen, M.D., Ph.D.

"Molecular Mechanisms Underlying Beige Adipocyte Dynamics in Response to Thermal Challenges"

Adipocytes are normally classified as white or brown, but a distinct type of fat cell, called the beige adipocyte, has recently been identified. These cells support thermogenesis and dissipate calories like brown adipocytes but reside within white adipose tissue regions, and express an overlapping but unique set of genes. They have recently been discovered in adult humans, holding therapeutic potential for obesity and diabetes. Beige adipocytes display dynamic population changes and cellular plasticity in response to temperature changes. Our long-term goal is to understand how to manipulate beige adipocyte number and activity for use in the therapy of metabolic diseases. It has been recently reported that beige adipocytes are newly differentiated from precursor cells upon cold exposure, and

they convert into white adipocyte-like cells in response to subsequent warming. However, mechanisms underlying such beige cell dynamics are poorly understood, in large part because the cellular heterogeneity of the adipose depot complicates the isolation and analysis of this specific minority cell type.

To elucidate mechanisms underlying beige adipocyte dynamics, we recently developed two mouse strains, UCP1-Cre and Rosa26fs-H2B-TRAP, which allow in vivo tracing of beige adipocytes and isolation of their transcripts and nuclei from a complex cellular mixture. My preliminary results using these strains suggest that beige adipocytes undergo one of two possible fates after warming: conversion into the white-like state or cell death. Also, this turnover process appears to be faster than has been reported for adipocytes generally, suggesting that beige adipocytes have a unique cell turnover mechanism. To address this, I propose two major aims. First, to determine cellular fate of beige adipocytes in response to warm conditions, I will analyze their fate and quantify turnover rate by tracing labeled beige adipocytes using immunohistochemistry and cell death assays. Second, to define molecular mechanisms that regulate beige adipocyte turnover, I will employ comprehensive transcriptional and epigenomic analyses to identify transcriptional pathways and downstream effectors that mediate beige adipocyte turnover during warming. These studies will advance our understanding of adipose biology, suggesting novel therapeutic strategies for obesity and diabetes.

Daniel Schmidt, Ph.D.

Massachusetts Institute of Technology

Mentor: Edward S. Boyden, Ph.D.

“Molecular Tools for Precision Control of Cellular Signaling”

Standard therapies for many cancers and neurodegenerative diseases are lacking due to a paucity of drug-able targets, side effects, and, most importantly, an incomplete understanding of disease biology. The complexity of ion channel and receptor signaling renders our understanding of cellular signal transduction in even the best-studied types of cells incomplete. Generating tools that allow cell-type specific, rapidly-perturbable pharmacology is therefore of great interest to many fields of medicine and biology.

We have addressed this need by designing and implementing a new set of optogenetic reagents. These reagents, called lumitoxins, combine a photoswitch and an ion channel-blocking peptide toxin to actuate specific endogenous ion channels. Lumitoxins enable cellular physiology to be perturbed in a fully genetically encoded and rapidly reversible way, without the need for exogenous chemical co-factors.

Going forward, our strategy is to build on the lumitoxin technology, to further enhance and validate it, and to develop derivative technologies that are based on these novel optogenetic reagents and concepts of protein engineering. With lumitoxin technology, by acting on ion channels and ligand-gated receptors, it may become possible to alter specific signaling pathways in a defined set of intact cells, and then to study their effects on normal and pathological biological functions. In this way we address unmet needs in human health by furthering our understanding of how cells sense, integrate and exchange information about their environment, how pathologic changes in these processes relate to disease, and how insights into the disease's biology can offer new therapeutic points of intervention.

Jianlong Sun, Ph.D.

Boston Children's Hospital

Mentor: Fernando Camargo, Ph.D.

“Clonal Analysis of Hematopoiesis in Aging”

Aging of the hematopoietic system is characterized by progressive decline in homeostatic maintenance and gradual loss of regenerative capacity following hematopoietic stress. This aging process is frequently associated with the onset of chronic anemia, the diminished adaptive immunity, and the elevated incidence of myeloproliferative diseases, all of which have been suggested to result from dysfunctions of aged hematopoietic stem cells (HSC). Although the functional alterations of HSCs during aging have been extensively characterized with bone marrow transplantation experiments, it is currently unknown how exactly HSCs behave in their native bone marrow niche during the natural process of aging, and how the potential changes of this primitive population will contribute to the deterioration of the hematopoietic system over time.

In order to understand the clonal dynamics of native hematopoiesis, our laboratory has established a novel mouse model where cells can be uniquely and genetically labeled in situ. Using this approach, we carried out longitudinal analysis of hematopoiesis at steady state, and found the successive contribution by hundreds to thousands of clones to the maintenance of blood homeostasis, which is in stark contrast to the oligoclonal behaviors following bone marrow transplantation. Our data implied that progenitors, rather than HSCs as previously believed, are the main drivers of unperturbed hematopoiesis. These findings not only uncovered distinct cellular mechanisms driving blood regeneration under different conditions, they also raise new questions with regard to the contributions of the dynamic progenitor populations in the aging process.

In the current application, I propose to investigate the impact of aging on the clonal behaviors of hematopoietic stem and progenitor cells in their native niche. Specifically, I plan to examine the exact changes that will occur to the complexity and dynamics of the progenitor and stem cells during the aging process (Aim 1). I will also determine the effect of aging on the regenerative capacity of these populations following multiple hematopoietic stresses (Aim 2). These proposed studies are built upon my previous work on clonal analysis, and will promise to bring in new insights to the aging hematopoietic system.

Piuter Tsvetkov, Ph.D.

Whitehead Institute for Biomedical Research

Mentor: Susan Lindquist, Ph.D.

“Hsp90 Regulation of Prion-Based Translation: Molecular Mechanisms and Evolutionary Conservation”

Prions are proteins that are able to convey epigenetic elements of cellular memory through conformational switching. Although originally associated with pathology, prion-like conformational switching is emerging as a novel mechanism for cellular regulation. The most abundant subgroup of cellular proteins predicted to have prion-like properties is the RNA-binding protein (RBP) class of proteins. The abundance of PrDs in RBPs is conserved from yeast to humans, suggesting a modular function for these domains. To date, little is known regarding the function or regulation of these PrDs. However, prion-based mechanisms can serve as a switch and a sensor to environmental cues, whereby the change in protein conformation alone is sufficient to induce an altered cellular response. In the case of RBPs, induced conformational switching could directly affect protein translation and cellular homeostasis. My goal is to explore the role of PrD-containing RBPs in regulating protein translation.

In yeast, prion propagation and inheritance is highly dependent on the chaperones Hsp104 and Hsp70. We have preliminary evidence that an additional chaperone, Hsp90, is a novel regulator of a protein translation phenotype that is prion in nature. In this project I intend to characterize the evolutionary conservation (between yeast and humans) of the role of the chaperone machinery in regulating RBPs. Initially, I will examine all known RBPs in human and yeast cells and determine whether they have prion-like characteristics by high-throughput biochemical analysis of aggregating propensities. Next I will determine which chaperones regulate prion conformational switching. In the yeast model I will use genetic and pharmacological perturbations. In human systems I will analyze the interactions of all RBPs with Hsp90, Hsp70 and their co-chaperones in hope of identifying and characterizing specific co-chaperones that mediate RBP prion switching utilizing the LUMIER assay, which is high-throughput platform developed in the Lindquist lab.

I believe that the integration of the experimental results obtained in the yeast model together with the high-throughput data from the mammalian system will provide us with an integrative picture of what might be an ancient prion-based network of translation regulation that may be relevant to many other essential cellular pathways.

Songyu Wang, Ph.D.

Harvard Medical School

Mentor: Tom Rapoport, Ph.D.

“Mechanisms Involved in the Formation of the ER Network and Nuclear Envelope”

The broad goal of this project is to understand how organelles are shaped, in particular, the endoplasmic reticulum (ER) and the nuclear envelope (NE). We will use *Xenopus* egg extracts to address these questions. Previous experiments have identified proteins that shape the tubules (RTNs and DP1) and mediate the fusion of ER membranes (ATLs). Now, we will address how these proteins and a new protein identified recently, termed lunapark (LNP), collaborate to form an ER network. The first aim is to test a possible role of LNP in stabilizing three-way junctions between ER tubules. Our preliminary data show that LNP inactivation induces tubule-to-sheet transition of the ER network. A cytosolic factor seems to be involved. The morphological changes are strikingly similar to those seen during the transition between interphase and mitosis. Our goal is to identify the cytosolic factor and to elucidate how LNP inactivation leads to the changes in ER morphology. This will also provide insight into the mechanism of tubule-to-sheet transition during mitosis. A second aim is to test whether ATL activity is required for the function of the tubule-stabilizing proteins (RTNs and DP1). We have made the surprising observation that ATL function is not only required for network formation, but also for its maintenance. Inactivation of ATL leads to the rapid disassembly of the ER network into small vesicles and short tubules. We hypothesize that many three-way junctions are actually tethered by ATL, rather than fused, and that ATL activity is required for the tubule-stabilizing function of the RTNs and DP1. We propose several experiments to test these hypotheses. The third goal is to clarify the mechanism of NE formation. We have recently reported that there are multiple fusion steps during NE assembly, including one that requires the function of ER SNAREs. Additional experiments show that importin beta and Ran play a role in NE formation. We suggest that a fusion factor is bound to importin beta in a RanGTP-dependent manner. Our goal is to identify the factor, clarify the connection with ER SNAREs, and characterize different vesicle populations required for NE formation.

Yinyuan Wu, Ph.D.

Boston Children's Hospital

Mentor: Xi He, Ph.D.

“Phosphorylation Recognition and Wnt Signaling Complex Assemblies by the Intrinsically Disordered Scaffold Protein Axin”

Wnt signaling through the transcriptional coactivator beta-catenin (the canonical Wnt pathway) has pivotal roles in embryonic development, stem cell regulation, and cancer and disease pathogenesis. Understanding/targeting the Wnt pathway may offer new therapeutic opportunities. The Axin scaffolding protein is a tumor suppressor and acts to degrade beta-catenin, whereas the Wnt receptor LRP6 inactivates Axin to stabilize beta-catenin. Axin binds to activated/phosphorylated LRP6 (p-LRP6), constituting a key initiation step in Wnt signaling; Axin phosphorylation further regulates Axin/p-LRP6 interaction. Mechanisms by which the Axin/p-LRP6 complex is assembled and Axin phosphorylation regulates this assembly are poorly understood. Intriguingly Axin belongs to intrinsically disordered proteins (IDPs), which account for more than a third of the eukaryotic proteome and are essential for most or all cellular function. IDPs lack a fixed 3D structure and therefore provide unique features including flexibility and evolvability for dynamic protein interactions. Although the role of IDPs in protein-protein interactions is well documented, whether an IDP can function as a module for recognition of post-translational modifications such as phosphorylation is unknown. This may be in part a result of the dogmatic view that protein phosphorylation is recognized by “domains” that have defined 3D structures such as the archetypal SH2 domain. I have acquired preliminary and exciting data suggesting that Axin/p-LRP6 interaction represents the first example of phosphorylation recognition by an IDP, thus departing from the paradigm of phosphorylation recognition by structured protein domains. In this application I propose (i) to characterize the mechanism by which Axin recognizes p-LRP6; (ii) to investigate the mechanism by which Axin phosphorylation regulates the Axin/p-LRP6 complex assembly; and (iii) to identify compounds that disrupt the Axin/p-LRP6 complex, thereby blocking Wnt signaling. My studies will provide insights into the Axin tumor suppressor function and Wnt signal transduction, reveal principles governing IDPs in recognition of phosphorylation and possibly other post-translational modifications, and broaden our understanding of IDPs in biological regulation. My experiments may further facilitate therapeutic development for cancers with excessive Wnt signaling.

Martin Wuehr, Ph.D.

Harvard Medical School

Mentor: Steven Gygi, Ph.D.

“Proteomics of Nucleocytoplasmic Partitioning”

The defining eukaryotic organelle, the nucleus, is bound by the nuclear envelope, which separates nuclear from cytoplasmic activities and allows for complex regulation. The nucleocytoplasmic distribution often encodes critical information during development, stress response, and general cell signaling. Despite the nucleus's central role, its molecular composition, how it adapts to perturbations, and how nuclear organization is related to disease is still poorly understood.

I chose the giant *Xenopus* oocytes as a primary model to study nucleocytoplasmic partitioning since it allows for physical isolation of the nucleus, avoiding protein loss or gain during isolation. Using two different methods of accurate quantitative proteomics, I found an unexpected trimodal distribution. Most proteins are exclusively located in nucleus or cytoplasm with a third subset approximately equally partitioned.

I will probe the selective barrier properties of the nuclear envelope. In apparent contradiction to the current model for nuclear pore permeability, my data indicates that many small proteins are sharply partitioned between nucleus and cytoplasm. I will explore the selectivity of the nuclear pore on a proteome-wide scale by injecting a prokaryotic proteome (passive to nuclear transport) into the cytoplasm of a eukaryotic cell. I then will measure the proteins' equilibration times with quantitative proteomics. As an alternative hypothesis, I will test if the majority of small proteins is bound in large protein complexes and therefore impermeable to nuclear pores.

Secondly, I will ask if substrate affinities to importins or exportins predict nucleocytoplasmic partitioning. Importins' and exportins' substrate affinity changes upon binding of Ran-GTP, which is restricted to the nucleus. I will quantify the Ran-GTP dependent binding affinity of substrates for all importins and exportins. An immediate application of this data will be to evaluate and improve algorithms to predict a protein's affinity to import/export receptors and subcellular localization.

By combining innovative mass-spectrometry based proteomics and classical biological tools, I have laid the foundation to study nucleocytoplasmic partitioning and the underlying mechanisms with molecular resolution on a proteome-wide level. The results will provide us with insight into cellular organization and may provide opportunities to treat nuclear miss-regulations often found in cancer and other diseases.

Dong Yan, Ph.D.

Harvard Medical School

Mentor: Norbert Perrimon, Ph.D.

“Splicing Regulation of Drosophila Germline Stem Cell Self-renewal and Signaling Transduction”

Stem cells possess the remarkable capacity to generate two cells of distinct fate upon division, with one cell retaining the stem cell state and the other cell destined to terminally differentiate. Although mammalian cell culture approaches have provided insight in this process, it is more desirable to study stem cells in their native environment, where they receive signals and interact with the stem cell niche. To understand the molecular basis of stem cell self-renewal in vivo, I used *Drosophila* germline stem cell (GSC) as a model system and performed a large-scale RNAi screen covering ~25% of the *Drosophila* genome. I identified 366 genes required for GSC maintenance or differentiation, allowing me to construct a comprehensive network for GSC regulation.

I performed a complex-enrichment analysis and identified several protein complexes and decided to focus on RNA splicing genes due to their impressive phenotypes upon knockdown. In particular, *spenito* (*nito*), identified from my RNAi screen, gives a strong stem-cell-tumor phenotype. Its human ortholog, OTT was originally identified from infants with acute myeloid leukemia (AML). Chromosomal translocation, resulting in fusion of OTT with another gene MAL, leads to leukemia but the mechanism is poorly understood. Recently, I made the surprising observation that Nito regulates sex determination by affecting the splicing of the master sex determination gene *sex-lethal* (*sxl*). Moreover, I found that Nito regulates Dpp/BMP signaling pathway that is essential for growth and patterning during development. I propose to analyze the mechanisms by which Nito, as well as other splicing genes, controls GSC self-renewal and signal transduction during development. Specifically, I will: Aim 1: Characterize the mechanism by which Nito regulates *sxl* splicing; Aim 2: Determine how Nito controls Dpp signal transduction pathway; and Aim 3: Study the functions of other splicing genes identified from my unbiased genetic screen, such as the exon junction complex (EJC). By combining molecular, cellular and genetic approaches together with deep-sequencing technique, I hope to establish the mechanism by

which Nito regulates both sex identity and tissue growth, uncover the targets of its splicing function, and identify potential new therapeutic targets for OTT-mediated AML.

Wen Yang, Ph.D.

Harvard Medical School

Mentor: Marcia Haigis, Ph.D.

“Mitochondrial Energy Status Regulate SIRT3 Interactome and Function”

SIRT3 is the major mitochondrial deacetylase, which has been established to have a wide spectrum of substrates in mitochondria. This protein plays an important role in many diseases, including neuron degenerative diseases and cancer. Although over 20 different substrates have been identified, little is known about how this enzyme is regulated and how it preferentially binds to some substrates rather than others, under different conditions, to facilitate its multiple regulatory roles in mitochondria. By constructing and analyzing the mitochondrial sirtuin network, we found that SIRT3 exhibits a unique strong physical association with mitochondrial ATP synthase (Complex V). More importantly, we further discovered that this binding is affected by mitochondrial membrane potential and Complex V function. We hypothesize that SIRT3 plays an important role in energy metabolism and the existence of a feedback loop through Complex V, which regulates SIRT3 function to determine its mitochondrial distribution and interactome. We further propose that this regulation is governed by mitochondrial energy status. Specifically, Complex V holds SIRT3 under normal condition but releases SIRT3 into the mitochondrial matrix with a decrease in membrane potential.

The goal of this research is to test this hypothesis by first investigating how membrane potential and Complex V activity regulate the interactome of SIRT3; then we will decipher the binding mechanism between SIRT3 and Complex V, and last we will investigate how this binding balances mitochondria energy status through changing of SIRT3 interactome.

Taken together, this study may reveal a novel mechanism of post translational regulation of SIRT3 as well as a new function for Complex V besides ATP synthesis. By studying SIRT3 from a different angle, the proposed research can increase our understanding of this major mitochondrial deacetylase. Therefore this study should be of great interest to the sirtuin field, as well as to the general mitochondrial bioenergetics field. In long term, knowledge obtained from our work may benefit drug development targeting SIRT3. Considering SIRT3 may not be the only enzyme regulated in this manner, this study may also open a new window for studies on mitochondrial function and mitochondrial-related diseases.

Xiaochang Zhang, Ph.D.

Boston Children's Hospital

Mentor: Christopher A. Walsh, M.D., Ph.D.

“Mutations in QARS, Encoding GlutaminyI-tRNA Synthetase, Cause Progressive Microcephaly, Cerebral-Cerebellar Atrophy and Intractable Seizures”

Microcephaly is a congenital or early-childhood neurodevelopmental disorder that an individual's head circumference is significantly smaller than expected for age and gender. Progressive microcephaly associated with diffuse cerebral-cerebellar atrophy and epilepsy is a heterogeneous condition and accompanies various neurodegenerative diseases. We identified four children from two unrelated Caucasian families to display progressive microcephaly, diffuse cerebral-cerebellar atrophy and

devastating seizures. Through whole exome sequencing, we identified QARS as the only candidate causative gene in each family. These results lead to our first hypothesis that recessive mutations in QARS cause progressive microcephaly and intractable seizures. Specific Aim 1: to determine the role of QARS mutations in progressive microcephaly by modeling the human syndrome in zebrafish and genetic complimentary tests. We found that QARS is highly expressed in the developing human and zebrafish brains, and that *qars* zebrafish morphants and mutants display almost identical small brain, small eye and uncoordinated movement phenotypes. I propose to determine the pathogenicity of individual human QARS mutation through introducing them into zebrafish morphants and measuring the rescuing effects of brain and eye sizes. The effect of glutamine supplement will also be tested in morphants injected with mutant QARS mRNAs. The small brain phenotype in zebrafish morphant and mutants may be consequences of either defective cell proliferation or premature cell death, and these possibilities will be determined by immunohistochemistry assays on brain sections. Specific Aim 2: to investigate the molecular mechanisms how human QARS mutations impair QARS protein functions. QARS encodes the glutamyl-tRNA synthetase, which conducts aminoacylation reactions and precisely attaches glutamine to correct tRNAs for faithful protein translation. We found that QARS aminoacylation activities are significantly reduced in cell lines derived from affected children and their QARS heterozygous parents when compared to healthy controls, with affected children's the lowest. This leads to our second hypothesis that each QARS mutations impair QARS aminoacylation activity. I propose to purify individual QARS mutant proteins and test their aminoacylation activity using the same assay as was done with patient cell lines. I'll also express individual mutant QARS cDNA in tissue culture cells and examine their interaction with the multiple synthetase complexes.

2013 Grant Recipients

Jamie Doyle, Ph.D.

Tufts Medical Center

Mentor: Alan S. Kopin, M.D.

"Generation of Novel Probes to Explore the Role of Mrgprs in Pruritus"

There is an unmet need for novel strategies to treat pruritus (itch). Itch is defined as an unpleasant sensation that leads to a desire to scratch and is a symptom associated with both skin diseases as well as many diseases that affect other organ systems. Pruritus has a dramatic effect on quality of life of individuals suffering from this relatively poorly understood condition. Of particular concern, psychological manifestations including difficulties with daily activities, sleep disturbances, and disruptions in the quality of social life are reported among individuals suffering from chronic pruritus. Very few treatment options for pruritus exist and although there have been many advances with regards to understanding the transmission of itch sensations, there still remain large gaps in defining and characterizing the pathways that mediate this disease. Furthermore, better tools are needed to probe the interaction between neurofibers, target cell types, and the mediators of itch in model systems.

With enhanced knowledge of the molecular basis of itch, selected G protein-coupled receptors (GPCRs) have emerged as promising therapeutic targets. Histamine receptors are one class of GPCRs that have long been associated with itch. The subset of patients with histamine dependent itch can be successfully treated with histamine receptor antagonists. Outside this realm, many questions remain. Recently, the Mrgpr (Mrg/SNSR) family of orphan GPCRs has received attention due to their expression

in primary sensory neurons. Of particular clinical interest, human MrgprX1 has been implicated in modulating histamine independent itch.

Despite this exciting progress, few pharmacological tools exist to explore Mrgpr receptors, in particular MrgprX1, as modulators of pruritus. Therefore, the goal of this proposal is to utilize a well-established strategy developed in our laboratory to generate and characterize novel probes for this receptor. Prior success in another disease area has been demonstrated using the proposed strategy to generate stable, long-acting modulators of GPCRs that show therapeutic utility in vivo. The overall impact of our proposed efforts with Mrgprs will include (i) the development of novel pharmacological tools for dissecting mechanisms involved in Mrgpr mediated itch and (ii) taking initial steps towards developing a new class of therapeutics for pruritus.

Alejo Efeyan, Ph.D.

Whitehead Institute for Biomedical Research

Mentor: David M. Sabatini, M.D., Ph.D.

“Elucidating a Non Canonical Mechanism of mTORC1 Activation”

The mammalian target of rapamycin complex 1 (mTORC1) signaling pathway drives cell growth and anabolism by its ability to integrate signals from growth factors and nutrients. mTORC1 activity is deregulated in several complex diseases, as cancer, type 2 diabetes and aging. Hence, exploring the mechanisms that lead to mTORC1 deregulation is key for improving our understanding of the pathogenesis of these diseases. We recently described the Rag family of GTPases as key for mTORC1 activation by amino acids, through a mechanism that requires mTORC1 shuttling to the lysosomal surface. In our efforts to generate mice with loss and gain of function alleles for the Rag GTPases, we have established a series of immortalized cell lines derived from mice that lack RagA. Although mTORC1 activity is barely detectable in RagA-deficient embryos, cell lines derived from them show reactivated mTORC1 signaling, which is now insensitive to amino acids withdrawal, but sensitive to growth factors withdrawal. Intriguingly, in these cell lines, mTORC1, although active, does not localize to lysosomes. This altered localization does not fit with current paradigm of mTORC1 regulation indicating that an unknown mechanism of mTORC1 activation is at work. Based on a combination of transcriptional profiling and proteomic-based approaches, we plan to find the genes responsible for the lysosomal-independent activation of mTORC1 and to understand how this occurs. In particular, we will aim for the following goals:

Aim 1. Characterization of the amino acid-independent activation of mTORC1 in RagA-null cells.

Aim 2. Identification of candidate genes responsible of this novel signaling mechanism, by two complementary approaches: 1) Transcriptional profiling of RagA-null cells; and 2) Identification of novel mTORC1 interacting proteins in RagA-null cells.

After validation of the candidates in cell culture (Aim 3), we will take advantage of RagA-null livers to study in vivo the consequences of altering their expression.

Aim 4. In vivo knock-down and overexpression of validated genes.

Further elucidation of the molecular mechanism leading to mTORC1 desensitization to amino acid withdrawal, together with the identification of potentially druggable targets within this pathway, may pave the way for novel therapeutic approaches aimed to target mTORC1.

Kimberley Ellis, Ph.D.

Tufts University School of Medicine

Mentor: Andrew Camilli, Ph.D.

“A Novel Bacteriophage Resistance Mechanism in Vibrio Cholerae”

Vibrio cholerae is the causative agent of cholera, an acute diarrhoeal disease that is endemic in over 50 countries and also causes devastating epidemics. Bacteriophages that specifically prey on *V. cholerae* have been hypothesized to contribute to the seasonality of cholera epidemics, particularly in the endemic region of Bangladesh. The most predominant group of phages found in cholera patients are the ICP1 related phages, which have sustained an association with *V. cholerae* O1 for over a decade. We hypothesize that ICP1-mediated predation of *V. cholerae* O1 has directed the selective emergence of pathogenic clones that circulate to cause epidemic disease. We have identified a novel anti-ICP1 resistance mechanism, designated a phage inducible chromosomal island-like element (PLE), which is found in many ICP1-resistant clones that were isolated from patients since 2005. Some ICP1 related phages have since evolved a counter-resistance mechanism, which effectively targets the PLE for destruction, however we currently do not understand how the PLE exerts its anti-ICP1 effect. Preliminary data suggests that the *V. cholerae* PLE represents a novel phage resistance mechanism; ICP1 infection of an individual cell triggers the PLE to undergo an excision-replication-packaging (ERP) cycle which effectively blocks ICP1 replication, but still results in cell death in order for the PLE to be effectively packaged and spread to neighboring cells. The net result is elimination of ICP1 from the surrounding environment, and we predict, better preparedness of the population as a whole against future ICP1 attack. In this proposal I describe experiments that will fulfill two main aims: Aim 1: To determine the biological parameters of the ERP cycle of *V. cholerae* PLE1. Aim 2: To determine the mechanism of ICP1 interference by *V. cholerae* PLE1. We will use quantitative PCR, mass spectrometry and transduction assays to address the features of the ERP cycle. Additionally we propose to use a mutational approach to identify which of the 23 PLE1 encoded genes contribute to its anti-ICP1 activity. The long-term goal of this proposal is an increased understanding of the dynamic between ICP1 phage and their pathogenic host, which will provide insight into epidemic occurrence and ultimately intervention.

Eugene Gladyshev, Ph.D.

Harvard University

Mentor: Nancy Kleckner, Ph.D.

“DNA Homology Sensing in the Absence of Double-Strand Breaks”

The ability of two homologous DNA molecules to recognize one another is fundamental to sexual reproduction and accurate DNA repair, and may also underlie other chromatin processes, such as regulation of allele-specific transcription. While the known mechanism of direct homology recognition is initiated by a double-strand break (DSB) in one of the participating DNA molecules, the existence of an alternative, break-independent pathway is suggested by several unrelated phenomena that include somatic chromosome pairing in *Drosophila*, meiotic chromosome pairing prior to or in the absence of

recombination in diverse species, and transient pairing of X chromosomes prior to X-inactivation in mammals. The basis for such break-independent pairing remains one of the central unsolved mysteries of chromosome biology, with proposed mechanisms including direct DNA/DNA contacts, RNA-mediated co-localization, DNA-guided protein/protein interactions

I chose to characterize the break-independent recognition of DNA homology in the fungus *Neurospora crassa*, where the phenomenon of RIP (Repeat Induced Point mutation) has provided the strongest support for the existence of direct sequence-specific interactions between intact DNA molecules. I found that DNA pairing in RIP is initiated within a short region of homology (<25 bp) and stabilized into a productive interaction by the cooperative alignment of many trinucleotides (>13-14) spaced evenly with a period of 11 bp. My working hypothesis is that this trinucleotide engagement involves a stacked quadruplex structure. While such DNA/DNA interactions are geometrically possible, they almost certainly require additional factors to form a stable complex. I propose to identify factors involved in RIP by a combination of genetic and biochemical approaches. Specifically: I will probe the possible role of Rad51, a molecule known to mediate a triplet-directed homology search during recombination. I will identify and characterize dominant gain-of-function mutations that increase RIP. I will also identify proteins that interact with the NcrID methyltransferase, the only factor now known to be directly involved in RIP. Once the molecular mechanism of RIP is elucidated, I hope to apply this understanding to a wider range of questions regarding the structural dynamics of chromatin in *Neurospora* and other model organisms.

Nitya Jain, Ph.D.

Massachusetts General Hospital

Mentor: Brian Seed, Ph.D.

“Understanding Neonatal Immunity: The Influence of Gut Microbial Colonization on Neonatal Adaptive Immunity”

A hallmark of adaptive immunity is the ability to mount protective antigen-specific responses to foreign agents while preventing deleterious responses to the hosts' own tissue. Autoimmune diseases arise due to a breakdown in tolerance mechanisms that keep pathogenic self-reactive T cells in check. The neonatal period is a unique ontological stage that is characterized by a largely immature immune system prone to tolerogenic responses. While beneficial for thwarting inappropriate autoreactivity, the lackluster immune responses in neonates has poor prognosis for successful pathogen-specific immunity as well as vaccinations. Interestingly, while newborns and infants are more susceptible to infectious diseases, infants in developed countries have become increasingly sensitive to developing autoimmunity such as Type 1 Diabetes (T1D) as well as allergies and asthma.

Alterations in intestinal microbiota or dysbiosis have been associated with the precipitation of several diseases such as T1D and IBD in adults. While dysbiosis can be brought about by various factors such as feeding regimens and antibiotic exposure, dramatic changes in gut microbial compositions also occur naturally during the post-natal period. Microbial colonization of the sterile fetal gut at birth and the establishment of a symbiont microbial community are critical for the development and maturation of

the neonatal immune system. Whether neonatal adaptive immunity perceives changes to the intestinal microbial environment and how it impacts peripheral T cell tolerance is largely unknown. Understanding how the neonatal immune system first interacts with and normally tolerates fluctuations in the microbiota will help dissect the events of pediatric autoimmunity resulting from genuine dysbiosis. Further, mechanistic insights into the evolution of neonatal T cell responses will aid in the improved design of infant vaccine strategies as well as immune-directed therapies.

Using multiple approaches including multi-color flow cytometry, in vivo manipulations of cell populations in mono-colonized germ-free mice, and cutting-edge single cell RNA sequencing technology, we will methodically characterize the neonatal T cell response to evolving gut microbiota, test the autoimmune potential of peri-natal T cells and comprehensively map the molecular profiles and developmental trajectories of neonatal thymic precursors that influence peripheral tolerance to intestinal antigens and microbiota.

Elinor Karlsson, Ph.D.

Harvard University

Mentor: Pardis Sabeti, D.Phil. M.D.

“From Ancient History to Modern Medicine: Selection, Disease, and Genome Function”

The revolution in genomics has yielded vast datasets of full genome sequences, new methods for rapid functional analysis, and discoveries of thousands of genes associated with common human diseases. However, pinpointing and functionally validating causal variants has proven to be much more difficult. One powerful, but often overlooked, tool for understanding the biology of the human genome is analysis of our recent evolutionary history. As we traveled out of Africa into a wide range of new environments, we experienced dramatic alterations in diet and disease risk. The rapid rise in frequency of newly advantageous genomic variants left distinctive signatures in the genome of the affected population, spotlighting important functional loci, yet providing few clues to their function. By pairing tests for selection with phenotype association, I will connect those loci to particular traits, and by then integrating high-throughput functional assays, I will elucidate their effects on cellular mechanisms.

I will focus on a phenotype and population for which we have historical, epidemiological and genetic evidence consistent with strong selective pressure to develop this approach: cholera susceptibility in Bangladesh. Cholera is an ancient, common and deadly disease in the Ganges River Delta, and genetics is known to influence susceptibility. I propose to develop a high-throughput computational and experimental framework to rapidly identify, pinpoint, and functionally elucidate biologically important human mutations under selection by integrating three different types of genomic information: (1) natural selection; (2) disease association; and (3) high-throughput functional screens. I will then validate this approach by functionally testing top candidates and pinpointing the adaptive variants.

By using large, publically available genomic datasets, I propose to develop a tool that is trait-independent and can be applied to many human diseases where associated variants may have been subject to selective pressure, including infectious disease susceptibility, autoimmune diseases, and metabolic diseases. With evidence mounting that much (probably most) of human adaptation has occurred in regulatory regions, not in coding regions of genes, this work is critically important, allowing us to leverage the power of natural selection to identify and elucidate genetic variants altering genome function.

Evangelos Kiskinis, Ph.D.

Harvard University

Mentor: Kevin Eggan, Ph.D.

“Investigating molecular mechanism in genetic forms of ALS using patient-specific iPSCs”

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that affects motor neurons in the brain and spinal cord. The vast majority of ALS is sporadic in nature while familial transmission accounts for approximately 10% of cases with mutations in an expanding number of functionally distinct but ubiquitously expressed genes. Approximately 60% of all familial cases can be accounted for by mutations in SOD1 and C9ORF72. Understanding the mechanisms that cause ALS and the discovery of treatments that might cure it have been hampered by the inaccessibility of human motor neurons. One important unresolved question in ALS and in all neurological disease is whether individuals with distinct mutations suffer through common degenerative mechanisms. Patient-specific induced pluripotent stem (iPS) cells, which are generated by reprogramming from readily available adult cells such as skin cells, provide an invaluable tool for in vitro disease modeling and could be used to address these questions.

Our preliminary data shows that iPS cell--derived motor neurons harboring mutations in the SOD1 gene degenerate in a manner reminiscent of the disease in patients, recapitulating key aspects of ALS. We propose to utilize this novel cellular model to address two outstanding questions in ALS with broader implications for the understanding of neurological disease. Do distinct familial mutations act through common molecular pathways to cause ALS? Why are motor neurons selectively vulnerable to the effects of these mutations, in genes that are widely expressed? In order to address the first question we will examine whether shared molecular and functional changes occur in motor neurons that harbor mutations in the two most common familial ALS genes, SOD1 and C9ORF72. To understand their selective vulnerability in ALS we will compare the effect of the SOD1A4V mutation in motor neurons to its effect in distinct neuronal subtypes, which are resistant to degeneration in this condition. These experiments will provide an insight into the functional defects of human motor neurons in the vast majority of familial ALS and into the characteristics that infer their selective vulnerability. If successful, our studies will illuminate broadly relevant, potential routes for therapeutic intervention.

Jordan Krall, Ph.D.

Whitehead Institute for Biomedical Research

Mentor: Robert Weinberg, Ph.D.

“The Systemic Impact of Wounding on Tumor Immunity and Metastatic Outgrowth”

The vast majority of cancer deaths are caused by the growth of metastases in vital organs, far from the site of the initial growth. Because tumor cells can spread through the body early in tumor development, surgical removal of the primary tumor is often not curative. The disseminated cancer cells usually remain dormant for extended periods of time, and the stimuli that induce these cells to renew proliferation are poorly understood. I am investigating the hypothesis that wounding and wound-healing associated with surgical primary tumor resection have systemic effects that trigger the outgrowth of dormant metastases. Specifically, I am investigating whether wounding can trigger the growth of distant

metastases by inducing systemic immune-suppression that allows the dormant cells to escape from immune-mediated dormancy. Recent advances have demonstrated that many, if not all, tumors display immunogenic properties which activate anti-tumor immune surveillance mechanisms. In order to grow at a primary or secondary site, the tumor cells must escape immune regulation, either by acquiring immune-suppressive properties themselves, or by recruiting stromal cells that generate an immune-suppressive microenvironment. My preliminary studies have demonstrated that wounding a mouse can inhibit the T cell-dependent rejection of distant immunogenic tumors. This phenomenon appears to involve the systemic mobilization of monocytes, cells that are commonly recruited to sites of inflammation and tissue damage where, among many functions, they can restore homeostasis by restraining T cell responses. In this proposal, I aim to understand the systemic mechanism by which wounding can inhibit the anti-tumor immune response. I will first analyze the impact of wounding on the activity of T cells in the microenvironment of distant immunogenic tumors. I then aim to determine the specific role of mobilized monocytes in inhibiting tumor-specific T cells at distant sites. In the future, I will extend the study to investigate the systemic impact of wounding in established models of metastasis, in which micrometastases are restrained by tumor-specific T cells. Ultimately, I hope to uncover systemic mechanisms of immune suppression that can be targeted in cancer patients shortly after tumor resection in order to mitigate the adverse effects of wounding on dormant metastases.

Dengke Ma, Ph.D.

Massachusetts Institute of Technology

Mentor: H. Robert Horvitz, Ph.D.

“Molecular and Neural Mechanisms of a Hypoxia-Inducible-Factor (HIF) Regulated C. Elegans Behavior”

Oxygen deprivation followed by reoxygenation causes pathologic responses in numerous medical conditions, including ischemic reperfusion injury and myocardial infarction. The discovery of the *C. elegans* gene *egl-9*, which encodes an O₂-sensing hydroxylase that modifies and destabilizes the hypoxia-inducible-factor-1 transcription factor, has led to the identification of an evolutionarily conserved pathway central for O₂ homeostasis in organisms ranging from nematodes to humans. The inhibition of mammalian homologs of EGL-9 strongly protects from myocardial ischemia and reperfusion injury. EGL-9 and HIF-1 are required for a *C. elegans* behavioral response to reoxygenation (the O₂-ON response) that is analogous to mammalian stroke-reperfusion injury responses. The molecular and neural mechanisms of the O₂-ON response are unknown. Key factors that act together with EGL-9 and HIF-1 in controlling the O₂-ON response are likely to define conserved mediators and modulators of hypoxia/reperfusion injury in humans.

From a series of genetic screens, I have recently discovered new components of the EGL-9/HIF-1 pathway and also isolated *C. elegans* mutants that define additional regulators of the O₂-ON response, some of which likely act downstream of HIF-1. The overall goal of this project is to analyze the genes defined by these mutants and determine the molecular and neural mechanisms of the O₂-ON response. In Aim 1, I will analyze genes that function as effectors of the EGL-9/HIF-1 pathway in regulating the O₂-ON response. In Aim 2, I will determine how EGL-25, a novel *C. elegans* homolog of progesterin and AdipoQ receptors that I have found to be required for the O₂-ON response, controls this response and interacts with the EGL-9/HIF-1 pathway. In Aim 3, I will identify the key sensory neurons and circuit responsible for the O₂-ON response and investigate how EGL-9 and EGL-25 control the O₂-ON response by modulating the sensory neural circuit function.

Using combined molecular, cellular and behavioral analyses together with powerful genetic screens, I hope to discover novel regulators and effectors of the EGL-9/HIF-1 pathway, establish mechanisms by which EGL-9 and EGL-25 control the O₂-ON response, and identify potential new therapeutic targets for the treatment of human ischemic disorders, including stroke-reperfusion injury and myocardial infarction.

Ana Maldonado, Ph.D.

University of Massachusetts Medical School

Mentor: Beth McCormick, Ph.D.

“A novel platform for a vaccine against gastrointestinal bacterial diseases”

This proposal addresses the problem of developing a suitable vaccine for diarrheal illness. Every year there are about two billion cases of diarrheal disease and millions of deaths. Ninety-nine percent of these cases occur in developing countries, where diarrhea is the second-leading cause of death in children. Although bacterial-induced diarrhea caused by Salmonella result in more than 2 millions of deaths annually, there is not an effective vaccine against this pathogen. Efforts to develop vaccines for Salmonella have been hampered by: i) the difficulty in creating live vaccine strains that colonize efficiently without triggering overt disease, and ii) the inability of mutant or killed vaccine strains to generate a fully neutralizing mucosal immune response.

Thus, we propose to overcome the limitations of prior vaccine strategies by implementing a novel approach. The platform for this strategy is based on our discovery that the pathogenesis of Salmonella depends on conserved caspase-3 motifs found within key virulence proteins. Mutation of the caspase-3 cleavage motif in these virulence proteins dramatically reduces the ability of the organism to cause disease, while conserving its ability to colonize the intestine. We posit that mutation of caspase-3 motifs in virulence proteins of Salmonella is a compelling strategy to create new attenuated vaccine strains that can colonize the host without causing disease, while generating a protective immune response. We will test this hypothesis by:

- 1) Determining the ability of attenuated Salmonella strains to provide immunological protection in a murine model of salmonellosis.
- 2) Comparing the immune response generated in mice as well as that observed in naturally infected human patients, to validate the potential of our vaccine to translate to human applications.

We expect these studies will provide the framework for a novel vaccine formulation that could have widespread utility in developing countries with a high impact on global health.

Francesco Marangoni, Ph.D.

Massachusetts General Hospital

Mentor: Thorsten Mempel, MD PhD

“Intravital Analysis of NFAT Signaling Dynamics In Tumor-Associated T Regulatory Cells And Its Role In Promoting Local Immunological Tolerance To Cancer”

The immune system can mount an effective response against cancer, but its efficacy is severely hampered by immunoregulatory mechanisms acting in the tumor-draining lymph nodes and within the tumor tissue. T regulatory cells are key to establish immune tolerance to tumor, and their suppressive activities are likely regulated in similar ways to the effector activities of conventional T cells. Therefore, therapeutically targeting T regulatory cells to specifically disable their functions may provide a novel therapeutic strategy to maximize the anticancer effect of the immune response.

T cell receptor triggering and the relocation of the transcription factor NFAT from the cytoplasm to the nucleus are key for the acquisition of suppressive function by T regulatory cells (Tang et al., J Exp Med 2004; Wu et al., Cell 2006). However, the anatomic location, the causative cellular interactions, and kinetics of NFAT activation in T regulatory cells have never been investigated.

Here we hypothesize that NFAT activation in T regulatory cells drives their expansion within tumor-draining lymph nodes and migration to as well as accumulation in the tumor tissue, which crucially contributes to the establishment of local immune tolerance. We propose to test this hypothesis through a novel technology that allows us to visualize and quantify the dynamics of NFAT signaling in T cells in live anesthetized mice through multiphoton intravital microscopy (Marangoni et al., Immunity, in press). In addition, genetic gain- and loss-of-function experiments will help us to identify the precise functional consequences of NFAT activation in T regulatory cells. Specific modulation of the NFAT signaling pathway in T regulatory cells, also explored in the proposal, may provide a therapeutic platform to overcome immunological tolerance to cancer.

Nalu Navarro Alvarez, M.D., Ph.D.

Massachusetts General Hospital

Mentor: James Markmann MD, PhD

“Innate Immune system over activation impairs Liver regeneration”

Nearly 20% of patients on the wait list for liver transplantation die before a donor liver becomes available. Although the unique ability of the liver to regenerate makes living and split liver donation possible, the risk of small for size syndrome (SFSS), or death due to insufficient mass and failure of the remaining liver to regenerate, has limited their applicability. Successful liver regeneration is the summation of interacting pathways, and a key player is the innate immune system. We believe that modulation of innate immunity will promote liver regeneration, allowing smaller segment transplantation and effectively expanding the donor pool. The long-term goal of my research is to understand how activation of the innate immune system by liver injury, decreased hepatocyte mass, and bridge therapies impact liver regeneration.

In Aim 1, I will employ a non-human primate model of SFSS by 90% hepatectomy established in our lab, to dissect the role of the different innate immune cell populations in liver regeneration. I will test the hypothesis that inhibiting over activation of the innate immune system will limit hepatocyte injury and promote regeneration, and I will compare immune cell activation and liver regeneration in baboons that have undergone 90% hepatectomy with and without immunosuppression. Innate immune activation will be analyzed by flow cytometry, histology, serum cytokine levels and liver damage and regeneration measured by standard clinical laboratory parameters, histology, Ki67 and TUNEL staining, liver weight measurements, and immunofluorescence for cell cycle proteins.

We have also developed an auxiliary liver xenotransplantation model as temporary support for SFSS. In Aim 2, I will utilize this system to examine the possibility that additional activation of the innate immune system by the introduction of xenotissue impairs native liver regeneration, and I will explore how this is balanced by the presence of supplemental hepatocyte function. We will compare both native and xenoliver injury and recovery in animals that undergo 90% hepatectomy only, xenotransplant only, and 90% hepatectomy plus xenotransplant.

The proposed aims represent first steps in a research program geared to revealing how and why the over activation of the innate immune system affects liver regeneration.

Timothy O'Leary, Ph.D.

Brandeis University

Mentor: Eve Marder, Ph.D.

“Degeneracy in Ion Channel Function and its Relation to Nervous System Robustness and Flexibility”

Nervous systems are inherently variable: the components that make up neurons and neural circuits vary between organisms of the same species and vary within the same organism over time. How can reliable nervous system function -- which is essential for life -- emerge from such variability? One important and reliable feature of nervous system function is that it can be modulated to generate different patterns of electrical activity that control behaviors such as sleep, feeding, attention and motor activity. Modulation is mediated by endogenous substances such as peptides and monoamines that alter the signaling properties of subsets of ion channels which determine the electrical properties of neurons. However, experiments have shown that ion channel expression in single neurons is highly variable and continually turning over to maintain some kind of 'target' activity. In addition, ion channel types often overlap in their functional properties within the same cell -- a phenomenon known as degeneracy. It is therefore something of a mystery that neuromodulators are capable of inducing reliable and physiologically important changes in the activity of neurons and networks in spite of ongoing 'homeostatic' tuning and the highly variable nature of ion channel expression.

This project will use computational methods to understand how reliable neuromodulation can be achieved in model neurons and networks that have biologically realistic properties. The models will be based on data from a well characterized model nervous system in which neuromodulation is known to be functionally important, the crustacean stomatogastric ganglion. A novel aspect will be a systematic investigation of degeneracy by constructing families of models that have membrane conductances that functionally overlap in a controlled way that mirrors experimentally-measured properties of ion channel subtypes. I will test the hypothesis that such degeneracy in fact facilitates reliable neuromodulation in self-regulating circuits. The overall goal is to understand if consistent neuromodulation is compatible with homeostatic regulation, or whether this requires novel mechanisms that are yet to be identified experimentally. This has important general consequences for understanding phenotypic variability and robustness in nervous systems.

Sharvan Sehrawat, Ph.D.

Whitehead Institute for Biomedical Research

Mentor: Hidde L. Ploegh, Ph.D.

“Function and differentiation of transnuclear CD8 T cells”

Herpesviruses and infectious agents such as *Toxoplasma gondii* cause significant morbidity in immunocompromised individuals, as is the case for those with HIV/AIDS. These pathogens cause persistent infections, the control of which requires T cells of appropriate specificity. A demonstration that antigen-specific CD8 T cells contribute to protection requires that these cells can be isolated in pure form and used in adoptive transfer experiments. In normal animals, the frequency of cells specific for a given antigen is very low and therefore a source of CD8 T cells of defined specificity, obtained from mice with a homogeneous T cell compartment, is required to study their contribution to protective immunity. Physiological models to explore the immune response to the antigen of *T. gondii* and ORF8 and ORF61 of the mouse gamma herpesvirus MHV68 (MHV68) were created by somatic cell nuclear transfer. These mice serve as a source of CD8 T cells of defined specificity, without relying on gene targeting or transgenesis. The functional properties and differentiation of CD8 TN T cells during persistent infections will be investigated using deep sequencing and other immunological methods. Follow up studies on some of the interesting hits obtained from RNA seq experiments will be performed to identify signatures of activated and memory cells. Additionally, how antigen specific CD8 T cell responses are affected by concurrent persistent infections will be investigated. The specific aims include:

1. Systematic analyses of the transcriptomes (RNAseq) of differentiating activated and memory CD8 T cells in order to identify their key molecular signatures, with definition of CD8 T cell subsets as a possible added dividend.
2. To assess the role of galectin-3 in differentiation of virus-specific CD8 T cells and in the outcome of gamma herpes viral infection.
3. To assess the influence of concurrent infection with *T. gondii* on the responsiveness of MHV-ORF8-specific CD8 T cells.

Viknesh Sivanathan, Ph.D.

Harvard Medical School

Mentor: Ann Hochschild, Ph.D

“A bacteria-based system for studying and uncovering amyloidogenic proteins”

The misfolding and aggregation of various proteins into highly ordered fibers termed amyloid is associated with a wide range of devastating human diseases that includes Alzheimer's, Parkinson's, Huntington's and the inevitably fatal prion diseases. Despite intensive scientific attention, many fundamental questions about the processes that trigger amyloid aggregation remain to be answered. With no effective therapies available to alter the course of amyloid-associated diseases, new experimental avenues are crucial. The objective of the proposed research is to mobilize bacterial genetics as a new experimental system to probe the behavior of amyloid proteins in a simplified cellular setting.

The proposed experimental system is based on our demonstration that a recombinant amyloidogenic protein can be targeted to the bacterial cell surface by exploiting a dedicated export pathway normally used for the export of two *E. coli* amyloidogenic proteins. Upon export to the cell surface, aggregation of the recombinant protein into the amyloid form can be monitored using a dye, Congo red (CR), which binds preferentially to amyloid aggregates. Therefore, *E. coli* cells grown on agar medium supplemented with CR form red colonies if amyloid fibers are being formed at the cell surface. By using colony color as a readout for the aggregation state of the exported protein, we propose to screen for small molecules

that can disrupt the formation of amyloid aggregates as means to explore new avenues for the development of therapeutics for amyloid-associated diseases. Additionally, based on the efficiency with which the export system facilitates conversion of amyloidogenic proteins from bacteria, yeast and human into the amyloid form, it will be developed as a simplified method for generating disease-related amyloid aggregates, which will serve as an essential tool for the study of amyloid aggregates. Finally, the colony-color phenotype will also be used to screen for novel amyloid-forming proteins, the identification and characterization of which will provide a better understanding of mechanisms for controlling amyloid aggregation.

Fei Wang, Ph.D.

Harvard University

Mentor: Vladimir Denic, Ph.D.

“The Mechanism of Tail-Anchored Membrane Protein Insertion into the Endoplasmic Reticulum”

Tail-anchored (TA) proteins are membrane proteins anchored on the cytosolic surface of cellular membranes by a single transmembrane domain at the C-terminus. This class of proteins includes SNAREs, which mediate vesicular transport, and members of the Bcl2 family, which mediate apoptosis. The majority of TA proteins are post-translationally targeted for insertion into the endoplasmic reticulum (ER) by a mechanism that has historically been difficult to pin down. In the past three years, I have made several prominent discoveries about this mechanism, which is called the GET pathway and is conserved from yeast to man. At the moment, the key unanswered question in the field is how the Get1/2 transmembrane complex mediates TA protein insertion. I have obtained preliminary evidence that the transmembrane regions of Get1/2 facilitate tail anchor entry into the lipid bilayer. Moreover, I have developed a complementing in vivo and in vitro yeast system for carrying out a structure-function analysis of Get1/2, which includes an engineered TA protein that arrests at the integration step of the GET pathway. Membrane protein biogenesis is still a poorly understood area of cell biology. In particular, the precise mechanism of membrane protein insertion by other pathways has been difficult to dissect because of the biochemically complex nature of the substrates and machineries involved. In published work, I have demonstrated that the biochemical requirements for TA protein insertion by the GET pathway are relatively simple. Thus, I am in an ideal position now to exploit this system to reveal new fundamental insights into how cells chaperone membrane proteins. While this research is very basic it also has direct relevance to human cancer chemotherapy. Specifically, the GET pathway is a major determinant of cisplatin resistance in mammalian cells. Moreover, GET pathway knockdown confers cisplatin sensitivity leading to enhanced cisplatin-induced cell death of many cancer types. Thus, my work will also improve our understanding of cancer chemoresistance and enable better pharmacological approaches for treating many human cancers.

Katja Weinacht, M.D., Ph.D.

Dana-Farber Cancer Institute

Mentor: Luigi Notarangelo, M.D.

“Reticular Dysgenesis: How a rare disease reveals new insights into myelopoiesis”

Reticular dysgenesis (RD) is one of the most profound forms of combined immunodeficiency characterized by severe congenital neutropenia (SCN) and impaired lymphocyte development. The origin of the neutropenia consists in a maturation arrest at the promyelocyte stage. Recently, the disease

has been linked to mutations in the gene encoding for Adenylate Kinase 2 (AK2). AK2 is a phosphokinase implicated in mitochondrial energy homeostasis. However, it remains unclear how defective expression and function of an ubiquitously expressed enzyme involved in energy metabolism would selectively manifest in defective myelopoiesis and lymphopoiesis. The mysterious pathogenesis of this rare disease makes it evident that fundamental principles of neutrophil development remain poorly understood. A significant obstacle to the study of rare diseases, like Reticular Dysgenesis, has been the inadequacy of animal models and limited availability of patient specimens.

The 2012 Nobel Prize in Medicine was awarded to Sir John Gurdon and Shinya Yamanaka for their discovery that somatic cells can be reprogrammed to induced pluripotent stem cells (iPSCs). The advent of reprogramming technology has presented us with the thus far unimaginable possibility to model human disease in a tissue culture dish.

We propose that induced pluripotent stem cells from various patients with different AK2 mutations can serve as a disease model for RD when differentiated in vitro along the myeloid lineage (aim 1). We further propose that the analysis of these cells for transcriptome, protein expression and nucleotide metabolite profile will enable us to understand how AK2 defects lead to a maturation arrest at the promyelocyte stage (aim 2A). We predict that this will allow us not only to solve the mystery of a devastating disease, but also yield new insights into the central pathways driving physiologic myelopoiesis (aim 2B).

As a proof of concept, we will gene-correct the disease specific iPSCs with the intent to demonstrate that gene repair restores normal neutrophil maturation in RD (aim 3). Such iPSC-derived, gene-corrected in-vitro differentiated cells may pave the way to future developments of "patient specific cellular therapy."

Bin Wu, Ph.D.

Boston Children's Hospital

Mentor: Sun Hur, Ph.D.

"Uncovering Mechanisms of MAVS Activation"

Pattern Recognition Receptors in the innate immune system serve as the first line of defense against pathogen infection by recognizing conserved molecular features commonly associated with pathogens. One important family of such receptors are viral RNA receptors, RIG-I and MDA5, which cooperate with their common adaptor, MAVS, to activate antiviral or inflammatory immune response. The interaction between RIG-I/MDA5 and MAVS represents a committed step in initiation of the innate immune response and is often subject to multiple layers of regulation from both the host and invading virus. Despite the importance, the molecular mechanism by which RIG-I and MDA5 interact with MAVS and link the upstream viral-detection events to downstream signaling events is yet unclear. This is partly due to challenges of analyzing protein aggregation or oligomerization, which occurs during signal activation, and seemingly transient interactions between RIG-I/MDA5 and MAVS. I here propose to investigate the assembly process and architecture of the oligomers of the signaling domains of MDA5 and MAVS, and their complex using an innovative "hybrid" approach that systematically integrates cellular assays, structural and biochemical analysis and computational modeling. I will first define the structural features of the signaling domain (CARD) of MAVS filaments using a combination of protein:protein crosslinking and surface mutagenesis (Aim 1A) and design constructs for crystallization for more detailed structural

analysis (Aim 1B). Using similar approaches, I will further define the oligomeric architecture of the signaling domain (2CARD) of MDA5 (Aim 2) and the complex of MDA5 2CARD:MAVS CARD (Aim 3). This project stems from my previous studies on the structure and biochemical mechanisms of how MDA5 recognizes viral RNAs (Wu et al, Cell, in press), but represents a further evolution into the downstream event involving the MDA5:MAVS interaction. I believe that this study will provide a solid biochemical foundation to obtain integrated understanding of the tripartite system of the receptor, ligand and adaptor and would offer novel insights into therapeutic strategies for treatment of viral infection, cancer or immune disorders.

2012 Grant Recipients

Madelyn Baez-Santiago, Ph.D.

Brandeis University

"Dynamic and Tonic Coding of Taste from the Brainstem to the Forebrain"

Brainstem taste regions take almost direct input from the mouth, and control some basic taste-driven behaviors. Thus it is reasonable to hypothesize that brainstem (specifically, the parabrachial nucleus of the pons, PbN) neurons might respond in a simple manner to activation of taste cells on the tongue--that is, they might respond to only one taste or another. However, the majority of input to PbN comes from forebrain regions such as gustatory cortex (GC), which processes tastes with complex 'temporal codes' signaling first the presence, then identity, and finally the desirability of tastes. Therefore it is also reasonable to hypothesize that PbN neurons may reflect this feedback, in the form of similarly temporally complex responses. Discerning whether PbN taste responses reflect simple ascending information, complex descending information, or some combination of the two--requires recording multiple neurons simultaneously in PbN and GC from conscious rats, bringing an extensive range of analyses to bear on these data, and directly assessing what happens when the connection between the brainstem and forebrain is disrupted. I will perform these experiments. First, I will record taste responses from PbN in conscious rats, evaluating the information available within the time courses of these responses (preliminary data demonstrates that these responses are temporally complex, even more so than those in GC). I will simultaneously record the responses of GC ensembles, enabling the first analysis of real-time interactions between forebrain and brainstem (using techniques already tested in our lab); I predict that these analyses will reveal coupled, information-rich temporal structure in the two regions. Next, I will use optogenetics and/or pharmacology to inactivate cortical feedback while recording from PbN, to directly test how GC influences PbN taste responses. These experiments will clarify how neural processing of tastes occurs in real time. Understanding how quality, concentration and hedonic values of taste are processed in the brain may also shed light on the neural underpinnings of eating disorders such as anorexia, bulimia, pathologies resulting from treatments to cancer, and aversions and craving that appear during pregnancy.

Matthew Banghart, Ph.D.

Harvard Medical School

"Compartment-Specific Signaling by Striatal Opioid Peptides"

Opioid neuropeptides are abundant in the mammalian striatum, a brain region important for motor function, yet a role for endogenous opioids in locomotion has not been established. Striatal opioid peptides and their receptors are spatially distributed according to the boundaries of distinct microcircuits that receive inputs from either limbic or sensorimotor cortical areas, but the functional significance of this compartmentalization is not understood. The main objective of this study is to understand how endogenous opioids alter circuit function in the striatum and how they affect locomotive behavior. I propose that opioid peptides signal within and between these compartments and that these messages are important for incentive-based motor learning and recall. To test these possibilities, I will first anatomically define and functionally characterize the synaptic inputs from different areas of the cortex to each striatal compartment. This will be achieved using viral tracing methods and electrophysiological recordings with optogenetic photostimulation in acute brain slices. Next, the cellular and synaptic actions of opioid peptides will be determined in the context of compartmental boundaries using electrophysiological and optical recordings. These efforts will be facilitated by photochemical tools that I previously developed for releasing opioid peptides in brain tissue with high spatiotemporal precision. A major goal of these experiments is to understand how the integrative actions of opioid peptides across striatal network components modulate net output from the circuit and how the limbic and sensorimotor microcircuits are differentially affected. To put these cellular phenomena into context, the involvement of opioid peptides in locomotive behaviors will be explored. I will determine if activity patterns observed in vivo are capable of driving peptide release in brain slices using optogenetic photostimulation. Finally, I will interfere with endogenous striatal opioid signaling during motor learning assays and attempt to pinpoint the location and timing of the pertinent activity. Collectively, these studies should uncover a novel, unified mechanism for opioidergic modulation of a neural circuit that is essential for motor function.

Hannah Blitzblau, Ph.D.

Whitehead Institute for Biomedical Research

"Telomere-Directed DNA Fragmentation and Genome Stability in Meiosis"

Sexually reproducing organisms make gametes using meiosis, a cell division specialized to reduce ploidy by separating each set of homologous chromosomes. In most organisms, faithful homolog segregation relies on a physical connection established by controlled DNA breakage and exchange of chromosomal segments called crossover (CO) repair. Errors in this process can cause genome abnormalities such as chromosome loss, gain or rearrangement, leading to numerous human disorders. Meiotic cells are faced with a conundrum; they must introduce enough DNA double-strand breaks (DSBs) into the genome to ensure that each chromosome pair makes at least one CO, yet limit the potential damage to the genetic blueprint of their offspring.

To understand how this problem is solved, we previously devised a technique to measure meiotic DSBs across the yeast genome. We observed an intriguing non-random distribution of DSBs; they occurred far more frequently than expected in large ~100-kb zones close to the ends of chromosomes, yet were depleted directly adjacent to chromosome ends and repetitive DNA. I hypothesize that the telomere directed DSB targeting (TDDT) increases DSB levels close to chromosome ends to ensure that all chromosomes receive the CO necessary to segregate during meiosis, but this also places the cells at risk, as COs too close to chromosome ends often fail to hold homologs together. Moreover, DSBs in the telomere repeat-sequences leave cells at risk for non-allelic homologous recombination and genome rearrangement, possibly explaining the silencing of DSB formation immediately adjacent to telomeres. I

have identified mutants that affect TDDT, and will use them to understand the function and regulatory mechanisms of TDDT. Specifically, I will:

1. Measure the contribution of TDDT to CO levels and chromosome segregation.
2. Define the attributes of chromosome ends responsible for TDDT and DSB repression.
3. Elucidate the mechanism of TDDT by mapping DSB factor binding, nuclear position and chromosome configuration in mutants that eliminate TDDT.

Marc Gershow, Ph.D.

Harvard University

"Olfactory Processing in the Drosophila Larva"

In no other organ is the relation between structure and function understood as poorly as it is in the brain. Although our understanding of individual neurons is quite sophisticated, we are just beginning to understand how interconnected groups of neurons work together to process information. The complexity and relative inaccessibility of the mammalian brain complicates our ability to study neural circuits in higher animals. The *Drosophila* larva, on the other hand, has few neurons but performs complex behaviors. Further, thanks to recent advances in genetics, protein engineering, and microscopy, and the larva's transparent cuticle, we can optically activate, suppress, and visualize the activity of the neurons of a freely behaving animal. These properties make the larva an ideal model system in which to link structure and function of complete neural circuits.

I will study the olfactory circuitry of the *Drosophila* larva. How do larvae recognize odors, determine if they are attractive or repulsive, and then react appropriately? In order to move towards or away from an odor source, the larva may move forward, backward, or stop, sweep its head to one side or the other, move straight or curve. The rules by which the larva changes its motor output in response to sensory input in order to achieve a goal represent a navigational strategy. I will study the navigational strategies of larvae responding to a variety of odors to reveal details about the organization of the olfactory circuit and the relation of receptor activity to motor behavior.

I will take advantage of the genetic tools available in *Drosophila* and the larva's transparency to image the responses of odorant receptor neurons in the larva's dorsal organ (its 'nose') as a first step to understanding how odors are represented in the brain, and I will link the patterns of neural activity and the behaviors evoked by odor presentation. Finally, to further elucidate the rules linking sensory input to motor output, I will use light activated ion channels to directly manipulate receptor neuron activity to drive navigational responses.

Alon Goren, Ph.D.

Broad Institute of MIT and Harvard

"Chromatin Regulatory Circuits in Mammalian Pre-implantation Development"

During early mammalian development, embryonic cells go through major changes as they transform from the zygote to the inner cell mass (ICM). Decoding the epigenetic regulatory systems orchestrating pre-implantation would shed light on several key biological pathways, both in normal setting and disease. Furthermore, these pluripotent cells serve as a model system particularly suitable for epigenetic studies, due to the important role these mechanisms play in development.

Despite the importance of pre-implantation development, the concerted spatio-temporal programs controlling this process, and more specifically the regulation of chromatin organization, are poorly understood. For example, the exact involvement of Chromatin Regulators (CRs) in setting or maintaining pluripotency, or the role of histone modifications play during early embryogenesis, is poorly understood.

Here, I propose to use genomic strategy to study chromatin regulation during early embryogenesis, combining in-vivo ICMs and in-vitro mouse embryonic stem cells (mESC) model systems. I will initiate this study by measurements of detailed molecular profiles of ICMs and mESCs. These data include genome-wide profiles of key histone modifications in ICMs. Together with public expression maps of mouse pre-implantation samples, these data will allow me to evaluate and focus on the aspects of the in vivo biological system that are recapitulated by the mESCs model. To address the challenges posed by the small number of cells in early embryos, I will use emerging technologies I developed. Finally, I will use a novel approach we established in human cells to generate maps of CR binding in mESCs. I will integrate these data into a model of chromatin regulation and CR combinatorial features during early development. This model will provide testable hypotheses for future perturbation studies; e.g. by shRNAs in mESCs.

The goal of my proposal is to identify and characterize chromatin regulatory mechanisms in early development. This study will promote understanding of developmental pathologies and cases of aberrant chromatin structures, such as those that are found in cancer. Additionally, my studies can allow better understanding of the principals governing pre-implantation embryogenesis; hence, have clinical relevance for reproductive medicine with potential to ultimately impact in vitro fertilization as well as early diagnostics or screening.

Kirk Jensen, Ph.D.

Massachusetts Institute of Technology

"How a Wide Spread Mammalian Parasite Dampens Intestinal Inflammation Through the Injection of a Potent Anti-inflammatory Effector Kinase"

Toxoplasma has spread throughout the mammalian kingdom to the extent that a third of the human population are chronic carriers. Reactivation of latent infection in immune compromised individuals, such as HIV AIDS patients, or acute congenital infection in the developing fetus, can lead to inflammatory related pathologies and even death. In mouse models of toxoplasmosis, the small intestine becomes severely inflamed following natural oral infection and bears striking resemblance to the human inflammatory bowel disease, Crohn's. Since Toxoplasma infection can spread through consumption of encysted tissue from chronically infected animals, it is hypothesized that Toxoplasma has developed ways to manipulate intestinal pathology to promote host survival. We have recently identified a polymorphic Toxoplasma kinase, ROP16 that is injected into the host cell and directly activates the STAT3 and STAT6 transcription factors. Once activated, these transcription factors induce the expression of a battery of genes controlling immune function and inflammatory responses, often in an anti-inflammatory manner. Interestingly, we found that when a pathogenic strain of Toxoplasma expresses versions of the ROP16 kinase that activate the STAT3/6 transcription factors, intestinal pathology is suppressed and host survival was enhanced. However, the mechanism by which ROP16 mediates these effects is not known.

In order to identify host factors that are activated by ROP16 and dampen intestinal inflammation, in AIM 1 we propose to use a recently described technique that allows *Toxoplasma* to inject the Cre recombinase into infected cells. Using this parasite strain to infect mice that contain loxP sites flanking candidate genes, we will induce the specific deletion of ROP16 target genes in infected cells during a natural oral infection. In AIM 2, we seek to understand the specific cell types manipulated by *Toxoplasma* in the Peyer's patch and how ROP16 works in these cells to achieve protective immunity early in infection. Thus, this proposal will identify specific host genes and cell types that mediate protection against *Toxoplasma*, and broaden our understanding of host pathways that dampen inflammatory responses in the small intestine.

Seokhee Kim, Ph.D.

Massachusetts Institute of Technology

"Regulation of Antagonistic Protease and Chaperone Activities in an Enzyme Required for Virulence"

Cells in all organisms have evolved quality control systems of proteases and chaperones to deal with the potentially toxic effects of protein misfolding. Degradation by proteases and refolding by chaperones are usually performed by different protein machines, which frequently are ATP dependent. However, DegP, a member of the highly conserved HtrA family, functions both as an ATP-independent protease and as a chaperone in the bacterial periplasm. Goal of my research is to understand the molecular mechanisms that allow a single protein to perform these apparently antagonistic functions in a regulated fashion.

Intriguingly, misfolded protein substrates trigger a dramatic structural transformation in which proteolytically inactive DegP hexamers assemble into active cage-like structures with 12, 18, 24, or 30 subunits. To probe molecular mechanism, I have developed biochemical assays that monitor DegP activation, cage assembly, substrate binding, and substrate cleavage. Studies using these assays, mutant enzymes and crystallography implicate an equilibrium between inactive and active conformations of DegP subunits as well as substrate-stabilized assembly into cages as potential regulatory mechanisms. I have also identified a DegP mutant, whose intracellular expression results in unregulated proteolysis and a dominant lethal phenotype.

Here, I propose to test whether cage formation and the control of conformational equilibrium are the major regulatory mechanisms that regulate DegP proteolysis and/or chaperone function or if additional mechanisms exist, including ones mediated by interactions with specific substrates. To address these questions, I will construct DegP variants with combinations of mutations that affect specific functions and assay their activities in vitro and in vivo. I will also screen or select for novel mutations that increase or suppress cytotoxicity in cells and that alter chaperone function. By studying the biochemical activities of these variants, their structures, and their interactions with model substrates, I will formulate and test molecular mechanisms of DegP function and regulation. I anticipate that the information and understanding that emerge from these studies will provide paradigms for the HtrA enzyme family, will aid in the development of antibiotics that target DegP, and will give mechanistic insights into homologs that are implicated in human disease.

Dudley Lamming, Ph.D.

Whitehead Institute for Biomedical Research

"Mammalian Target of Rapamycin Complex 2 (mTORC2) Signaling in the Control of Glucose Homeostasis and Aging"

The mechanistic target of rapamycin (mTOR) is a widely conserved protein kinase that plays a central role in linking nutrient signaling to cell growth and proliferation. mTOR is a member of two complexes, mTORC1 and mTORC2, each of which regulates distinct cellular targets. Recent studies have demonstrated that treatment with rapamycin, originally thought to be a specific inhibitor of mTORC1, can extend the lifespan of yeast, flies, and mice, but the mechanism that underlies this extension is still unknown. Recently, the Sabatini laboratory discovered that chronic treatment with rapamycin disrupts mTORC2, suggesting a possible role for this complex in the lifespan extension brought on by rapamycin treatment. In this proposal, I outline studies to identify novel substrates of mTORC2, examine the consequences of mTORC2 disruption on longevity, and determine the role played by mTORC2 in the response to calorie restriction.

Recent studies have begun to explore the crucial role played by mTORC2 in regulating organismal physiology, yet only a few direct substrates of mTORC2 have been characterized. In an effort to find physiologically relevant mTORC2 substrates, I will use a proteomics-based approach to characterize the mTORC2-regulated hepatic phosphoproteome. Further, I will examine how the regulation of these substrates by mTORC2 signaling changes with age.

To determine the effects of mTORC2 disruption on health, glucose homeostasis, and lifespan, I will use three genetic mouse models in which Rictor (an essential component of mTORC2) is depleted. I will use mice heterozygous for a Rictor null mutation, mice which specifically lack hepatic Rictor, and mice in which Rictor is deleted from all tissues in adult mice using a tamoxifen-inducible system. I will also determine how mTORC2 and aging interact to regulate health and glucose homeostasis.

Calorie restriction (CR) is an intervention in which caloric intake is reduced while maintaining adequate nutrition, extends lifespan in many organisms, including mice and primates. While the mechanism by which CR promotes longevity is unclear, CR promotes glucose tolerance and insulin sensitivity. I will determine if mTORC2 is required for the positive effects of CR on glucose homeostasis.

Lukas Neukomm, Ph.D.

University of Massachusetts Medical School

"Genetic Pathways Mediating Axon Death"

Wallerian degeneration is a conserved phenomenon where, upon axon transection, the distal portion of the axon degenerates. This was thought to be due to the lack of trophic support provided by the cell body. However, recent work directly challenges this view: First, long-term axon survival of severed axons is provided by the neomorphic WldS molecule. Second, our lab has shown that loss-of-function mutation in *Drosophila* dSarm blocks the degeneration of severed axons for the life span of the fly. These

remarkable observations demonstrate that axon death is a genetically programmed mechanism, but the vast majority of axon death genes remain to be identified.

The long-term goal of this proposal is to define the molecular basis of axon death signaling. However, a lack of efficient screening methods to identify axon death genes has made this phenomenon refractory to incisive analyses. In preliminary work, I have established a powerful yet simple new genetic approach -- the *Drosophila* wing blade -- that will allow me to rapidly identify mutants that block the degeneration of severed axons. The efficiency of this approach relies on: the use of mosaic analysis for F1-based screening; a simple and highly reproducible axotomy; and the ability to score axonal integrity with single axon resolution *in vivo*. Using this approach, I have already discovered 5 new mutants; 3 suppress axonal degeneration, and 2 mutants exhibit normal axon degeneration but cellular debris persists.

In aim 1, I will use the wing blade to perform a genome wide saturation screen for mutants that suppress axon degeneration. In aim 2, I will identify and characterize the already isolated axon death mutants. These aims represent a bold attempt to genetically attack the question of how axons promote their own destruction.

My findings will substantially advance our knowledge of the biology of axon death. In addition, the results of my studies will improve our understanding of the nature of axon and synaptic loss -- a hallmark of every neurodegenerative disease and peripheral neuropathy. Importantly, each newly-identified axon death gene provides a novel potential drug target for intervention in neurodegenerative disease.

Buck Samuel, Ph.D.

Massachusetts General Hospital

"Molecular Examination of the Microbial Influences on Host Health"

During my postdoc in the Ruvkun Lab, I have developed the already genetically tractable, high-throughput amenable and microbially 'tuned' nematode *Caenorhaditis elegans* into a system for interrogating beneficial microbe-host interactions. Contrary to popular views, microbes are not simply as a source of food for *C. elegans*. In their natural habitats (rotting fruits and vegetation), I have demonstrated that wild *C. elegans* adults harbor microbial communities in their guts. To explore this community more comprehensively, collaborators and I have sampled several field seasons of habitats to define the composition of this gut microbiota and the habitats as a whole by using metagenomic sequencing of community rDNA. I have found that wild *C. elegans* strains are genetically adapted to respond to these microbes. Similarly, they are more fit (better prepared) to respond to stressful microbes isolated from their habitats compared to *E. coli*-adapted lab *C. elegans*. Finally, using a combination of sequencing, comparative genomics, and biochemical approaches, I have demonstrated that *C. elegans* tunes its metabolism to both evolutionarily conserved and novel microbial products, specifically microbial RNA. This simple and natural system is well suited to expedite the identification and further characterization of the host and microbial signals and pathways that affect host health.

In the final 2-3 years of my postdoc, I plan to publish 2 papers based on the preliminary data presented here. The first is a description of the microbial ecology of natural *C. elegans* habitats and how population growth is affected by community composition. The second paper provides the first comprehensive look of the microbial determinants of host health from a metabolic perspective. From the studies proposed herein, I expect a third paper focused on a genetic examination of the host

pathways that are responsive to microbes in *C. elegans* that will provide a solid foundation for the establishment of my own research group. Ultimately, I intend to center my research career on the interrogation of host microbial interaction pathways that affect host health using *C. elegans* and a variety of other systems.

Xu Tan, Ph.D.

Brigham and Women's Hospital

"Multiplex Screen for Interacting Compounds Targeting Hepatitis C Virus"

Human diseases often involve more than one mechanism and signaling pathway. Consequently multiple therapeutic interventions are often needed to treat a disease. Our goal for this proposal is to develop a method that allows systematical identification of effective compound combinations and apply this method to identify drugs targeting Hepatitis C virus, which causes over 10,000 deaths each year in the US and presents an urgent need for better therapies. To that end, we use a pooled screening strategy to overcome the problem of the large number of combinations, which increases exponentially with the number of compounds. We carefully selected 1000 FDA approved drugs or clinically tested compounds and constructed a library that consists of pools of 10 compounds that covering all the possible pair-wise combinations. We named it the MUSIC library (Multiplex Screen for Interacting Compounds). We validated this novel screening platform by discovering a number of compound pairs that act synergistically to inhibit HIV replication. The major focus of the proposal is to screen for anti-Hepatitis C virus (HCV) drug combinations. We aim to: 1) screen the MUSIC library on the fully infectious genotype 2a HCV strain JFH1 to identify drug combinations that inhibit HCV replication and validate the hits on the genotype 1 HCV replicons systems 2) mechanistically characterize the hits that show broad anti-HCV activity by RNAi knock-down of putative targets, gene expression profiling, SILAC mass spectrometry coupled with pull down assay etc. The Charles King Fellowship will allow me to gain experience with virology, especially HCV virology. With these training, in my own laboratory in the future, I will continue in the direction of applying high throughput technology to pathogenic diseases that cause serious human health problems.

Ekaterina Toropova, Ph.D.

Harvard University

"Structural Basis for the Regulation of the Molecular Motor Dynein by Lis1"

Cytoplasmic dynein uses the energy from ATP hydrolysis to step along microtubule tracks in eukaryotic cells. It is one of the most versatile molecular motors known, with critical roles in powering transport of diverse cargos, constructing the cell division machinery, and polarizing molecules during development. Remarkably, there is just one cytoplasmic dynein responsible for all of its diverse cellular tasks. It therefore relies on interactions with regulating proteins to achieve specificity of function but very little is known about these co-factors and how regulation is achieved. Recent data show that dynein's motile behavior is altered by Lis1, a ubiquitous dynein co-factor associated with a severe brain development disorder in humans (lissencephaly).

The objective of this project is to determine structurally and mechanistically, how dynein is regulated by Lis1. I will use a combination of cryo-electron microscopy, docking of known crystal structures and biochemistry to address the following specific questions: 1) how does Lis1 affect the structure of dynein

in solution and when bound to microtubules? Can Lis1 constrain dynein in a particular structural state? 2) Which structural elements of dynein does Lis1 engage? What does this tell us about how Lis1 affects the flow of structural changes in dynein to alter its function? 3) Does Lis1 undergo conformational changes to engage dynein? By providing answers to these questions, this work will begin to provide mechanistic insight into how dynein's motile behavior is regulated to meet its wealth of functions in living cells.

In the longer term these studies could provide a foundation for developing lissencephaly treatments, and aid development of synthetic applications for dynein, such as drug delivery systems.

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 V α 14;J α 18 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 J α 18^{-/-} 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 J α 18^{-/-} 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 Monopolin 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 monopolin 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^{1/2}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 proteins of the HR pathway. Using a fluorescence 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 Sapieha, 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”

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

Dana-Farber Cancer Institute

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

M. Golam Mohi, Ph.D.

Beth Israel Deaconess Medical Center

“Role of Shp2 and its Binding Protein Gab2 in Leukemogenesis”

Adrian Salic, Ph.D.

Harvard Medical School

“Novel Regulators of the Kinetochore-Microtubule Interaction”

Haihong Shen, Ph.D.

University of Massachusetts Medical School

“Alternative splicing mechanism of spinal muscular atrophy (SMA)”

Efstratios 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”