

## 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”

Key Words: Gut Microbiome, Genome Mining, Metabolism, Choline, Mechanistic Enzymology, Drug Development

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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 health 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 molecule 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”

Key Words: Cancer, Ubiquitin, Proteolysis, Cell Cycle

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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”

Key Words: Epithelial–Mesenchymal Transition, Invasive Cancer, Chemical Biology

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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”

Key Words: DNA Damage Tolerance, Translesion Synthesis, Single-Molecule

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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*”

Key Words: Aging, Activity-Based Proteomics, Mass Spectrometry, *C. Elegans*

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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.