Smith Family Foundation: Odyssey Award

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2020 Award Recipients

- **Yonatan Grad, M.D., Ph.D.**
  Melvin J. and Geraldine L. Glimcher Assistant Professor of Immunology and Infectious Diseases
  Harvard T.H. Chan School of Public Health

"Mechanisms of Host-pathogen Interactions from Conditionally Essential and Deleterious Genes"

Key Words: Infectious diseases, Host-pathogen interactions, Population genomics, Evolution, Neisseria gonorrhoeae

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Given high and increasing incidence and extensive antibiotic resistance, the obligate human pathogen Neisseria gonorrhoeae has become a critical public health problem. Despite being one of the most common human bacterial infections, little is understood about how N. gonorrhoeae adapts to the environments of the mucosal niches it infects—the urethra, cervix, pharynx, and rectum. This is due in part to lack of methods to investigate N. gonorrhoeae phenotypes in humans, which are the key source of data because there is no animal model system that demonstrably replicates human niches.

To fill this gap in knowledge, vast datasets from many thousands of clinical specimens with associated data on the anatomical site of infection and genome sequence now enable us to identify genes essential and deleterious to N. gonorrhoeae growth in each anatomic site it infects. Using computational and experimental methods, we have discovered in our preliminary work that many genes previously thought to be essential in fact have prevalent loss-of-function alleles, and that for many of these genes the loss-of-function alleles are associated with specific sites of infection.

But which genes and pathways are selected at each site, and what are the host selective pressures? Our central hypothesis is that host niche-specific factors select for conditionally essential and against conditionally deleterious genes in N. gonorrhoeae. In this research proposal, we will leverage our large dataset of genome sequences from >12,000 clinical isolates and (1) use statistical association methods as the equivalent of a "natural screen" to identify conditionally essential and deleterious genes and use experimental methods to define the growth conditions that favor the loss-of-function and the wildtype forms, and (2) use these variants to learn the host selective pressures shaping N. gonorrhoeae evolution, starting with the pressures from cervical site of infection. The results of the proposed studies will open up a new approach to understanding host niche pressures, with methods that can be applied to all N. gonorrhoeae niches. More broadly, these approaches can be applied to other bacteria host-pathogen relationships and aid in identifying target genes and pathways for novel site-specific therapies and treatment strategies.

- **Gene-Wei Li, Ph.D.**
  Assistant Professor of Biology
  Massachusetts Institute of Technology

"Breakaway Transcription as an Alternative Paradigm for Bacterial Gene Expression"

Key Words: Transcription, Translation, Bacterial Gene Regulation, Breakaway Transcription

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Coupled transcription and translation is considered a defining feature of bacterial gene expression. In the well-studied Escherichia coli, the pioneering ribosome can both physically associate and kinetically coordinate with the RNA polymerase (RNAP), forming a signal-integration hub for co-transcriptional regulation. However, recent data from my lab led to an unintentional finding that transcription-translation coupling – together with its broad functional consequences – is not a fundamental characteristic of the central dogma in other bacterial species. Instead, there may exist an alternative paradigm which we termed "breakaway transcription", whereby the RNAPs dramatically outpace pioneering ribosomes.

The long-term objective of this project is to understand how breakaway transcription determines genome-specific regulatory mechanisms across the bacterial domain of life. We hypothesize that, by uncoupling RNAP from ribosome movements on nascent mRNAs, breakaway transcription could broadly enable RNA-based regulation that substitutes translation-based mRNA surveillance and control. Elucidating such basic mechanisms of gene expression will provide foundational knowledge for understanding the regulatory genomes of many bacteria that are relevant to human health, including gut commensals, pathogens, and antibiotics producers.

In the proposed research, we will comprehensively characterize the consequence and extent of this novel paradigm. In Aim 1, we will determine the regulatory mechanisms enabled and disabled by breakaway transcription in the Gram-positive bacterium Bacillus subtilis, which we have shown to have a much faster speed of RNAP compared to ribosomes. Specifically, we will leverage our high-resolution transcriptomic methods developed in-house to determine how co-transcriptional regulation by both ribosomes and Rho-dependent termination is achieved. In Aim 2, we will map the phylogenetic distribution of breakaway transcription. Using a novel genomic signature, we will leverage the wealth of information about bacterial genome sequences to determine the phyla that follow this alternative paradigm of gene expression. Together, these investigations will redefine the landscape of possible modes of co-transcriptional regulation and establish an important milestone in the studies of bacterial gene expression.

- **Chenxiang Lin, Ph.D.**
  Associate Professor of Cell Biology
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  "Tension-loaded DNA Nanodevices for Structural Determination of Mechanosensitive Proteins"

  Key Words: DNA nanotechnology, Mechanotransduction, Focal adhesion, Cryo-EM, Force spectroscopy, Vinculin

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Cells use complex protein machineries to sense the mechanical properties of the environment and convert the mechanical forces into biochemical signals that influence the cells' decisions to grow, migrate, and communicate. Many proteins responsible for sensing and translating the mechanical stimuli (mechano-transduction) change their structures and biological functions under tensional stress. Current methods used to study these proteins and their complexes lack the ability to map the structures of a protein (or a protein complex) with high resolution under precisely defined tensions. To address this technical challenge, we propose to build nanomechanical devices made of DNA that pull the protein of interest with programmable force. We will explore a variety of device designs to optimize the structural homogeneity of the device, protein loading efficiency, and precision of force control. The tension across the protein will be reported as Förster resonance energy transfer (FRET) signals by a built-in molecular sensor, and the protein structure will be solved with domain-level details by means of cryo-electron microscopy. Therefore, we will be able to directly couple the conformational transitions of a protein to the exact tension that it experiences. We will use vinculin, a well-studied protein that functions as a force transmitter at cell-extracellular matrix adhesions, as a model molecule for testing our prototype mechanical devices (Aim 1). By subjecting vinculin to a range of physiologically relevant tensions (0–10 pN), we will obtain data pertaining to vinculin conformations and ligand-binding behaviors to establish the utility of the DNA nanodevices (Aim 2). In the long term, we expect the project to deliver an adaptable platform for the structural analyses and functional study of proteins under tension, thus furthering our understanding of the cell mechanotransduction and signaling. Because exposure to abnormal forces or miscommunication of mechanical stimuli by cells is linked to a number of diseases such as arteriosclerosis and cancer, better understanding of the underlying molecular mechanisms will open up new therapeutic opportunities for mechanotransduction-related diseases.

- **Meenakshi Rao, M.D., Ph.D.**
  Assistant Professor of Pediatrics
  Boston Children's Hospital

"Glial Phagocytosis in Intestinal Homeostasis"

Key Words: Glia, Enteric Nervous System, Cellular Neurobiology, Inflammatory Bowel Disease, Efferocytosis

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Advances in glial biology have revolutionized the understanding of brain development and disease. The largest populations of glial cells outside the brain are in the digestive tract and are called enteric glia. Enteric glia are profoundly understudied and much remains unknown about their normal functions and underlying diversity. Identifying the normal roles of enteric glia in gastrointestinal homeostasis is essential for understanding how the nervous system regulates gut functions and uncovering how glial dysfunction contributes to digestive disease. This proposal will test the hypothesis that enteric glia have phagocytic capabilities and that one of their key homeostatic functions is to engulf and dispose of apoptotic cells in the gut.

Prompt clearance of dying cells is crucial in all tissues to prevent secondary damage to surrounding cells. Apoptotic cells exhibit specific "eat-me" signals on their surfaces that are recognized by receptor proteins on phagocytes. Receptor activation triggers the cytoskeletal changes within phagocytes that mediate apoptotic cell engulfment and corpse clearance. Our preliminary data show that enteric glia express a key receptor for recognizing "eat-me" signals on apoptotic cells, MEGF10, as well as downstream molecular machinery important for cell engulfment. Furthermore, we have found that enteric glia are capable of engulfing apoptotic cells in vitro. The objective of this proposal is to build on these exciting observations by moving into ex vivo and in vivo experiments that will determine which cells are the targets of phagocytic enteric glia, if this phenomenon is MEGF10-dependent, and to what extent glial phagocytosis normally limits tissue injury. In Aim 1, we will establish and utilize a novel live imaging preparation in dual reporter mice to visualize the phagocytic capabilities of enteric glia in their native microenvironments. In Aim 2, we will disable MEGF10 function in vitro and in vivo to determine to what extent enteric glial engulfment of apoptotic cells is MEGF10-dependent and important for limiting tissue injury in intestinal homeostasis. This work will establish a new conceptual framework for understanding glial function in the gut with broad implications for this and other tissues in both health and disease.

- **Mansi Srivastava, Ph.D.**
  Associate Professor
  Harvard University

"Comparing Development and Regeneration to Uncover Mechanisms for Maintaining Regenerative Ability in Adult Animals"

Key Words: Embryonic development, Pluripotency, Chromatin regulation, Epigenome, Regeneration

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Though great advances have been made in uncovering molecular pathways that maintain pluripotency and regulate differentiation in vertebrate cells in culture, these undifferentiated, pluripotent stem cells cannot be maintained and differentiated in adult animals to facilitate regeneration. We are driven by the observation that despite having limited regeneration capacities in adulthood, vertebrates do maintain undifferentiated cells in embryos and are also able to recover from cell loss in a process called regulative development. The long-term goal of this work is to obtain a mechanistic understanding of how pluripotent stem cells made in early embryos can be maintained into adulthood. Our lab used a new research organism, the acoel Hofstenia miamia, which can regenerate any missing cell type and both adult animals and early embryos have pluripotent cells. Whereas these features are present in other regeneration model systems such as planarians and hydra, the rationale for choosing this new model system is that Hofstenia produces manipulable and accessible embryos in large numbers, providing a unique system where embryonic development can be studied and transgenic tools are available. This project will ask two questions about how Hofstenia development and regeneration compare 1) how genes involved in regeneration in adults operate and control each other during development, and, 2) how the genomes of cells in adult animals are maintained in undifferentiated/pluripotent states (and whether these states are the same as or different from embryonic cell states). We will study chromatin states in embryonic cells capable of regulative development and compare to those in adult stem cells, and identify factors that are needed for maintaining embryonic states into adulthood. Our work will reveal genetic pathways that can be used to maintain pluripotent cells into adulthood, which can inform the development of new applications in human regenerative medicine. We will do this work via two specific aims:

Aim 1: Determine the cellular contexts and network topologies of regeneration pathways in embryos
Aim 2: Identify chromatin states and corresponding regulatory factors associated with regulative development