Smith Family Awards Program for Excellence in Biomedical Research
2015 Award Recipients

- **Michael Crickmore, Ph.D.**
  Assistant Professor of Neurology, Harvard Medical School
  FM Kirby Neurobiology Center, Boston Children's Hospital

"Molecular and Circuit Analyses of a Motivational State"

Key Words: Neurobiology, Motivation, Dopamine

The long-term goal of my lab is to understand the molecular and circuit mechanisms that impart behavioral flexibility onto sensory-motor circuitry. We primarily study the mating behaviors of male Drosophila melanogaster because much of the underlying sensory-motor circuitry has been characterized, allowing us to ask how activity is controlled at many different processing stages. In the preliminary data for this proposal, we identify and begin to characterize a small group of dopaminergic neurons that is responsible for gating the transformation of sensory information about females into the motor outputs that comprise male courtship behavior. We show that the activity of these dopamine neurons is tuned to reflect the reproductive capacity of the male and is used to instruct mating drive. The dopamine neurons gate the flow of activity through a node that lies at the sensory-motor interface of courtship circuitry. We propose a thorough investigation of the molecular and circuit mechanisms underlying two aspects of this regulation: 1) How the dopamine neurons of the male are tuned to reflect reproductive capacity and 2) How different dopamine levels instruct different levels of mating drive. We will use the advanced genetic techniques of the fly and the extensive literature on courtship circuitry to gain an understanding of these phenomena at a depth not achievable in most systems. We anticipate that the results will establish new principles of behavioral regulation that will be broadly generalizable. Already, the central role of dopamine in motivating mating behavior argues for conservation of mechanism with the many mammalian behaviors to which dopaminergic regulation is central.

- **Stephanie Dougan, Ph.D.**
  Assistant Professor, Microbiology and Immunobiology
  Dana-Farber Cancer Institute

  "In Situ Delivery of Neoantigens and Targeted Radiation in a Therapeutic Vaccine for Pancreatic Cancer"

  Key Words: Immunotherapy, Radiation, Pancreatic Cancer, CD4 T cells, CD8 T cells, Neoantigens, Anti-CD40, Immune-based Therapies, Orthotopic models, Cancer Vaccines

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Pancreatic cancer is one of the deadliest cancers, and forms dense, fibrotic masses that preclude adequate drug delivery. We hope to extend the promise of immunotherapy to pancreatic cancer, a disease that has not yet benefited from current immunotherapy. Our proposal aims to demonstrate in mice, the success of a directly implantable therapeutic cancer vaccine combined with targeted radiation, with the goal of eliciting neoantigen-specific CD4 and CD8 T cell responses to pancreatic cancer.

Aim 1: Predict the neoantigens expressed by Panc02 cells and validate which epitopes stimulate CD4 and CD8 T cell responses in a mouse model of pancreatic cancer. We have generated a library of synthetic neoantigen peptides predicted to bind MHC. These peptides will be pulsed onto dendritic cells and used to stimulate T cells from mice vaccinated with Panc02-GVAX. We will validate antigenicity of each epitope by assessing T cell production of cytokines.

Aim 2: Use intratumoral injection with innate immune agonists and anti-CD40 to deliver neoantigen based vaccines to mice bearing pancreatic cancer. We will induce the recruitment and differentiation of large numbers of dendritic cells, which when activated by anti-CD40, will prime a robust T cell response which may lead to tumor regression. We will evaluate tumor size and composition of tumor infiltrates by flow cytometry.

Aim 3: Combine validated neoantigen peptides with targeted radiation therapy. In collaboration with radiation oncologist Wilfred Ngwa, we will directly implant radiosensitizing gold nanoparticles, innate immune agonists and anti-CD40 to generate tumor vaccines in-situ. Currently, this approach relies on irradiated, dying tumor cells as a source of antigen. Addition of neoantigens may broaden the repertoire of responding T cells and enhance the efficacy of this approach. We will examine neoantigen-specific CD4 and CD8 T cells to determine the effects of radiation on development of an integrated effector and memory T cell response.

• **Yonatan Grad, M.D., Ph.D.**
Assistant Professor of Immunology and Infectious Diseases
Harvard School of Public Health

"Genetic Networks of Antibiotic Resistance in Neisseria gonorrhoeae"

Key Words: Antibiotic resistance, Population biology, Genomics, Gene networks, Neisseria gonorrhoeae, TnSeq

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Antibiotic-resistant bacteria emerge from previously susceptible populations through mutations and acquisition of resistance determinants. To outcompete susceptible bacteria, resistant strains must be able to integrate these changes and their associated fitness costs, adapting to the multiple conditions and contexts in which the bacterial populations grow. My lab is working towards understanding the mechanisms of how, from the immense genomic diversity within bacterial species, strains of resistant bacteria emerge and spread, with the aim of developing novel strategies to prevent and treat resistant infections. We approach this problem by integrating large-scale population genomics with computational and experimental methods to identify the genetic determinants of resistance and the networks that support acquisition and maintenance of resistance. This strategy overcomes many limitations of traditional methods of studying single lab strains and lack of knowledge of the natural genetic diversity of a species. In this proposal, we focus on the emergence of resistance in Neisseria gonorrhoeae, using this clinically important pathogen (the cause of gonorrhea and >100 million infections globally each year) as a model system and for the development of methods that are broadly applicable to bacterial pathogens. With the recent appearance of cephalosporin-resistant strains, the impending threat of widespread gonorrhea resistant to the last-line treatment regimen prompted the CDC to identify resistant N. gonorrhoeae as one of the top three resistance threats. Here, we leverage our unique collection of >1100 clinical isolates with known resistance phenotypes and genome sequences to define the genetic network underlying resistance to two important classes of antibiotics. We have three aims: (1) define alleles highly associated with resistant and susceptible populations using computational analysis of the genome sequences; (2) determine loci that modulate the function of resistance determinants by screening mutants; and (3) validate the candidate loci identified in the first two aims using representative clinical isolates.

- **Andrew Kruse, Ph.D.**
  Assistant Professor of Biological Chemistry and Molecular Pharmacology
  Harvard Medical School

  "A New Approach to Targeting GPCRs"

  Key Words: G protein-coupled receptor, GPCR, signal transduction, antibody, yeast display, combinatorial biology

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G protein-coupled receptors (GPCRs) are the largest family of transmembrane proteins in humans, and they play critical roles in neurotransmission, cardiovascular biology, and metabolic homeostasis. Due to their central role in human physiology, these receptors have become some of the most important targets for the treatment of disease. While many GPCRs have been effectively targeted by small molecule drugs, others have resisted decades of effort at therapeutic development. In particular, peptide and protein receptors have proven exceptionally recalcitrant, with no small molecule ligands available for most such receptors. These include important potential drug targets such as the apelin receptor, parathyroid hormone receptor, GLP-1 receptor, and many others. Clearly, new approaches are needed.

We will address this challenge by developing new methods for modulating GPCR signaling using functional antibody fragments. In previous work, my colleagues and I used antibody fragments in structural studies of acetylcholine receptors. Now, my group is poised to adapt and extend this approach, developing new methods in antibody discovery and applying these methods to examine GPCR structure, function, and signaling biology.

Aim 1. Generalize antibody methods. Development of functional antibodies for GPCRs remains difficult and unreliable. My group is working to develop general methods and combinatorial libraries to enable rapid and straightforward production of functional antibodies.

Aim 2. Develop "biased" agonists. Many of the most important unanswered questions in GPCR signaling surround the phenomenon of "biased" signaling, in which a receptor activates one pathway to the exclusion of others. Using a modified yeast display procedure, we will perform functional selections to create biased agonists and examine the molecular basis of biased signaling.

Aim 3. Determine the mechanisms of antibody signaling. Antibody fragments have proven invaluable tools in GPCR structural biology, and we will use X-ray crystallography to characterize the molecular mechanisms for signaling activity of functional antibodies.

- **Gabriela Schlau-Cohen, Ph.D.**
  Assistant Professor of Chemistry
  Massachusetts Institute of Technology

"A Biophysical Toolkit to Explore Bacterial Chemotaxis"

Key Words: Single-molecule spectroscopy, Membrane biophysics, Signal transduction

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Bacteria have evolved sophisticated molecular machinery that allows them to move in response to chemicals in their environment. This functionality is known as chemotaxis. Notably, chemotaxis is the method by which infectious bacteria target favorable conditions for host colonization. Identifying the mechanisms behind this targeting would pave the way for an entirely new strategy to combat bacterial infection. Interruption of the initial targeting event could provide the optimal combination of selective and early intervention. In this targeting event, membrane-bound receptors bind ligands and transmit information across the membrane. However, the mechanisms by which receptors function remain largely unknown. Traditional techniques lack spatial resolution or require non-physiological, frozen samples. As a result, receptor motions have remained inaccessible.

The goal of this proposal is to develop and deploy new biophysical methods that uncover the molecular mechanisms of chemotactic receptors. A new model membrane platform containing fluorescently-labeled receptors will allow physiological functionality to be interrogated without a background of extraneous cellular processes. This platform allows a powerful optical technique, single-molecule spectroscopy, to resolve conformational changes in individual proteins. With a technique known as single-molecule fluorescence resonance energy transfer (smFRET), the efficiency of energy transfer serves as a spectroscopic ruler between two points of interest on the protein structure. The proposed work leverages this spatial resolution to monitor the molecular motions of the receptors upon active state formation and ligand binding. In contrast to previous work, both large-scale motions, such as protein dissociation, and small-scale motions, such as receptor tilting, will be visible. These results will build a mechanistic understanding of receptor function.

The combination of single-molecule and biochemical tools developed here opens a window into nanoscale molecular machinery. More generally, this combination enables a new class of biophysical studies to elucidate membrane protein functionality and, potentially, to drive new biomedical approaches.

- **Radhika Subramanian, Ph.D.**
  Assistant Molecular Biologist and Assistant Professor of Genetics
  Massachusetts General Hospital

  "Building a Cilium for Intercellular Signaling: in Vitro Reconstitution of the Hedgehog Signaling Pathway"

  Key Words: Cilium, Hedgehog signaling, Microtubules, In vitro reconstitution, TIRF microscopy, Single-molecule methods

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The Hedgehog (Hh) pathway is a major intercellular signaling pathway important for embryonic development and adult tissue homeostasis. Errors in Hh signaling are linked to several newborn birth defects (e.g. skeletal malformations and craniofacial defects) and associated with multiple tumors (e.g. basal cell carcinoma and medulloblastoma). An important aspect of vertebrate Hh signaling is the strict requirement of a microtubule-based organelle known as the primary cilium. While we now have a wealth of data on the 'parts-list' of proteins involved in the Hh signaling, the molecular mechanisms underlying cilia-mediated signal transduction remains poorly understood. Our overall goal is to fill this major gap and provide a biochemical framework for the Hh signaling pathway by reconstituting key reactions of this pathway from its components. For this research, we will build on our experience in integrating single-molecule imaging methods with structural and cell biological tools. In this proposal, we will focus on a conserved protein module of the Hh signal transduction pathway composed of the motor protein Kif7, the transcription factor Gli and its negative regulator Sufu. The proper localization and regulation of these proteins at a specialized subcellular site at the cilia tip is critical for Hh signal transduction. However, this aspect of the signaling pathway remains poorly understood. To fill this gap in our knowledge, we will: (i) Elucidate the molecular mechanisms underlying the Kif7-mediated assembly of the cilia-tip compartment, (ii) Define the dynamic mechanisms that recruit and regulate the Hh transducers Gli and Sufu at the cilia tip, and (iii) Investigate the regulatory mechanisms that modulate Kif7 activity in response to Hh signal. We expect that our findings will not only advance our understanding of the basic biology of the Hh pathway but also shed light on how mutations in pathway components contribute to developmental disorders and human cancers.

Smith Family Award Program for Excellence in Biomedical Research
2014 Award Recipients

Neil Ganem, Ph.D.
Assistant Professor of Pharmacology and Medicine, Departments of Pharmacology and Medicine
Boston University School of Medicine

"Maintenance of Genome Stability by the Hippo Tumor Suppressor Pathway"

Scientific Abstract

Genome instability, broadly defined as the persistent acquisition of both numerical and structural chromosome aberrations, is a hallmark of solid tumors cells and is known to facilitate tumor initiation, progression, and relapse. Consequently, genome instability confers poor clinical prognosis. To become genomically unstable, cells must not only generate chromosome abnormalities, but also adapt to overcome the tumor suppression mechanisms that normally act to restrain their proliferation. Over the past decade, intensive efforts have focused on identifying the underlying mechanisms that generate the numerical and structural chromosome abnormalities found in cancer cells. By contrast, our understanding as to how cancer cells adapt to tolerate this ongoing instability in order to survive and proliferate remains starkly incomplete. Recent studies have demonstrated that non-transformed cells possess mechanisms that sense abnormalities in mitosis and/or chromosome number and ultimately trigger irreversible cell cycle arrest. Our preliminary data suggest that the Hippo pathway plays a critical role in this process, and this proposal will test the hypothesis that the Hippo pathway maintains genome stability. This work has the potential to uncover new therapeutic avenues designed to selectively kill genomically unstable cancer cells while sparing the normal cells from which they originated.

Aim #1: We will test the hypothesis that the Hippo pathway becomes progressively activated during prolonged mitosis, which is often indicative of an underlying mitotic defect, and that this hyper-activation represents a mechanism by which cells monitor the fidelity of cell division.

Aim #2: We will use multiple approaches to inactivate the Hippo pathway, both in vitro and in vivo, and assess the subsequent effects on genome stability, cell cycle progression, and p53 pathway activation.

Aim #3: We will test whether inactivation of potentially novel regulators of the Hippo pathway, as identified by recently completed RNAi and miRNA screens, promote genome instability and transformed growth.

Ya-Chieh Hsu, Ph.D.
Assistant Professor, Department of Stem Cell and Regenerative Biology
Harvard University

"Investigation of How Cell Types from Diverse Lineages Coordinate Changes and Behaviors during Regeneration"

Scientific Abstract

Mammalian organs are functional units composed of cells from multiple lineages. Our understanding of how each individual lineage is specified has increased dramatically in the past decade. However, each lineage is often viewed as a separate entity. As such, a major obstacle in tissue engineering has been to regenerate organs with precise compositions, arrangements, and integration of diverse lineages that ultimately enable function. A comprehensive analysis of how different lineages coordinate and influence one another during the regeneration process will provide important insights into overcoming this hurdle.

We use murine skin as an accessible paradigm to study this problem. Skin, the largest organ in mammals, protects us from insults, infection, dehydration, and enables thermoregulation. These multifaceted functions are accomplished by a rich diversity of cell types, necessitating multiple reservoirs of stem cells. Hair follicles, appendages of the epidermis, cycle between a regeneration phase (anagen) and a resting phase (telogen). Upon entry into anagen, hair follicle stem cells proliferate and generate their transit-amplifying cells (TACs), collectively known as the matrix. Distinct changes in surrounding lineages occur concurrently with matrix formation, raising the possibility that TACs from one lineage may orchestrate changes in neighboring lineages, thus promoting optimal tissue production collaboratively.

In this proposal, we take an integrative approach combining powerful tools of genetics, genomics, imaging, FACS, and histology to delineate alterations in three distinct lineages following hair follicle TAC production, and to define the molecular signals governing these changes. Completion of these aims will not only facilitate therapeutic interventions targeting skin disorders associated with these lineages, but also build tools and a framework for our future studies on skin homeostasis and cancer formation. In addition, findings from the proposed experiments might also lead to therapeutic advances in engineering fully functional organs.

Karla Kaun, Ph.D.
Assistant Professor of Neuroscience, Department of Neuroscience
Brown University

"Neuro-molecular Mechanisms Underlying Alcoholism"

Scientific Abstract

Alcohol is among the most widely used and abused drugs in the world. Unfortunately, there are few effective treatment options for alcohol-use disorders. It is imperative that we investigate the how alcohol affects the brain so we can identify and understand the genetic and molecular factors that affect alcoholism, and use this information to develop better pharmacological therapies. Intriguingly alcohol has both aversive and rewarding properties; however, the abiding memories of an intoxication experience are for alcohol's rewarding properties. In contrast, the memory for alcohol's aversive properties, such as a bitter taste or a resulting hangover, is often short-lived. Thus, the associated memory valence for alcohol reliably switches from negative to positive resulting in enduring preference. This intrinsic switch in emotional valence associated with memories for alcohol highlights the dynamic nature of memory and its malleability. Little is known, however, about the neuronal mechanisms underlying this consequential switch in alcohol memory valence.

The fruit fly, *Drosophila melanogaster*, is an ideal model system to investigate the neural and molecular substrates underlying alcoholism. These animals are similar to mammals in alcohol responses and memories, and there is a broad spectrum of genetic, and molecular tools available to manipulate behavior in vivo. The current genetic tools available in the fly provide the resolution to investigate alcohol memories in live, behaving animals at the molecular, single neuron, and individual circuit levels.

We describe a simple *Drosophila* model in which to investigate the neural and molecular mechanisms underlying a switch from aversive to appetitive alcohol memories. We have identified a simple circuit underlying this switch and identified a role for the Notch signaling pathway in mediating the switch in this circuit. We propose to elucidate how this circuit functions to result in this switch, and identify transcriptional targets of Notch that underlies the neuronal plasticity required for this switch. This proposed research provides an important step towards a functional understanding of the mechanisms underlying memory for alcohol intoxication associated with alcohol cravings.

Michael Lee, Ph.D.
Assistant Professor, Program in Systems Biology
University of Massachusetts Medical School

"Micro-Environmental Regulation of Network State and Drug Sensitivity in Triple-Negative Breast Cancer"

Scientific Abstract

Our ability to potently and selectively kill cancer cells is compromised by the tremendous heterogeneity displayed between individual subtypes of cancer, between individual patients within a single subtype, or even between the individual cells within a single tumor. To understand this complexity, most efforts have focused on the genetic lesions found in tumor cells, however, much less effort has been placed on understanding the role of non-genetic factors in therapeutic variability. The tumor microenvironment itself is very complex, featuring a heterogeneous composition of immune, inflammatory, and other stromal cell types. While it is generally believed that these normal cells play important roles in tumor biology, our understanding is incomplete, and in many cases data are conflicting. The central goal of this proposal is to understand crosstalk between malignant and non-malignant cells at a "systems" and network level. We hypothesize that heterogeneous interactions between tumor and non-tumor cells contribute to the phenotypic diversity seen following drug treatment. To identify meaningful interactions, as well as the mechanisms underlying the effects, we will utilize a high-throughput platform that we developed to quantify the state of signaling networks that control cell death. Quantitative measurements will be made at various intervals following drug exposure and this information will be used to create data-driven computational models to identify how microenvironment-induced changes in the signaling network state alter drug responses. Importantly, these data will be collected using cells grown in 2D co-culture and from primary patient derived tumors grown in a humanized mouse model. A novel computational framework will also be used to directly compare how the in vitro network state differs from the in vivo state. These results will aid in our understanding of how the tumor microenvironment alters drug responses, and will enhance our ability to predict more efficacious forms of therapy.

Rebecca Scheck, Ph.D.
Assistant Professor of Chemistry, Department of Chemistry
Tufts University

"Mapping the Uncharted: Encodable Chemistry to Discover the Role of Glycation in Disease"

Scientific Abstract

Protein posttranslational modification (PTM) networks have proven to be vast realms for drug discovery. Accordingly, our increasing appreciation for how these networks influence cellular function has had an enormous positive impact on the treatment and prevention of disease. Glycation is a non-enzymatic PTM that affects protein function and is linked to many pathologies, including diabetes, cancer, and neurodegenerative disorders. However, its exact role in biology remains poorly understood. This proposal confronts the unmet need for tools that can address non-enzymatic chemistry in vivo. This is accomplished through the development of encodable chemical tools that are used to study the biology of glycation. These studies will enable discovery within this uncharted posttranslational landscape, and will have an immediate impact on our understanding of how glycation influences human health and disease.

This proposal uses a chemical approach to reveal a molecular understanding of glycation and provide encodable tools to study its biology. In Aim 1, we identify the features of local environment that promote glycation. Our findings will provide rules to predict how selective glycation occurs, which will lead to the development of short, encodable, tags for the study of glycation in vivo. In Aim 2, we perform targeted studies that will uncover the biology of glycation. We develop encodable, fluorescent glycation sensors that are used to evaluate glyoxalase inhibition as a cancer therapy. We also identify the role of specific glycation products in influencing oncogenesis driven by the chaperone Hsp27. In Aim 3, we focus on defining how the glycation signal is communicated intracellularly. We will identify "reader" proteins that recognize glycation products and we test if, and how, specific products prevent degradation by the ubiquitin-proteasome system. Together, our studies to define this unexplored horizon will have a significant impact on our understanding and treatment of human disease.

Mario Suva, M.D., Ph.D.
Assistant Professor of Pathology, Department of Pathology
Massachusetts General Hospital

"Uncovering the Role of Epigenetic Programs in Glioblastoma"

Scientific Abstract

Glioblastoma is a complex high-grade infiltrating brain tumor affecting both adults and children that remains incurable. Two recent developments shed light on interplay between aberrant epigenetic programs, histone modifications and gliomagenesis: (1) the understanding that glioblastoma cells recapitulate aspects of neural developmental and maintain transcriptional programs reflecting their differentiation status, with primitive stem(like) cells (CSC) driving tumor propagation; (2) the identification of mutations in histone modifying enzymes, chromatin remodelers and histone genes in subsets of glioblastoma. Translating these novel findings into actionable vulnerabilities requires the systematic survey of epigenetic networks in defined glioblastoma models.

Specific aim 1: Targeting epigenetic stem-like programs in primary glioblastoma
In the recent past, our group has developed a research program focusing on the analysis of epigenetic programs in primary glioblastoma, unmasking a developmental hierarchy controlled by master transcription factors. We reconstructed a network model and revealed a key set of transcriptional targets and pivotal chromatin regulatory complexes as promising therapeutic targets. We propose to systematically disrupt this program, utilizing novel genome-editing technologies to highlight specific epigenetic vulnerabilities. We will utilize primary gliomaspheres models and assess the consequences of network disruption with extensive functional assays and genome-wide analyses.

Specific aim 2: Annotation of functional genomic elements in IDH1/ATRX and in H3F3A mutant glioblastoma.
In a second set of experiments, we want to acquire high-resolution global chromatin landscape of specific genetic subsets of glioblastoma driven by mutations directly affecting the epigenome. Our hypothesis is that these mutations alter the activities of regulatory elements and rewire cis-connectivities. Using primary cultures, we will map by chromatin immunoprecipitation and deep sequencing signature chromatin modifications that will allow the systematic identification and activity of promoters, gene bodies, and distal regulatory elements.

Over the long term, we predict that these approaches have high potential to significantly impact our understanding and treatment of brain tumors.

Smith Family Award Program for Excellence in Biomedical Research
2013 Award Recipients

Abhishek Chatterjee, Ph.D.
Assistant Professor, Department of Chemistry
Boston College

"A Chemical Toolset to Investigate and Engineer Viral Infection"

Scientific Abstract

Human viruses have evolved highly sophisticated cell-invasion strategies to establish infection. Our long-term goal is the elucidation of the multifaceted roles virus-associated proteins play during this process, using site-specifically installed novel chemical probes. Furthermore, using these uniquely reactive chemical handles to attach targeting agents onto the virus, we will develop a general strategy to modulate its infectivity towards different cell-types. A dearth of strategies for selective labeling of distinct proteins within a virus-particle with useful probes has significantly impeded the interrogation of their function. Traditional genetically encoded probes such as fluorescent proteins or peptide tags often perturb the delicate viral architecture, especially for non-enveloped viruses like adenovirus. We propose to develop technology for generating virus incorporating unique amino acids (UAAs) into a pre-defined site of a chosen viral protein, using adenovirus as the model system. UAAs are small, ensuring minimal disruption to the viral architecture, and engineered genetic machinery is available to introduce UAAs with a variety of useful chemical functionalities, such as bioorthogonal conjugation sites, fluorophores, photo-crosslinking agents, radiolabels and photo-caged amino acids. These unique UAA-encoded probes will be utilized for functional interrogation of distinct viral proteins during infection using a variety of bioanalytical techniques such as fluorescence microscopy, radioisotope tracing, and electron microscopy. These results would elucidate the mechanistic details of viral invasion into human cells, providing a platform for designing next generation antiviral therapeutics. Target-specific viruses are highly desirable for gene-therapy applications, and require the incorporation of targeting agents such as antibody-fragments onto the virus. Our technology will also provide precisely-positioned attachment sites for such targeting agents, overcoming limitations of current strategies: genetic fusion, which is limited to protein-based targeting agents and often perturbs virus stability; or non-specific chemical attachment, which randomly coats the virus surface, compromising functionally important sites.

Damon Clark, Ph.D.
Assistant Professor, Department of Molecular, Cellular and Developmental Biology
Yale University

"Dissecting Motor Pattern Generating Circuits in Walking Drosophila"

Scientific Abstract

Animals generate rhythmic behaviors such as walking by means of specialized neural circuits called central pattern generators (CPGs). Much is known about how CPGs create rhythmic activity, but we know far less about how CPG activity changes when, for instance, the animal wants to change direction, or how CPGs coordinate actual motor behavior. To address these questions, I propose to dissect the CPG circuitry in walking Drosophila, where I can take advantage of genetic tools that allow us to dissect and manipulate neural circuits. Because these tools are genetic, we can perform experiments in freely walking flies and investigate both the circuit's natural outputs (the fly's leg movements) and the circuit's response to natural inputs (commands from the fly's brain as it walks through a visual environment). We will investigate how CPG dynamics are shaped by command signals from higher brain centers in order to generate a desired behavior. Using a high-speed camera, we will quantify the repertoire of the fly's walking behavior, describing a full set of outputs of the CPG circuit. We can then relate those outputs to the behaviors elicited by visual stimuli to discover how movement patterns are structured by commands to the CPG. Further, by manipulating neurons in the fly's brain and spine-equivalent and by measuring the effects on its walking patterns, we can investigate how neurons in the CPG circuitry interact to generate those patterns. Finally, by measuring neural activity in walking flies, we can relate dynamic patterns of activity in the CPG circuit to patterns of leg coordination. We can thus investigate the connection between the fly's natural commands, CPG dynamics, and measured behavioral outputs. Our goal is to understand the principles by which CPG dynamics are structured by natural inputs, and the mechanisms that translate CPG dynamics into patterns of walking.

David Cox, Ph.D.
Assistant Professor of Molecular and Cellular Biology
Harvard University

"Learning How to See: a Biological and Computational Investigation of How Visual Cortex Learns and Develops"

Scientific Abstract

Our brain's visual system is an amazingly complex information processing system that transforms a flurry of photons arriving at the retina into a coherent understanding of objects and surfaces in the environment. We know that this feat is achieved through successive processing of visual inputs through a hierarchical cascade of cortical areas, and we know that visual experience and development play key roles in organizing visual cortex. However, our understanding of the role experience and development play in shaping cortical processing has thus far been largely limited to early visual cortex, mostly because we have lacked appropriate model systems and tools that enable us to probe the development of visual cortical population responses. Our group has recently done fundamental work to establish the rodent as a powerful new model system for studying higher-level visual processing, showing that rodents are capable of sophisticated visual object recognition behaviors and mapping object-evoked responses in previously uncharted regions of rodent cortex.

We propose to leverage these tools to make a concerted push on three fronts to understand the development and organization of later (or "higher") visual cortex:

Aim 1: Carefully characterize the normal progression of neuronal development in high-level visual cortex, by measuring neuronal population visual responses as a function of age in normally developing animals.

Aim 2: Measure the effect of fully experimentally-controlled, abnormal visual experience from birth on representations in visual cortex, testing how over-exposure to certain visual objects and deprivation of others affects high-level visual representations.

Aim 3: Test the hypothesis that temporal statistics are important for learning in high-level vision. Many computational theories of vision posit a key role for temporally coherent visual experience in building up normal visual representations in cortex. We will test these hypotheses by raising animals in full-controlled environments where temporal coherence is disrupted.

Jun Huh, Ph.D.
Assistant Professor, Department of Medicine, Division of Infectious Diseases
University of Massachusetts Medical School

"Bacterial Regulators of Immunity and their Modulation of Host Transcription Factors"

Scientific Abstract

The goal of this proposal is to elucidate microbial pathways that govern the development and modulate the function of pro- and anti- inflammatory immune cells in the mammalian gut. The former mediate host immune responses against pathogenic microorganisms, while the latter prevent hyper-inflammation, which may underlie pathologies like cancer or colitis. Recent data suggests that complex interactions exist between gut-residing microorganisms and host immune systems. For example, host cells sense molecular patterns commonly present in microorganisms and contain these organisms by secreting antimicrobial peptides. Some microorganisms, in turn, use multiple strategies to evade detection or modulate host immune responses. It is still not clear, however, how different microorganisms induce particular immune responses and how they regulate the amplitude and flavor of a given response.

I will investigate if nuclear hormone receptor (NhRs) activities in host immune cells are regulated by small molecule metabolites from gut-residing microorganisms. To do so, I will generate murine NhR reporter lines to monitor NhR activities, in the presence or absence of bacteria, in different locations of the mammalian gut. Using cell line NhR reporters and immune cell culture assays, I will test the potential of known microbial metabolites to regulate NhRs. Lastly, I aim to elucidate key bacterial enzymes or bacteria that contribute such metabolites for immune regulation. These studies may lead to the identification of novel regulatory mechanisms by which commensal or pathogenic microorganisms control host immune responses and point towards novel therapeutic strategies for treating multiple inflammatory diseases.

This proposal consists of three specific aims:

Aim 1: Development of mouse reporter lines to investigate in vivo NhR activities that are normally present in gut immune cells.

Aim 2: Identification of bacterial metabolites that regulate NhRs or other host transcription factors.

Aim 3: Identification of gut-residing bacteria and/or their enzymes essential for the synthesis of such metabolites.

Bernardo Lemos, Ph.D.
Assistant Professor of Environmental Epigenetics
Harvard School of Public Health

"Heterochromatin Variation, Bisphenol A sensitivity, and Environmental Epigenomics"

Scientific Abstract

Bisphenol A (BPA) can be found in commercially available plastics, resins, the internal lining of food, and beverage cans. The growing health concern arises for two reasons. Firstly, exposure to epigenetic modulators may have subtle but long term disruptive effects on biological networks that increase disease risk. Secondly, exposure may have long-lasting epigenetic consequences that increase disease susceptibility in the progeny of exposed parents. We will examine the interactions between BPA and the genome. Further context for this research emerges from our recent observations that highly repetitive, and poorly annotated, genomic segments of heterochromatin are functionally relevant and variable among individuals. Here we will identify regulatory networks that are perturbed upon BPA exposure and address their stability in the progeny and grandprogeny of exposed parents. Moreover, the research will identify key regulatory networks that confer higher individual sensitivity to BPA. We will specifically address whether abundant variation in heterochromatin segments might modify BPA toxicity transgenenerationally. We expect that understanding BPA-heterochromatin interactions will help trace disease causing alleles in heterochromatin tracts of human chromosomes. The research will provide novel insights to understand the epigenetics of BPA exposure and open new opportunities for expanding our approach to a variety of commonly encountered environmental pollutants and toxins.

Matthew Shoulders, Ph.D.
Assistant Professor of Chemistry
Massachusetts Institute of Technology

"Hijacking the Host Cell's Protein Homeostasis Network to Potentiate Viral Evolution"

Scientific Abstract

RNA viruses display high genetic variability, enabling them to expeditiously adapt to pressure by generating novel protein variants. The rapid exploration of amino acid space is beneficial for viral adaptation in the face of stress, but it comes with a penalty--the widespread production of viral proteins with reduced stability and/or an enhanced propensity to misfold. In the absence of autonomous protein folding machinery, we hypothesize that viruses hijack the host cell's protein homeostasis network to buffer destabilizing amino acid substitutions. This buffering permits the folding of viral protein variants that would otherwise be unfolded and non-functional, thereby potentiating viral evolution. As a corollary, we propose that the molecular composition and activities of the host cell's protein homeostasis network strongly impact viral evolutionary trajectories and the onset of antiviral drug resistance.

Our first objective is to test these hypotheses using state-of-the-art chemical methods that allow us to rapidly diversify viral populations while simultaneously modulating the host cell's protein folding capacity in well-defined ways. This 'chemical virology' strategy, will reveal how the host cell's protein homeostasis network is hijacked to support rapid viral adaptation under pressure. Our second objective is to elucidate the interplay between evolving viral proteins and the host cell's protein homeostasis network at the molecular level, identifying both the individual viral proteins and the mutations that most effectively hijack the host's protein folding machinery. Altogether, our efforts will provide important fundamental insights into host-potentiated viral evolution and adaptation, an improved understanding of how viral drug resistance develops, and could ultimately catalyze the development of first-in-class antiviral adjuvants that inhibit, for example, a host chaperone required for viral adaptation under pressure.

Jessica Whited, Ph.D.
Assistant Professor of Surgery, Department of Orthopedics
Brigham and Women's Hospital

"Understanding Information-Encoding During Salamander Limb Regeneration"

Scientific Abstract

Axolotl salamanders can regenerate numerous organs, including full limbs, throughout life. Axolotls regenerate precisely the portion of the limb that has been lost and must therefore have a mechanism for coding positional information along the proximal-distal (body to digit tip) axis throughout life. Limb regeneration occurs by activation of differentiated stump cells. These cells re-enter the cell cycle, collect at the tip of the stump beneath a specialized epidermis, and proliferate in a mass called a "blastema." In turn, blastema cells differentiate to give rise to nearly all internal tissues in the regenerated limb. The molecular and cellular biology behind this process is very poorly understood. A thorough understanding of axolotl limb regeneration would have important implications for regenerative biology and medicine.

Though most blastema cells are likely derived by dedifferentiation of stump tissues such as muscle, bone, cartilage, and others, we know very little about how these diverse starting cell types are cued to become blastema cells. How the ancestry of blastema cells impinges upon the descendent cell fate is a critical question, and some recent experiments indicate that very little transdifferentiation occurs during regeneration. Thus, blastema cells likely differ from one another in their lineage and regenerative potentials, and we hypothesize that transcriptional differences may underlie these properties. To test this hypothesis, we are performing RNA-seq on individual blastema cells, comparing the transcriptional profiles between them, and performing functional analyses. We are also investigating the molecular mechanism whereby blastemas encode positional information along the proximal-distal axis. We have uncovered six transcripts much more highly expressed in blastemas derived from proximal amputations compared to those derived from distal ones. We are testing the hypothesis that these transcripts can reprogram distal blastemas to proximal by retroviral infections *in vivo*.

Smith Family Award Program for Excellence in Biomedical Research
2012 Award Recipients

Mark Andermann, Ph.D.
Instructor of Medicine
Harvard Medical School

"Cortical Networks Guiding Hunger-Dependent Attention to Food Cues"

Scientific Abstract

Hunger selectively enhances attention to food-associated visual cues, often leading to excessive eating and refractory obesity. My laboratory seeks to define the neural networks that mediate hunger-dependent processing of food cues. While previous human neuroimaging studies have identified specific cortical brain areas that are selectivity activated by pictures of food stimuli when subjects are hungry, these studies lack the cellular resolution to dissect the basic microcircuits within these areas, a key first step to unlocking basic mechanisms and clinical targets.

The current proposal aims to characterize hunger influences on homologous brain regions in behaving m ice, using novel tools for mapping and manipulating brain activity in identified neurons. As in human imaging studies, we will physiologically characterize neural responses to food and non-food stimuli across a large number of mouse cortical areas using widefield calcium imaging, and how these visual responses are influenced by hungry and sated states (induced by fasting and re-feeding; Aim 1A). We will then zoom in to visualize activity of individual neurons using two-photon calcium imaging. By simultaneously imaging hundreds of neurons repeatedly across hours and days, we will assess the diversity of hunger-related modulation across neurons in different cortical layers and with different stimulus preferences (Aim 1B). We will then test whether the hunger-modulated neurons project to specific target areas and are sensitive to the reward-related neuromodulator, dopamine (Aim 2). In Aim 3, we will determine whether the effects of fasting and refeeding on cortical activity can be reproduced by rapid and reversible optogenetic activation of hypothalamic AgRP neurons known to trigger food-seeking.

Together, these experiments will provide the experimental basis for understanding the networks underlying hunger-dependent attention to food cues, a critical first step towards cell-type specific therapies targeting over-attention to food cues in obesity and other eating disorders.

Jessica Cardin, Ph.D.
Assistant Professor
Yale University

"GABAergic Contributions to Neural and Cognitive Deficits in a Genetic Model of Schizophrenia."

Scientific Abstract

Schizophrenia is a complex psychiatric disease involving dysregulation of basic cognitive abilities, although the underlying perturbations of neuronal function are poorly understood. Previous work on schizophrenia has largely relied on genetic models studied either at the single-cell level or in general behavioral tasks. However, two main hallmarks of this disease in humans are deficits in gamma oscillations, a phenomenon associated with organized neural network activity, and in sensory perception and cognition. Recent work has identified ERBB4 (ErbB4), a tyrosine kinase receptor coupled to the Ras-MEK-ERK pathway, as a strong candidate gene for schizophrenia. ErbB4 protein is expressed predominantly in GABAergic inhibitory interneurons. These cells regulate the magnitude and timing of network activity in the brain, placing them in a critical position to influence cognitive function. Mice lacking ErbB4 exhibit key behavioral deficits associated with schizophrenia, including impaired working memory and decreased prepulse inhibition. Studies in vitro suggest that disruptions of ErbB4 significantly alter the development of interneuron function, ultimately leading to decreased GABA release in the cerebral cortex. Importantly, human and animal studies have identified abnormal development of inhibitory signaling as a key feature of schizophrenia. Together, these findings suggest that ErbB4 plays a significant role in the development of inhibition in cortical circuits, and that its dysregulation may contribute to cognitive disturbances in schizophrenia. Despite these links, the precise role of ErbB4 signaling in the development and function of cortical circuits remains unclear, particularly in vivo. We will use a novel combination of genetic manipulation, dense in vivo electrophysiology, optical stimulation, and behavioral analysis to identify the role of ErbB4 signaling in regulating inhibitory function in cortical circuits in vivo. Our goal is to elucidate the neurodevelopmental function of ErbB4 and provide important context for understanding the role of impaired GABAergic inhibition in schizophrenia.

Ethan Garner, Ph.D.
Assistant Professor, Molecular and Cellular Biology
Harvard University

"Building a Mechanistic Understanding of Bacterial Cell Wall Growth using High-Resolution Dynamic Imaging"

Scientific Abstract

We will build a mechanistic understanding of bacterial growth by directly visualizing the spatial activity of enzymes that construct the cell wall. Bacteria grow in defined shapes by inserting new material into the peptidoglycan (PG) sacculus. The local, short-range activity of the enzymes building these shapes must be spatially regulated in order to create reproducible long-range structures. This regulation is controlled by cytoskeletal polymers, whose localization in vivo is modulated by their intrinsic polymerization kinetics. Thus, to understand how bacteria grow, we must understand; 1) How these polymers assemble 2) How these polymers regulate local PG synthesis, the cellular distribution of PG synthesis, and rate of cell growth, and 3) How the short-range enzymatic activities create emergent long-range shape.

We will use biochemical methods to elucidate the kinetics of these polymers in vitro, then probe how these properties set the distribution of PG synthesis in vivo using high-resolution imaging. We will dissect how these molecular machines assemble, function, and control growth rate by using in vivo single molecule dynamics as readouts of activity, and use the directed motions of these enzymes to understand how their local synthetic activities create ordered, reproducible shapes.

Aims:
Create a dynamic map of all proteins known to be involved in cell elongation by measuring their in vivo motions using single molecule imaging.

Creating quantitative high-resolution static maps of cell growth by determining the in vivo distribution of MreB filaments and PG synthesis sites with STORM.

Determine the assembly kinetics of MreB in vitro to explore how these dynamics set the distribution of PG sites in vivo; probe how perturbations of these dynamics affect cell growth.

Observe how rod shape and radially organized MreB motion emerges from unorganized, spherical cells.

Investigate how the cell modulates the rate of cell wall growth.

Elsie Sunderland, Ph.D.
Mark and Catherine Winkler Assistant Professor of Aquatic Science
Harvard School of Public Health

"Sources, Trends and Effects of Immunotoxic Perfluorinated Compounds"

Scientific Abstract

Many environmental toxicants disproportionally affect children due to hypersusceptibilty during critical developmental windows. Allergic diseases in children such as asthma and skin allergies are increasingly prevalent, affecting greater than 10% of the population. This work will investigate the immunotoxicity of a class of environmental contaminants (perfluorinated compounds - PFCs). Preliminary research shows PFC exposures are associated with severe declines in antibody production following vaccination, in many cases below clinically protective levels. PFCs have been produced in large quantities since the 1940s for manufacturing and consumer products (packaging, textiles) and are transported in wastewater and rivers where they eventually enter the global oceans, persist for thousands of years, and accumulate in marine food webs. These compounds are now detectable in aquatic environments and human sera globally. A decline in production of one the most prevalent PFCs (PFOS -- perfluorooctane suphonate) has led to concomitant increases in long-chained compounds. This study will investigate the compound specific immune responses elicited by exposures to individual PFCs and their isomers using existing birth cohorts in the Faroe Islands with extensive baseline epidemiological data. We will use antibody production in response to specific vaccine toxoids as an indicator of immune health. The specific goals of this study are to investigate: (a) Changes in antibody production against vaccine antigens in association with compound specific PFC exposures; (b) the relationship between serum PFCs in Faroese children and concentrations in marine foods; (c) temporal changes in exposures with shifts in global production; and (d) present and future risks for U.S. children given previously reported NHANES serum levels, benchmark doses established in this study, and global production trends. Ultimately, our goal is to better understand risks to vaccine effectiveness for U.S. children associated with present and expected future exposures to these compounds, which has broad implications for herd immunity.

Eduardo Torres, Ph.D.
Assistant Professor, Program in Gene Function and Expression
University of Massachusetts Medical School

"Role of Protein Turnover Pathways in Aneuploid Cells"

Scientific Abstract

Aneuploidy is defined as the state of having a chromosome number that is not a multiple of the haploid number. Although aneuploidy is a common feature of cancer cells, little is known about its consequences on cellular physiology. Whether aneuploidy is a result of, or plays a causative role in tumor initiation and/or maintenance remains unclear. Utilizing Saccharomyces cerevisiae as model organism, we have shown that aneuploidy confers a proliferative disadvantage and alters cellular metabolism independent of the identity of the extra chromosome. Subsequent studies in mammalian aneuploid cells have recapitulated these results. Recently, we discovered that aneuploid yeast cells quickly evolve and acquire specific genomic alterations leading to improved cellular fitness. Interestingly, several of these include mutations in genes known to regulate protein turnover pathways, such as the deubiquitinating enzyme (DUB) UBP6 and the E3 ligase RSP5. This proposal focuses on understanding how aneuploidy affects the proteome content of cells, and how mutations in UBP6 and RSP5 affect protein turnover pathways and help cells tolerate aneuploidy. We specifically aim to: 1) characterize and quantify the proteome of yeast cells in response to aneuploidy; 2) investigate the mechanisms by which loss of function of the deubiquitinating enzyme UBP6 helps cells tolerate aneuploidy; 3) study the effects of mutations in the E3 ligase RSP5 on the fitness of aneuploid cells. Accomplishing this project will provide the basis for studying aneuploidy in mammalian cells. Importantly, elucidating the mechanisms underlying the cellular response and adaptation to genomic imbalances will help explain the seemingly contradictory correlation of aneuploidy (a state of proliferative disadvantage), with cancer, a hallmark of which is aberrantly enhanced cellular proliferation. Furthermore, unveiling the role of protein turnover pathways in aneuploid cell physiology might reveal yet unidentified aneuploid cell vulnerabilities, leading to the potential future development of novel anti-cancer therapies.

Smith Family Award Program for Excellence in Biomedical Research
2011 Award Recipients

Emily Balskus, Ph.D.
Assistant Professor of Chemistry and Chemical Biology
Harvard University

"Understanding and Preventing the Production of Disease-Associated Metabolites by the Human Gut Microbiota"

Scientific Abstract

Humans live in symbiosis with numerous microorganisms that have been collectively termed the human microbiota. The composition and metabolic activities of this vast community impact heath in both positive and negative ways. Inhibiting harmful microbial metabolism with small molecule drugs, the long-term objective of this project, would constitute a new strategy for disease treatment and prevention. A major obstacle preventing implementation of this approach is the difficulty connecting microbiome sequence data to biochemical function.

We have developed a genome mining strategy that combines biosynthetic knowledge with bioinformatic analyses to identify disease-associated metabolic pathways in symbiotic microbes. In preliminary work, we have used this approach to discover a gene cluster that may encode a microbe-specific pathway for degradation of the essential nutrient choline, an activity that has been linked to non-alcoholic fatty liver disease, the metabolic disorder trimethylaminuria, and cardiovascular disease. Homologs of this cluster are found in genomes of gastrointestinal tract isolates, supporting the potential relevance of this pathway in the human gut.

The specific goals of this proposal are to understand the biochemistry of microbial choline degradation and its influence on host health. We will begin by confirming the link between our gene cluster and choline metabolism using both genetic knockout and heterologous expression approaches. We will also study the mechanism of choline cleavage in vitro and use this information to design small molecules inhibitors of choline metabolism. Additionally, we will characterize the choline degrading activity of human gut isolates both in pure culture and in a mouse model to better understand its influence on host health. Realization of these aims will not only facilitate the discovery of drugs targeting microbial choline degradation, but will also construct an experimental framework and collaborative network for future projects aimed at identifying, understanding, and preventing other harmful metabolic activities associated with the gut microbiota.

Jennifer Benanti, Ph.D.
Assistant Professor, Program in Gene Function and Expression
University of Massachusetts Medical School

"Mapping the Proteolytic Regulatory Network that Controls Cell Division"

Scientific Abstract

The goal of this proposal is to elucidate the proteolytic regulatory network that controls cell division and thus understand how the misregulation of protein expression contributes to the uncontrolled proliferation of cancer cells. Throughout the cell division cycle the proteome is actively remodeled by the opposing actions of ubiquitin ligases (E3s), which attach ubiquitin chains to protein substrates and target them for degradation in the proteasome, and deubiquitinating enzymes (DUBs), which cleave ubiquitin chains from proteins. The balance between ubiquitination and deubiquitination ensures that proteins are only expressed during the window of time when their functions are needed, thereby enforcing unidirectional progression through the cell cycle.

A comprehensive picture of how the cellular proteolytic regulatory network is wired is critical to fully understand cell cycle control. However, it has been difficult to examine connections between ubiquitination and deubiquitination pathways on a system-wide level because of the complexity of the human proteome. To overcome these hurdles, we will take advantage of the powerful genetic tools available in budding yeast to elucidate the proteolytic regulatory network that controls the cell cycle. We recently found that a large fraction of cell cycle proteins are not degraded by any single E3, and subsequently uncovered partially redundant functions of two E3s that control cell cycle entry. In the experiments proposed in Aim 1, we will carry out a genetic interaction screen among all E3s in yeast, in order to identify additional redundancies within the E3 network. We will then identify cell cycle proteins that are targeted by pairs of redundant E3s. In Aim 2 we will examine the contribution of DUBs to the proteolytic regulatory network. We will take genetic and biochemical approaches to identify DUBs that oppose E3 function and regulate the degradation of cell cycle proteins. Together, these experiments will shed light on how ubiquitination and deubiquitination pathways act together to coordinate the cell cycle. Ultimately, a greater understanding of the proteolytic regulatory network controlling the cell cycle will facilitate the development of therapies that more precisely target specific components of the ubiquitin-proteasome pathway, in order to block the hyperproliferation of cancer cells.

Piyush Gupta, Ph.D.
Member, Whitehead Institute and Assistant Professor of Biology, MIT
Whitehead Institute for Biomedical Research

"Functional Dissection of Invasive Mesenchymal Cancer Cells"

Scientific Abstract

The primary cause of cancer mortality is the development of therapy resistance and subsequent metastasis of invasive cancers. The acquisition of both of these phenotypes – drug resistance and invasive potential – can occur concomitantly in epithelial cancer cells by entry into a mesenchymal state of transdifferentiation. Given the central role of invasiveness and drug resistance in determining patient prognosis, an improved understanding of the signaling mechanisms that mediate the specification and survival of mesenchymal cancer cells is essential. Characterizing these mechanisms would further our knowledge of how tumors evade therapy and progress to metastatic disease, and would also facilitate the development of improved therapies.

Chemical molecules can serve as powerful tools that can facilitate the exploration of cellular processes. One promising approach to study invasive, mesenchymal cancer cells would be to develop small-molecule probes of their biology. To this end, we have previously developed a chemical screening strategy to identify chemical compounds that are selectively toxic to invasive, mesenchymal cancer cells that have undergone an epithelial-to-mesenchymal transition. Screening of over 301,000 compounds with this strategy has led to the identification of compounds with highly selective toxicity towards mesenchymal cancer cells.

In the proposed research, we use these unique and highly selective small molecules to probe the biology of invasive cancer cells that have undergone epithelial-to-mesenchymal transitions. We propose experiments that combine the selective chemical probes with shRNA genetic screens and quantitative proteomics in human cells. These studies apply an integrative approach to uncover genetic and physical components of the cellular circuitry that underlies the aggressive phenotypes associated with invasive mesenchymal cancer cells.

Joseph Loparo, Ph.D.
Assistant Professor of Biological Chemistry and Molecular Pharmacology
Harvard Medical School

"Exploring the Molecular Mechanisms of Translesion DNA Synthesis through Single-Molecule Microscopy"

Scientific Abstract

DNA damage that has evaded repair machinery serves as a potent block of DNA replication and can lead to cell death. Translesion (TLS) DNA polymerases alleviate this stress by carrying out synthesis across from blocking DNA lesions. Given their dramatically lower fidelity, the activity of TLS polymerases must be tightly regulated. Indeed, overexpression, loss of function mutations and misregulation of TLS polymerases have been implicated in a number of different cancers across a range of tissue types.

The mechanisms by which TLS polymerases are recruited to the replication fork, carry out translesion synthesis and then dissociate to allow for the replication machinery to regain control of DNA synthesis are largely undetermined. The goal of this proposal is to develop and apply novel single-molecule methods to study the molecular mechanisms of TLS. Building on single-molecule tools our laboratory has developed, we will probe the structure and function of the TLS replisome in real time. Single-molecule fluorescence imaging will be used to determine the composition, stoichiometry and molecular conformation of fluorescently labeled DNA polymerases at the replication fork. These measurements will be simultaneously correlated with the activity of these proteins in DNA synthesis through the nanomanipulation of individual DNA substrates.

We will apply this single-molecule approach to study two important mechanistic questions in TLS using the DNA polymerases of Escherichia coli as a model system. First, we examine how protein-protein interactions are responsible for mediating polymerase exchange between replicative and TLS polymerases. We will determine how the beta clamp, the processivity factor, recruits excess polymerases from solution and utilizes distinct interaction domains to control access of polymerases to the replication fork. Secondly, we will examine how other factors, especially those expressed during the SOS DNA damage response act to further regulate TLS.

Our work will provide new mechanistic insight into the functioning and regulation of translesion DNA synthesis. Additionally, the single-molecule tools developed will be foundational for our continuing efforts to understand structure-function relationships in the multiprotein machines that are involved in genome maintenance.

Eranthie Weerapana, Ph.D.
Assistant Professor of Chemistry
Boston College

"Activity-Based Proteomic Approaches to Investigate Aging in C. Elegans"

Scientific Abstract

The process of organismal aging is characterized by gradual physiological deterioration. The molecular mechanisms underlying the aging process are poorly understood, thereby hindering the development of therapeutics to delay the onset of aging and age-related degenerative diseases. The elucidation of protein activities dysregulated during the aging process is a vital step toward the discovery of signaling networks and metabolic pathways directly implicated in aging. Genetic studies in the model organism, Caenorhabditis elegans, generated a daf-2 mutant that has a significantly extended lifespan. The daf-2 gene mutant activates DAF-16, a transcription factor, which initiates gene expression changes that presumably mediate the life extension phenotype of this mutant. Identifying the protein activity changes that are downstream effects of DAF-16 activation will illuminate cellular pathways directly implicated in the aging process.

To complement the gene expression and protein abundance comparisons that have been performed in daf-2 and daf-2/daf-16 mutants, we propose to directly monitor protein activity changes using the tools of activity-based protein profiling. Conventional genomic and proteomic approaches that measure mRNA and protein abundance provide an inaccurate estimate of the activity state of these proteins due to the plethora of posttranslational modifications that serve to regulate protein activity in vivo. We will initially focus our efforts on cysteine-mediated protein activities that encompass a subset of diverse protein classes including proteases, kinases, ubiquitinating proteins and metabolic enzymes. Several of these proteins were identified in the previous genomic and proteomic studies and, additionally, the activities of these proteins are highly sensitive to the redox state of the cell, which is known to be perturbed in daf-2 mutants. We will apply chemical proteomic technologies to investigate and perturb these cysteine-mediated protein activities in daf-2 mutant nematodes. Utilizing the tools of activity-based protein profiling and chemical genetics, we will address the following two hypotheses: 1) dysregulated cysteine-mediated protein activities contribute to the aging process by affecting key signaling pathways and, 2) a chemical genetics approach to target cysteine-mediated activities will identify novel protein targets and lead molecules for drug discovery.

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"

Scientific Abstract

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"

Scientific Abstract

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."

Scientific Abstract

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:

Identify the actions of DA and NE on glutamatergic hippocampal and intracortical synapses in the PFC.
Determine the spatial scale over which synapses can be modulated independently.
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"

Scientific Abstract

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"

Scientific Abstract

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"

Scientific Abstract

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 mesages 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.