

## Smith Family Awards Program for Excellence in Biomedical Research

### 2010 Award Recipients

- **Sudha Biddinger, M.D., Ph.D.**

Assistant Professor of Pediatrics  
*Children's Hospital Boston*

“Regulation of the LDLR by Insulin and Diabetes”

Key Words: Diabetes, Insulin, Low-density-lipoprotein receptor, Dyslipidemia, Atherosclerosis

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The most important cause of death in diabetic patients is cardiovascular disease (CVD). However, the mechanisms by which diabetes causes CVD remain unclear. The low density lipoprotein receptor (LDLR) is a key regulator of serum cholesterol levels and CVD risk. Our preliminary data show that insulin regulates the LDLR in a diet-dependent manner that has not previously been described. In diabetic mice, LDLR receptor protein is normal on a chow diet, but decreased ten-fold on an atherogenic diet. Moreover, insulin appears to regulate the LDLR through both transcriptional and post-transcriptional mechanisms.

The overall goal of this proposal is to determine how insulin regulates the LDLR, and how this fails in the diabetic state. To do this, we will test, in vitro, the role of insulin in regulating LDLR transcription, mRNA stability, and protein turnover. In vivo, we will determine how diet and diabetes interact in the control of LDLR and its regulators, sterol regulatory element binding protein (SREBP)-2, which promotes LDLR transcription, as well as proprotein convertase subtilisin-like kexin type 9 (PCSK9) and inducible degrader of LDLR (Idol), which both promote LDLR degradation. In particular, we will determine the roles of SREBP-2, PCSK9 and Idol in mediating the effects of diabetes on LDLR protein on an atherogenic diet.

These studies will provide important insights into LDLR biology. Moreover, they will identify novel strategies and drug targets for increasing LDLR and lowering CVD risk in diabetic patients.

- **James Bradner, M.D.**

Instructor in Medicine

*Dana-Farber Cancer Institute*

“Chemical Inhibition of Bromodomains in Cancer”

Key Words: Epigenetics, Cancer, Bromodomain, Chemical biology

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Gene regulatory proteins are highly desirable targets for therapeutic development, yet perceptions regarding tractability have limited coordinated efforts in ligand discovery. Motivated by this challenge, we have assembled a collaborative, multi-disciplinary team of scientists to develop chemical probes modulating key effectors in transcriptional networks. In this proposal, we outline a chemical strategy selectively to target an emerging class of epigenetic proteins, called bromodomains.

Epigenetics classically defines the study of heritable phenotypes not genetically encoded in DNA. In cancer, epigenetic proteins are among the most promising and intently pursued targets in ligand discovery. Already, inhibitors of DNA methyltransferases and histone deacetylases have been established as impactful therapeutic agents, leading to regulatory approval by the FDA for use in hematologic malignancies. These events have prompted intense competition to develop inhibitors of chromatin-modifying enzymes, or so-called epigenetic "writers" and "erasers". Perhaps owing to perceptions regarding the feasibility of abrogating protein-protein interactions, small-molecule inhibitors of histone binding modules or epigenetic "readers" have not been described. Based on compelling, preliminary research emerging from our laboratory, we now propose to chemically optimize highly potent inhibitors of human bromodomains.

We present a chemical hypothesis regarding molecular recognition of twin bromodomain-containing proteins of the BET sub-family: BRD2, BRD3, BRD4 and BRDT. To test this hypothesis, we propose to prepare focused chemical libraries of small-molecule ligands based on the privileged scaffold of the thieno-1,4-diazepine. Target potency and selectivity will be determined using a novel, miniaturized biochemical assay platform capable of measuring acetylated histone tail binding by individual recombinant bromodomains. Finally, lead compounds will be studied for effects on cell proliferation, differentiation and maintenance of epigenetic memory in translational models of human carcinoma, seminoma and non-Hodgkin lymphoma.

- **Michael Higley, M.D., Ph.D.**

Assistant Professor of Neurobiology

*Yale University School of Medicine*

“Neuromodulation of Synaptic Transmission in the Prefrontal Cortex.”

Key Words: Dopamine, Norepinephrine, Glutamate, Calcium, 2-photon, Optogenetics, Spine

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Dysfunction of dopamine (DA) and norepinephrine (NE) signaling in the prefrontal cortex (PFC) is implicated in human neurological and psychiatric illnesses, including schizophrenia, attention deficit disorder, and major depression. Unfortunately, the cellular mechanisms of neuromodulatory action are poorly understood. Co-localization of DA or NE receptors with glutamatergic synapses on dendritic spines suggests that modulation may be extremely spatially precise, regulating individual synaptic contacts to selectively influence distinct populations of inputs. However, technical challenges have prevented direct testing of this hypothesis.

We propose to use a combination of electrophysiological and optical approaches to determine how neuromodulators, in particular DA and NE, regulate synaptic transmission in the PFC. We will combine electrophysiological recordings with 2-photon microscopy and calcium imaging to monitor synaptic activity in single dendritic spines. We will also use 2-photon glutamate uncaging and optogenetic manipulation to selectively stimulate targeted glutamatergic synapses and activate endogenous neuromodulatory signaling pathways. We will apply these tools to answer three fundamental questions: (1) Are the actions of DA and NE selective for specific synaptic inputs? (2) Are modulatory signaling pathways compartmentalized at individual synaptic inputs? (3) What are the functions of neuromodulators in regulating synapse-specific learning rules? To address these questions, we propose the following aims:

1. Identify the actions of DA and NE on glutamatergic hippocampal and intracortical synapses in the PFC.
2. Determine the spatial scale over which synapses can be modulated independently.
3. Determine the functional consequences of DA and NE modulation for synapse-specific long-term plasticity.

These experiments will test several hypotheses of synaptic regulation in the PFC and provide important context for understanding the molecular links between neuromodulator signaling and behavior. Our long-term objective is to elaborate the processes by which perturbation of neuromodulatory systems, and synaptic function in general, contribute to neuropsychiatric disease.

- **Steven McCarroll, Ph.D.**

Assistant Professor, Department of Genetics  
*Harvard Medical School*

“Finding the Human Genome's Missing Pieces”

Key Words: Human genome, Copy number variation, Structural variation, Deletion polymorphism, Complex disease genetics

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The goal of this project is to identify thousands of previously unknown human genome segments that are present in some but not all human genomes, and to relate such "missing pieces" of the human genome to variation in human phenotypes and risk of disease.

We will first systematically identify missing pieces of the human genome by analyzing in novel ways whole-genome sequence data derived from many humans and from select other species. We will molecularly characterize the genomic and population-genetic properties of these "missing pieces" by determining their allelic state in hundreds of human genomes. We will determine how these polymorphisms relate to well-characterized forms of genome variation. We will identify genetic markers for following these missing pieces of the human genome, then use these markers to evaluate how the presence or absence of the missing pieces relates to variation in hundreds of human phenotypes. We will also pursue experiments to evaluate the relationship of the genome's missing pieces to the histocompatibility barriers that complicate allogeneic transplantation in humans.

Our work will elucidate an emerging form of human genome variation and its relationship to variation in human phenotypes.

- **Matthew Vander Heiden, M.D., Ph.D.**

Assistant Professor

*Massachusetts Institute of Technology*

“The Impact of Pyruvate Kinase Activity on Cancer Cell Metabolism and Tumor Growth in Vivo”

Key Words: Cancer Metabolism, Pyruvate Kinase, PK-M2, Mouse Cancer Models, Cancer Therapy

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Cancer cells, unlike their normal counterparts, metabolize glucose by aerobic glycolysis. Although this phenomenon characterized by increased glycolysis with lactate production was described many years ago, how altered metabolism contributes to tumor growth remains controversial. Cancer cells require altered metabolism to efficiently incorporate nutrients such as glucose into biomass to support proliferation. Our understanding of how cancer cells meet these metabolic needs is based primarily on studies of cultured cells, and nutrient conditions in vitro differ significantly from those seen by tumor cells in vivo. Our long-term objective is to develop a comprehensive understanding on how cell metabolism is altered to accommodate anabolic synthesis and support the growth of tumors in vivo.

The M2 isoform of pyruvate kinase (PK-M2) promotes the metabolism of glucose by aerobic glycolysis, contributes to anabolic metabolism, and appears necessary for optimal tumor growth in vivo. Paradoxically, these effects of PK-M2 expression are associated with decreased pyruvate kinase enzyme activity. We aim to use genetically engineered mouse cancer models together with small molecules targeting PK-M2 to test the hypothesis that decreased pyruvate kinase activity promotes anabolic metabolism and cell proliferation in vivo. We have generated mice harboring conditional alleles for PK-M2 as well as the constitutively active PK-M1 to control the levels of pyruvate kinase activity in tissues. These mice will be crossed to mouse models of breast and lung cancer to determine the importance of decreased pyruvate kinase activity for tumor growth and metabolism in vivo. We have also developed small molecule activators of PK-M2 suitable for use in mice. These compounds will be used for experiments to understand the consequences of PK-M2 activation for tumor growth and metabolism. This work will advance our understanding of glycolytic regulation in cancer, and determine whether pyruvate kinase activation is a viable strategy to treat cancer.

- **Shobha Vasudevan, Ph.D.**

Assistant Professor, Assistant Geneticist  
*Massachusetts General Hospital*

“Regulation of Quiescence by MicroRNAs”

Key Words: MicroRNAs, Cancer Quiescence, Target Purification, MicroRNP, Hes1, G0

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The primary goal of this study is to characterize the roles of microRNAs, non-coding RNA gene expression regulators, in quiescent (G0) cancer cells and identify their target messages that maintain quiescence. Tumor populations selectively promote cells that adapt to unfavorable environments by entering quiescence, a reversible arrest state to escape permanent senescence or apoptosis, outcomes of tumor-negative environments. Quiescence regulators remain largely undiscovered despite the significance of G0 in cancers. Based on our data demonstrating the importance of microRNP (microribonucleoprotein) regulation in G0, we propose that quiescent cancer populations are maintained in part by altered microRNA expression and targets, permitting synthesis of quiescence specific factors, necessary for maintenance of G0. A related important focus is to investigate the interconnected regulation of microRNP expression and modifications in quiescence to reveal the intricate feedback control of these key quiescence regulators that results in perpetuation of the G0 state.

Aim I: To biochemically isolate functional microRNPs from quiescent cancer cells to identify G0-specific microRNAs, factors and associated targets.

Aim II: To validate the targets identified and investigate the requirement for microRNA-controlled expression to maintain quiescence.

Aim III: To elucidate the components of the microRNP complex required to maintain G0 and characterize the regulation of microRNPs by the quiescent state.

An investigation of microRNAs and their targets in maintaining quiescent cancer cells will lead to a greater understanding of the underlying mechanisms responsible for recurrence of tumors. These data will provide the basis for future development of antisense approaches against the characterized microRNAs and targets in G0 cancer cells as a potential, selective therapeutic avenue to manipulate the quiescent cancer cell state. Unraveling regulated microRNA functions in clinically important, cancer-associated cell states will provide extensive insights into the versatility of gene expression control and potential therapeutic approaches, significantly impacting a wide range of cancers.

## 2009 Award Recipients

- **Laurie Boyer, Ph.D.**

Assistant Professor

*Massachusetts Institute of Technology*

“Non-coding RNA as a Regulator of Cell Fate through Functional Interaction with Polycomb Group Proteins”

Key Words: Non-Coding RNA, Polycomb Group Proteins, Chromatin, Stem Cells, Cell Fate

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The goal of this proposal is to elucidate the principles that govern the maintenance of cell identity, a critical process for normal development as loss of cell identity is associated with many diseases including cancer. While it is known that cell diversity is specified in large part by gene expression programs, how changes are made to these global patterns during lineage commitment is not well understood. I have a long-standing interest in exploring the molecular basis of embryonic stem (ES) cell pluripotency and in determining the mechanistic principles that control cell fate changes as a function of gene regulation. To this end, my lab has recently developed a multi-disciplinary approach in human ES cells to investigate the interplay between Polycomb group (PcG) proteins, which are important modifiers of developmental gene expression patterns by regulating chromatin structure, and non-coding RNA molecules, which have been newly suggested to influence genome function and differentiation potential through interaction with this set of regulators.

We expect that this work will lead to novel insights into how developmental decisions are controlled at the molecular level in ES cells. Given the prominent role of PcG proteins in cancer progression, the identification of RNA molecules that collaborate with these factors may also lead to the discovery of novel drug targets for therapeutics.

- **Timothy Gardner, Ph.D.**

Assistant Professor

*Boston University*

“Exploring the Function of Sleep in Neural Circuit Formation”

Key Words: Sleep, In-vivo Imaging, Neural Circuit, Behavior, Transgenic

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Newborn babies spend the majority of their time sleeping. During this extended sleep, patterns of spontaneous neural activity may play a critical role in the development of the brain, but the scientific understanding of this process is at an early stage of investigation. In developing neurons, what forms of anatomical changes occur in sleep? Dendrogenesis? Formation of new synapses? If patterns of activity in sleep are altered, what are the consequences for the development of cells and circuits? What behavioral changes are manifest in waking hours? Answers to these questions may influence how hospitals care for premature infants, could lead to improvements in anti-depressants, or methods of enhancing the flexibility of the aging brain.

Songbirds are a valuable model organism to address many of these questions. In sleep, songbirds spontaneously activate their song motor program. We seek to observe the pattern of this spontaneous activity through in-vivo calcium imaging, and to alter this pattern of activity, examining its role in the maturation of song.

Changes to the patterns of spontaneous replay in sleep can be introduced by infusion of neurotoxins that silence specific stages of the song vocal pathway, or by activation of electrodes implanted in the song system. While altering patterns of neural activity in sleeping birds, we will examine the impact on the maturation of newborn neurons (labeled through viral infection of neural progenitor cells). We will also examine the impact of sleep replay perturbation on song behavior expressed in waking hours. This work has the potential to move beyond correlations in the study of spontaneous activity in sleep. Our goal is to form a more direct test of its role in neural circuit formation.

- **Joshua Kritzer, Ph.D., M.Sc.**

Assistant Professor

*Tufts University*

“Targeting Hsp90 with Small Cyclic Peptides”

Key Words: Cancer, Screening, Peptides, Drug Discovery

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This project aims to discover and characterize small cyclic peptides that modulate the function of the validated cancer target Hsp90. These molecules would provide drug leads, and would also help reveal the roles of Hsp90 in cancer and how we might more effectively target it for cancer therapies.

Heat-shock protein 90, or Hsp90, is a highly conserved chaperone protein. Hsp90 is absolutely required for proper folding of many of the major drivers of oncogenesis, tumor maintenance, invasion and metastasis. Inhibitors of Hsp90 have shown promise in clinical trials but broad application of this anticancer strategy has been limited by side effects and toxicity. There remains a need for molecules that more subtly alter Hsp90 function without completely inhibiting it, in order to minimize these problems. The discovery and characterization of such "Hsp90 modulators" will help us further understand the role of Hsp90 in cancer, and will provide a first step towards more potent and selective Hsp90-based therapies.

During my postdoctoral work, I developed a novel method of screening large libraries of cyclic peptides, an underexplored class of molecules that can modulate protein function. The first two specific aims of the project employ two independent screening strategies, both performed in living cells: one will uncover inhibitors of specific protein-protein interactions of Hsp90, and another will uncover molecules that bind Hsp90 and alter its structure. The third specific aim uses site-directed mutagenesis, binding assays, and application to cell culture to characterize cyclic peptides uncovered in the first two aims. These experiments will provide immediate insight into how Hsp90 can be targeted to kill cancer cells without general toxicity.

Overall, the cyclic peptide strategy is uniquely poised to deliver new drug leads as well as to provide answers to critical questions about targeting Hsp90 as a broad therapeutic strategy for cancer.

- **Hongwei Wang, Ph.D.**

Assistant Professor

*Yale University*

“Structure and Mechanism of the Human RISC–Loading Complex”

Key Words: RNA Interference, RISC, Dicer, Argonaute, TRBP, siRNA, miRNA, Cryo–Electron Microscopy

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We aim to understand the molecular mechanism and regulation of the biogenesis and maturation of small RNAs in the RNA interference and its related RNA–induced silencing pathways. The goal of this proposal is to delineate the structure of the human RISC–loading complex (RLC) and capture the conformational changes that it undergoes in generating small RNAs. The structural information garnered from our work by cryo–electron microscopy (cryo–EM) will provide important mechanistic insights into the process of small RNA maturation within the RLC. Here we describe studies under two specific aims to dissect the structure and function of the RLC.

**Specific Aim 1: Reconstruction of the RLC ensemble at high resolution.** We have determined the coarse shape of the RLC at a resolution of about 30 Angstroms using negative staining EM. We will perform cryo–EM on the ensemble to define its fine structure and components' interactions more precisely. **Aim 1a: Reconstructions of different RLC conformers.** We will perform three–dimensional heterogeneity image analysis to reveal the RLC's structural variation. **Aim 1b: Improve the resolution of the RLC reconstruction.** We will explore sample preparation conditions to further stabilize the complex and use Phase–plate electron microscope to enhance image contrast.

**Specific Aim 2: Assemble a dynamic map of the RLC during its functional cycle.** That the RNA molecule needs to undergo dramatic geometric changes during its maturation process dictates a dynamic behavior of the RLC. **Aim 2a: Define the thermodynamic and kinetic properties of the RLC.** We will employ Surface Plasmon Resonance and fluorescence resonance energy transfer spectroscopy to characterize the interactions between the complex's components under different processing steps. **Aim 2b: Visualize the RLC's structural changes at different RNA processing steps.** We will exploit intra–complex interaction information to guide our electron microscopy of RLC at various states to reveal the RISC–loading process.

- **Florian Winau, M.D.**  
Assistant Professor  
*Immune Disease Institute*

“Hepatic Stellate Cells Instruct Lymphoid Progenitors to Home to the Thymus”

Key Words: Hematopoietic Stem Cells, Liver, T cell Development

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This project investigates the function of hepatic stellate cells to instruct lymphoid progenitors to home to the thymus. Hepatic stellate cells are star-shaped cells located in the liver, and they play an important role in the generation of liver fibrosis. Moreover, stellate cells store vitamin A and produce retinoic acid (RA), which induces the expression of the gut homing receptor CCR9 on T cells. Based on our findings that stellate cells induce CCR9 receptors on T cells for migration to the gut, and considering that CCR9 is important for the homing of lymphoid progenitors to the thymus, we propose that stellate cells also induce CCR9 instruction of bone marrow precursors to seed the thymus. Our preliminary data demonstrate that stellate cells induce CCR9 instruction on lymphoid progenitors in vitro. Aim of this project is to show that stellate cells in the liver induce thymus homing of progenitor cells in vivo. For this purpose, we developed a mouse model of stellate cell depletion. Transfer of progenitor populations upon stellate cell-depleted mice will elucidate whether thymus homing of bone marrow precursors is impaired in stellate cell deficiency. Accordingly, we will track transferred progenitor cells in the liver for analysis of stellate cell-dependent CCR9 induction. Subsequently, we will analyze the effects on thymopoiesis when stellate cells are lacking.

This project will reveal an unexpected relationship between liver and thymus, with stellate cells directing the stream of lymphoid precursors from the bone marrow to the thymus.

The mechanism of stellate cell-mediated progenitor instruction will lead to a better understanding and potential modulation of thymus engraftment after bone marrow transplantation, as well as possible treatment of patients with thymus-dependent T cell deficiencies.

## 2008 Award Recipients

**Zolt Arany, M.D., Ph.D.**

Beth Israel Deaconess Medical Center

**"Peroxisome Proliferator-Activated Receptor Gamma 1alpha (PGC-1alpha) and Cardiac Angiogenesis"**

Cardiac hypertrophy and remodeling in response to chronic hemodynamic load eventually leads to heart failure, through still incompletely understood mechanisms. Microvascular density initially increases during remodeling, keeping pace of the hypertrophy. At some point, however, cardiac vessel content decreases. Recent evidence strongly suggests that this microvascular rarefaction is partly causative to the ensuing development of heart failure. Precisely how the initial angiogenic response and the subsequent vessel dropout happen remain unclear. We have recently described a potent angiogenic pathway in skeletal muscle myocytes, involving Peroxisome Proliferator-Activated Receptor Gamma Coactivator (PGC)-1alpha, a transcriptional coactivator that is inducible by various stimuli and dominantly regulates a number of metabolic pathways in different tissues. PGC-1alpha acts independently of the well-studied Hypoxia Inducible Factor (HIF) pathway and induces VEGF, angiogenesis, and protection against limb ischemia. The expression of PGC-1alpha is repressed in a number of models of heart failure, and PGC-1alpha -/- mice develop accelerated heart failure in response to pressure overload-induced hypertrophy, though how this cardioprotection occurs is still not clear. The hypothesis is proposed here that PGC-1alpha contributes to adaptive angiogenesis during remodeling, and that inappropriate down-regulation of PGC-1alpha contributes to microvascular dropout and the development of heart failure. Specific aims for this project are to investigate the angiogenic pathway regulated by PGC-1alpha in cultured cardiomyocytes, and to test if PGC-1alpha is required for normal cardiac vasculature and for the angiogenic response to pressure overload in vivo. The long-term objective is to establish the role of PGC-1alpha in cardiac angiogenesis and its potential as a therapeutic target.

**Suzanne Paradis, Ph.D.**

Brandeis University

**"Dissecting the Function of Semaphorin 4B in Excitatory and Inhibitory Synapse Formation"**

The complex circuitry of the mammalian brain enables the execution of fundamental cognitive processes such as learning, speech, and memory. Synapses are specialized sites of cell-cell contact that mediate communication between cells in the nervous system. A given neuron, through its synaptic connections, either excites or inhibits other neurons in the circuit and the correct balance of excitation and inhibition must be established for proper circuit function. Aberrant changes in the balance of excitation and inhibition can have pathological consequences for circuit function as demonstrated by the manifestation of devastating neurological impairments, including epilepsy and autism spectrum disorders. A major limitation to our understanding of neural circuit connectivity is a lack of understanding of the molecular mechanisms which govern synapse formation.

To address this gap in knowledge, we have developed a novel, forward genetic RNA interference (RNAi)-based screen in cultured hippocampal neurons that has identified new molecules required for synapse formation. Using this approach, we discovered that RNAi-mediated knockdown of a class 4 Semaphorin, Sema4B, in cultured hippocampal neurons leads to a decrease in the density of glutamatergic and GABAergic synapses. These findings identify Sema4B as one of only a few molecules described thus far that regulates both glutamatergic and GABAergic synapse development and suggest that Sema4B could be a key regulator of the balance of excitatory and inhibitory inputs onto a neuron.

The experiments outlined in this proposal will elucidate the role of Sema4B in synapse formation and neural circuit development. First, we will identify the step in synapse formation that requires the function of Sema4B. Second, we will begin to define the intracellular signaling pathways downstream of Sema4B in synapse formation. Lastly, we will test the hypothesis that additional class 4 Semaphorins are playing a role in regulating the balance of excitation and inhibition in the nervous system.

**John Rinn, Ph.D.**

Beth Israel Deaconess Medical Center

**"Large Intergenic Non-Coding RNAs in Cancer"**

The post genomic era has revealed an abundance of intriguing RNA molecules with no prior annotations. These regions share many of the same properties as protein coding genes -- they are large, spliced, poly-adenylated, transcribed by RNA Polymerase II, with highly conserved promoters and exonic structures -- yet have no potential to encode a sensible amino-acid sequence.

Thousands of these large intergenic non-coding RNAs (lincRNAs) have been detected, but very few have been functionally characterized. Those that have, demonstrate powerful and diverse biological roles. For example, XIST and HOTAIR are two lincRNAs involved in the epigenetic regulation of broad chromatin domains in cis and trans respectively. These two examples have opened up the genome to a whole new form of regulation where a lincRNA can traverse the genome to target a specific locus of DNA. Our lab has established novel computational and experimental methods to identify thousands of highly conserved lincRNAs genome-wide. Including an informatics pipeline that assigns putative functions to each lincRNA. Our analysis has revealed an interesting new class of lincRNAs that are likely to function as either "Onco-lincRNAs" or "Tumor-suppressor lincRNAs".

We recently developed a lincRNA/mRNA expression array to comprehensively identify lincRNAs misregulated across a diverse spectrum of cancer types. The putative functional roles of candidate "Onco-lincRNAs" and "Tumor-suppressor lincRNAs" in either tumor initiation and/or progression are being experimentally verified through classic loss of function screens in a variety of well-established cancer models.

lincRNAs could herald a new paradigm in our understanding of cellular transformation and/or metastasis. Defining the roles of these RNA molecules in cancer could open up new avenues for better diagnostics and therapeutics. With our new experimental and functional genomics pipelines combined with RNAi screening, we can now comprehensively and functionally characterize the roles of lincRNAs in human cancer.

**Tobias Ritter, Ph.D.**

Harvard University

**"Approach to Access Positron-Emission-Tomography (PET) Tracers for Molecular Imaging"**

Positron-emission tomography (PET) is a powerful, non-invasive tool to image biological processes in vivo. Currently, PET finds applications in oncology, neurological disease research, gene therapy, and drug development. Many promising [fluorine-18]-PET tracers for the above mentioned applications are currently inaccessible because no general chemical late-stage fluorination reaction is available to make them. Within this grant application we propose to develop a conceptually different, general solution to the synthesis of novel PET tracers by carbon-fluorine bond formation that is based on transition metal chemistry recently developed in our lab. Additionally, we will prepare three promising, previously inaccessible, PET tracers using our new reaction that may find immediate biomedical applications in cancer, Alzheimer's, Parkinson's, and depression. The biomedical evaluation of the PET tracers will be performed in collaboration with the group of Prof. Weisleder at Massachusetts General Hospital by molecular PET imaging. If successful, our new chemical approach will significantly increase the number of readily available, highly desired PET tracers, which currently cannot be efficiently accessed.

The proposed research cuts across traditional boundaries of scientific disciplines using novel transition-metal chemistry that may have an immediate and significant impact on molecular imaging in medicine.

**Beth Stevens, Ph.D.**

Children's Hospital Boston

**"The Role of the Complement Cascade in Eliminating Synapses during Development and Disease"**

Synaptic pruning is essential for the precise wiring of the developing brain, but what if the same mechanisms that normally eliminate inappropriate synapses during development are reactivated to trigger destructive synapse loss in the adult brain? We recently identified an unexpected role for proteins of the innate immune system in CNS synapse elimination. We found that astrocytes upregulate neuronal expression of complement C1q, the initiating protein of the classical complement cascade. C1q, along with downstream complement protein C3, binds to CNS synapses and is required for developmental synapse elimination. As the primary role of the complement cascade in the immune system is to eliminate dead cells, pathogens, and debris by "tagging" them for rapid removal, we propose to test the hypothesis that complement may be similarly "tagging" synapses for elimination in the brain. Importantly, we found that C1q becomes aberrantly upregulated and is relocalized to synapses in a mouse model of glaucoma prior to RGC neurodegeneration.

Our findings suggest that this normal developmental mechanism of complement-mediated synapse elimination becomes aberrantly reactivated in the adult CNS at early stages of neurodegenerative disease. Thus, understanding how complement normally eliminates synapses during development could provide valuable insight into how synapse loss and cell death can be prevented in neurodegenerative diseases. One of the major aims of this project is to develop an in vitro model of CNS synapse elimination in order to dissect the mechanisms by which complement mediates synapse elimination. We will use transgenic and reporter mice in combination with reagents that activate or disrupt the complement cascade to address several mechanistic questions including: Do microglia phagocytose complement-tagged synapses? What is the identity of the astrocyte-derived signal that upregulates C1q in neurons? Which synapses are targeted by C1q? Is complement expression, activation or synaptic localization regulated by neural activity?

## 2007 Grant Recipients

**Iain Cheeseman, Ph.D.**

*Member, Whitehead Institute for Biomedical Research*

*Assistant Professor of Biology, MIT*

*“Proteomic and Functional Dissection of the Human Kinetochore”*

**Scientific Abstract, Key Words: Kinetochore**

A fundamental aspect of cell division is the accurate distribution of genetic material, which is packaged into chromosomes, to each new cell. A key player in chromosome segregation is the kinetochore – a specialized organelle that forms on each sister chromatid to act as the primary chromosomal attachment site for microtubule polymers. Errors in chromosome segregation result in aneuploidy, which in turn can contribute to tumorigenesis. Thus, in addition to the essential role for the kinetochore during mitosis in segregating chromosomes, the kinetochore itself represents an important target for the diagnosis and treatment of human diseases including cancer. Indeed, several drugs targeting the kinetochore are currently in clinical trials.

Although the central importance of the kinetochore during mitosis has long been appreciated, the molecular basis for its many activities remains poorly understood. In particular, defining the molecular composition of this structure has proven a major challenge, and it is highly likely that additional kinetochore proteins remain to be discovered. Thus, identifying and functionally characterizing the full complement of proteins at the human kinetochore is an important goal. During my graduate and post-doctoral work, I developed methods for the biochemical affinity purification of kinetochore proteins. Although the strategies that I initiated have proven highly successful for identifying new human kinetochore proteins and defining kinetochore organization, this approach must now be extended to the entire human kinetochore. I propose to use funds from a Smith Family New Investigator Award to conduct a proteomic analysis of the human kinetochore. New proteins identified by proteomics will be validated to confirm their identity as kinetochore components, and analyzed to determine their contributions to kinetochore function. Overall, these studies will be targeted at defining the complete composition of the human kinetochore and will lay the groundwork for future studies on kinetochore function.

**Alexei Degterev, Ph.D.**

*Assistant Professor of Biochemistry*

*Tufts University*

*"Molecular mechanism and oncogenic regulation of cellular necrosis"*

**Scientific Abstract, Key Words:** *necrosis, necroptosis, cell death, apoptosis, transformation*

Necrosis, a catastrophic cell death caused by overwhelming stress, is a major contributor to human disease. Very little effort, however, has been made to develop specific therapies targeting pathologic necrosis due to its perceived uncontrolled nature. This key notion has been recently challenged by the discovery that cellular necrosis can result from the activation of the intrinsic cellular regulatory pathways, suggesting that necrosis can be specifically targeted for inhibition. As a direct demonstration of the feasibility of this approach, we have recently developed five potent and selective small molecule inhibitors of such regulated necrosis, or "necroptosis," induced by tumor necrosis factor family of death domain receptors. We have used one of these molecules, necrostatin-1, to directly establish that necroptosis is a major component of the acute pathologic injury *in vivo* in cases of ischemic brain damage, brain trauma and acute liver damage. Furthermore, recent evidence suggests that changes in cellular sensitivity to necroptosis could be an intrinsic part of the process of oncogenic transformation by SV40 large T antigen. Discovery of necrostatins potentially provides a unique opportunity to develop novel necrosis-specific therapies.

We and others have recently identified a number of cellular factors contributing to the activation of necroptosis in tissue culture. In addition, we have discovered the molecular target of necrostatin-1 activity in the cells. We propose to expand on these studies by exploring further the signaling mechanisms underlying cellular activation of necroptosis. We propose to perform a comprehensive analysis of the necroptosis signaling network to define temporal course and interrelationship between different steps in necroptosis activation. We will further use these findings to investigate the mechanism of necroptosis inhibition by large T to determine whether necroptotic pathway plays a tumor suppressive role.

**Jianmin Gao, Ph.D.**

*Assistant Professor, Department of Chemistry*

*Boston College*

*"Understanding beta sheet structure within the membrane bilayer"*

**Scientific Abstract, Key words:** *protein folding, membrane protein, beta-barrel, OmpX, VDAC, thermodynamics, unnatural amino acids*

The long-term goal of this project is to define and quantify the energetic factors that govern beta-sheet structure formation within the membrane bilayer. Compared with soluble proteins, the folding mechanism of membrane proteins, particularly beta-structured membrane proteins, is poorly understood. We propose to use the outer membrane protein OmpX from *Escherichia coli* as a tractable model system to understand membrane beta-sheets. As the smallest membrane beta-barrel known to date, OmpX is amenable to chemical synthesis, allowing the incorporation of strategically designed probes that would reveal the basic principles underlying the beta-structure formation in membranes.

The knowledge learned will provide guidelines for predicting membrane protein structures and disease-related conformational changes. Particularly, an increasing number of beta-barrel membrane proteins, such as voltage dependent anion channels (VDACs) in the outer membrane of mitochondria, have been indicated as potential targets of anti-cancer therapeutics. Improved understanding of their stability and potential conformational changes will add our knowledge in cancer biology and facilitate drug design. To accomplish the proposed goal, we will pursue the following specific aims:

Aim 1. Develop the chemical synthesis and semi-synthesis of OmpX.

Aim 2. Quantify the energetic role of polar groups in OmpX folding utilizing nonpolar isosteres.

Aim 3. Investigate the physical basis of the aromatic girdles observed in membrane embedded beta-barrel employing designed unnatural amino acids.

**Timothy Graham, M.D.**

*Assistant Professor of Medicine, Harvard Medical School*

*Beth Israel Deaconess Medical Center*

*“Stimulated by Retinoic Acid-6 (Stra6) and Insulin Resistance”*

**Scientific Abstract, Key Words:** *Type 2 diabetes, obesity, metabolic syndrome, insulin resistance, adipokines*

Type 2 diabetes, obesity, and other insulin resistant states are associated with loss of GLUT4 expression selectively in adipose tissue. Tissue-specific gene knockout of GLUT4 expression in adipose tissue leads to increased expression of serum retinol binding protein (RBP4) in adipose tissue and increased levels of circulating RBP4 in serum. Increasing serum RBP4 in mice causes whole body insulin resistance with specific inhibitory effects evident in muscle and liver.

RBP4 is the sole specific transport protein for retinol (Vitamin A) in blood, but it is not yet known whether altered delivery or metabolism of retinol mediates insulin resistance caused by RBP4. In fact, little is known at all about the cellular mechanisms by which elevated serum RBP4 causes insulin resistance. The first high affinity receptor for RBP4 was recently identified. This protein, known as Stra6, binds RBP4 and mediates uptake of RBP4-bound retinol into cells. Stra6 is expressed in a variety of tissues, including skeletal muscle and adipose tissue, but not in liver. Stra6 has been proposed to act as a channel for mediating retinol influx, but this has not been formally tested. Stra6 contains features that suggest it may activate intracellular signal transduction upon RBP4 binding, including structural similarities to G protein coupled receptors and a predicted Src-homology 2 domain and phosphorylation site critical for Stra6 biological function during organ development in humans.

The long-term objective is to establish whether RBP4–Stra6 interactions cause insulin resistance and risk for type 2 diabetes. Specific aims of the proposed project are to determine whether Stra6 acts as a signal-transducing RBP4 receptor and to determine whether binding of RBP4 to Stra6 causes insulin resistance using *in vitro* cell culture models and an *in vivo* mouse model of insulin action in muscle.

**Andreas Hochwagen, Ph.D.**

*Whitehead Fellow*

*Whitehead Institute for Biomedical Research*

*"Investigating the Role of Checkpoint Adaptation in Anti-Cancer Drug Resistance"*

**Scientific Abstract, Key words:** *DNA damage checkpoint, adaptation, genome instability, drug resistance, bleomycin*

Drug resistance of tumors is recognized as a chief cause of failure of chemotherapeutic treatment. A variety of mechanisms, including increased drug export, drug metabolism, and up-regulation of repair pathways can contribute to drug resistance. The proposed project aims to test the hypothesis that weakening of the checkpoint response, which normally translates a drug-induced DNA damage signal into a cell cycle arrest, may be another way to increase resistance to cancer drugs. Since checkpoints normally protect genome integrity, we predict that this mode of resistance would be associated with substantial chromosome instability.

This proposal is based on our observation in budding yeast, that mutants lacking two putative checkpoint regulators exhibit increased resistance to the DNA damage inducing anti-cancer drug bleomycin. Given that one of these factors acts as an inhibitor of checkpoint adaptation in meiotic cells, we will determine whether these mutants are able to prematurely adapt to bleomycin-induced DNA breaks. Roles of these factors in drug export and DNA repair will be excluded by analyzing the response to drug-independent DNA damage and by introducing persistent DNA breaks. The role of other adaptation factors in mediating bleomycin resistance will be evaluated, and the organization of the involved molecular pathways will be determined through analysis of double mutants and biochemical interactions. In addition, new factors involved in this mode of bleomycin resistance will be identified through genetic screens, biochemical purifications, and by cloning as yet uncharacterized suppressor mutations. Finally, we will use chromosome loss and chromosome rearrangement assays to test the prediction that bleomycin resistance of these mutants will cause substantial genome instability.

Both resistance to anti-cancer drugs and chromosome instability are hallmarks of tumor progression. Thus, by promoting both processes, adaptation mechanisms may be potent factors in tumor development.

**Ann Sheehy, Ph.D.**

*Assistant Professor, Department of Biology*

*College of the Holy Cross*

*"Harnessing the power of the anti-HIV protein, APOBEC3G"*

**Scientific Abstract, Key Words:** *APOBEC3G, HIV-1, viral restriction*

The role of human APOBEC3G (hA3G) as an innate cellular defense protein that functions as a protective host factor restricting the replication of HIV-1 is of significant interest. Although its viral suppressive activity is effectively countered by the HIV-1 Vif protein during a natural infection, there are experimental indications and data from patient cohorts suggesting that elevation of hA3G expression may result in an invigorated ability to limit viral infection. Our long-term objective is to provide insight into the cellular regulation of *hA3G* gene expression and elucidate important molecular details of the Vif: hA3G regulatory circuit in an effort to harness the power of this cellular defense and contribute to the development of a potent therapeutic counterattack to HIV-1 replication.

Specifically, we will define the promoter of *hA3G* and characterize the regulatory sequences driving *hA3G* gene expression within the cells targeted by HIV-1. The transcription factor binding sites that are critical to promoter activity will be delineated utilizing bioinformatics and experiments using reporter gene constructs. Detailing precise binding sites will lead to a definitive identification of the specific transcription factors involved in hA3G regulation.

We will also examine the molecular details of hA3G protein function via a comprehensive mutagenesis approach. We will complete a broad structure-function analysis of the hA3G protein by performing an alanine-scanning mutagenesis across the entire protein. The resulting mutant proteins will be evaluated for expression stability, antiviral function, enzymatic activity and packaging into virions. This experimental undertaking is purposefully designed to characterize the specific amino acids, motifs and domains within hA3G that are important for the myriad of protein interactions that impact its function. This global approach is expected to reveal regions of hA3G that have not been previously identified as critical to antiviral function, thereby potentially providing additional targets for rational drug design.

**Kimberly Stegmaier, M.D.**

*Assistant Professor of Pediatrics, Harvard Medical School*

*Department of Pediatric Oncology*

*Dana-Farber Cancer Institute*

*"Chemical Genomic Approach to Modulating Oncogenic Transcription Factors"*

**Scientific Abstract, Key Words:** *acute leukemia; small molecule library screening; transcription factor; chemical genomic; MLL*

Despite progress in understanding cancer pathogenesis, many challenges impede drug discovery. This proposal will address the following critical issues: 1. drug discovery for the "undruggable" target (i.e. transcription factors) and 2. elucidation of the protein target of discovered compounds. We will use the identification of modulators of mixed lineage leukemia (MLL) oncogenic translocations as a model organized by the following specific aims:

***Aim 1. Identify small molecule modulators of MLL-AF9.***

***Aim 2. Determine the biological effects of confirmed hits.***

***Aim 3. Determine the protein target of confirmed hits.***

To overcome limitations to small molecule screening, we developed Gene Expression-based High-throughput Screening (GE-HTS). With this approach, any cellular state of interest can be defined by a gene expression signature and small molecules screened for the ability to modulate this signature. In Aim 1, a signature for MLL-AF9 off will be developed with RNA interference (RNAi). Bioactive and diversity oriented synthesis (DOS) collections will be screened by GE-HTS for compounds inducing an MLL-AF9 off signature. In Aim 2, several assays will determine the biological activity of confirmed hits: viability, cell cycle, apoptosis, transformation, differentiation, and genome-wide expression studies. In Aim 3, we will use three approaches to identify the protein target of confirmed hits: proteomic, genomic, and genetic. The proteomic approach will leverage DOS chemistry and new quantitative affinity pull-down methods. The genomic approach will rely on a pattern-matching algorithm to query a reference collection of complex gene expression signatures from human cell lines treated with bioactive small molecules. Candidate proteins identified in the proteomic and genomic approaches will be evaluated with RNAi and overexpression genetic studies. These studies should elucidate mechanisms of MLL-associated leukemogenesis and identify therapeutic leads with direct clinical relevance to MLL-associated leukemia. Furthermore, this work has broad relevance to modulating any cancer-promoting transcription factor.

**Naoshige (Nao) Uchida, Ph.D.**

*Assistant Professor, Department of Molecular and Cellular Biology*

*Center for Brain Science*

*Harvard University*

*"Elucidating Neural Circuits Involved In Decision-Making In Rodents"*

**Scientific Abstract, Key Words:** *decision-making, behavior, basal ganglia, olfaction, rat, mouse*

Our goal is to understand neural circuit mechanisms underlying decision-making using rodent models. Research into how decisions are made has only recently begun to uncover the neural bases. These experiments have been done primarily on monkeys. For mechanistic as well as biomedical questions, however, it would be desirable to be able to study decision-making in rodents, especially mice, in which emerging molecular and genetic tools can be applied more efficiently. We have recently developed an experimental system for studying decision-making in rodents and utilize it in this project.

Animals' actions are controlled by at least two distinct mechanisms: one is goal-directed, rational and flexible while another is stereotyped or stimulus-bound, seen as habit or skilled actions. However, how these two distinct modes of actions are generated is not fully understood. Different lines of research suggest that the striatum, the main input structure of the basal ganglia, is a key structure for controlling both modes of action. Recent behavioral studies suggested that the specific parts of the striatum, the dorsomedial and dorsolateral regions, are involved in goal-directed behavior and habit, respectively. We hypothesize that cognitive information, such as uncertainty and prediction about rewards provided by the prefrontal areas, is essential for the dorsomedial striatum to exert its functions for goal-directed, flexible behaviors. First, we will examine neurophysiological differences between the two areas of the striatum during the performance of odor-guided decision-making tasks. Second, we will test the importance of specific corticostriatal interactions by blocking specific regions of the prefrontal cortex. We will first use local pharmacology to block the activity but, in parallel, we will use molecular tools to obtain better control over temporal and regional specificity.

Deeper understanding of the role of cortical-basal ganglia networks on decision-making will provide crucial insights into various neurological diseases as well as psychiatric disorders.

## **2006 Grant Recipients**

### **Daniel Chase, Ph.D.**

*University of Massachusetts, Amherst*

“Electrophysiological Analysis of Novel Dopamine Signaling Mechanisms *in C. elegans*”

### **Matthew Freedman, M.D.**

*Dana-Farber Cancer Institute*

“A Genome-Wide Admixture Scan to Identify Genetic Determinants of Basal-Like Breast Cancer”

### **Marc-Jan Gubbels, Ph.D.**

*Boston College*

“Dissection of *Toxoplasma gondii* Cell Division, an Opportunistic Pathogen Causing Myocarditis and Encephalitis”

### **Peter Reddien, Ph.D.**

*Whitehead Institute for Biomedical Research*

“Regulation of Stem Cells for Regeneration in Planarians”

### **Yong-Xu Wang, Ph.D.**

*University of Massachusetts Medical School*

“Transcriptional Control of Hepatic Gluconeogenesis by Twist-1”

### **Yong Xiong, Ph.D.**

*Yale University*

“Mechanisms of HIV Suppression by Human Antiviral Protein APOBEC3G and HIV’s Countermeasures”

## **2005 Grant Recipients**

### **Jeanne Hardy, Ph.D.**

*University of Massachusetts Amherst*

“Designing Allosteric Switches in Caspases”

### **Galit Lahav, Ph.D.**

*Harvard Medical School*

“p53 Dynamics and Control of Cell Fate”

### **Matthew J. LaVoie, Ph.D.**

*Brigham and Women's Hospital*

“The Role of Protein Modification and Dysfunction in Parkinson's Disease”

**Christopher Passaglia, Ph.D.**

*Boston University*

“Retinal Coding of Visual Information in Glaucomatous Eyes”

**Sandra Ryeom, Ph.D.**

*Children's Hospital Boston*

“Cancer Protection by the Down Syndrome Candidate Region-1 Gene”

**Adrian Salic, Ph.D.**

*Harvard Medical School*

“Chromosome Segregation in Vertebrates: A Multidisciplinary Approach to Understanding Kinetochore Function and Centromeric Cohesion”

**Shannon Turley, Ph.D.**

*Dana-Farber Cancer Institute*

“Targeting I $\kappa$ B Kinase in Dendritic Cells to Prevent Pancreatic Cancer”

**2004 Grant Recipients**

**Scott A. Armstrong, M.D., Ph.D.**

*Children's Hospital Boston*

“Multi-Step Pathogenesis of MLL-Dependent Leukemia”

**Sean Elliot, Ph.D.**

*Boston University*

“Thiorexdoxins, Thioredoxin Reductase and the Mechanism of Oxidative Stress”

**Marc R. Freeman, Ph.D.**

*University of Massachusetts Medical School*

“Characterizing Core Components of the Glial Cell Machinery”

**Anthony G. Letai, M.D., Ph.D.**

*Dana-Farber Cancer Institute*

“Decoding BCL-2 Family Interactions to Promote Cancer Cell Death”

**Amy J. Wagers, Ph.D.**

*Joslin Diabetes Center*

“Intrinsic and Extrinsic Regulators of Hematopoietic Stem Cell Function”

**Rachel Wilson, Ph.D.**

*Harvard Medical School*

“Synaptic Mechanisms of Chemical Stimulus Encoding”

**Lee Zou, Ph.D.**

*Massachusetts General Hospital*

“Sensing and Signaling DNA Damage in Human Cells”

**2003 Grant Recipients**

**Francis Ka-Ming Chan, Ph.D.**

*University of Massachusetts Medical School*

“TNF-Induced Programmed Cell Death in Immune Responses”

**James J. Chou, Ph.D.**

*Harvard Medical School*

“Structural Study of the Membrane-Associated State of BID for Understanding BID-Induced Damage of Mitochondrial Wall during Apoptosis”

**Patrick T. Ellinor, M.D., Ph.D.**

*Massachusetts General Hospital*

“The Genetics of Atrial Fibrillation: Exploration of Endophenotypes”

**Lisa Goodrich, Ph.D.**

*Harvard Medical School*

“Analysis of Genes Important for Hearing and Balance”

**Fotini Gounari, Ph.D., D.Sc.**

*Tufts-New England Medical Center*

“Thymocyte Transformation in the DN to DP Transition”

**Nathan D. Lawson, Ph.D.**

*University of Massachusetts Medical School*

“Genetic Dissection of the Vascular Endothelial Growth Factor Signaling Pathway”

**Sarah O'Connor, Ph.D.**

*Massachusetts Institute of Technology*

“Human Biosynthesis of Alkaloids: Chemical Inducers of Parkinson's Disease”