- **Lucas Farnung, Ph.D.**
Assistant Professor of Cell Biology
President & Fellows of Harvard College

"Elucidating the Mechanism of Co-transcriptional Histone Mark Deposition by the Histone Methyltransferase SETD2"

Key Words: Transcription, Chromatin, Cancer, Structural Biology, Biochemistry, Epigenetics

Transcription is the first step in the expression of our genetic information and is a primary determinant of cell fate and identity. How genes are switched on and off and how this process is regulated by RNA polymerase II, the enzyme that transcribes most protein coding genes, has wide implications in healthy and diseased cells. During transcription, RNA polymerase II must traverse through the genome that is compacted into a structure called chromatin. Importantly, chromatin and its fundamental unit, the nucleosome, impose a barrier to transcription. To allow transcription through chromatin, RNA polymerase II requires additional transcription elongation factors and the presence of post-translational modifications on nucleosomes that modify the level of chromatin compaction. Significantly, the precise mechanism of transcription through chromatin remains elusive. The Farnung Lab combines biochemical, biophysical, and structural biology approaches to elucidate how RNA polymerase II and chromatin interface. In the proposed work, we will investigate three fundamental aspects of chromatin transcription by 1) investigating how nucleosomes are retained on the DNA during transcription, 2) developing a new assay to study the coupling of transcription and methylation of histone H3 by the methyltransferase SETD2, and 3) determining the structural basis of co-transcriptional histone mark deposition using a new experimental paradigm termed visual biochemistry.
This work will identify and dissect molecular rules of chromatin transcription and clarify how its dysregulation results in the emergence of diseases such as cancer.

- **Ana Fiszbein, Ph.D.**
Assistant Professor of Biology
Boston University

"Evolution and Regulation of Hybrid Exons"

Key Words: Transcription, Splicing, Gene Regulation, Transcription Start and Termination Sites

---

The architecture of mammalian genes enables the production of multiple transcripts by using alternative promoters, alternative termination sites, and differentially spliced exons, which greatly expand the coding capacity of our genomes. We recently discovered that exon splicing can activate cryptic promoters located nearby and that these new promoters often arise near annotated internal exons, creating "hybrid" exons that can be used as both first and internal exons in different transcripts. The regulation of these processes has profound impacts on gene expression, and yet the specific mechanisms are poorly understood. Indeed, key gaps in our understanding of co-transcriptional gene regulation include the specific mechanisms and the trans-factors involved in the functional coupling between transcription and splicing. Here, we will use hybrid exons as a unique natural system where transcription and splicing are intrinsically coupled to study their inter-regulation, evolution, and mode of action. We will combine genetic, molecular, and genomic techniques with high-throughput and computational analyses to address two key aspects of co-transcriptional gene regulation. First, we will focus on how transcription and splicing are functionally linked in hybrid exons and identify key cis- and trans-factors involved in the splicing-dependent activation of promoters. Then, we will explore the genomic features of hybrid exons in human transcriptomes and how those features evolved. Our research will result in insights crucial to uncovering the molecular events that cumulatively establish co-transcriptional gene regulatory networks. Ultimately, our findings will lead to the development of new computational tools to predict gene regulatory networks and design molecules to control gene expression with therapeutic benefits.

- **Christine Grienberger, Ph.D.**
Assistant Professor of Biology
Brandeis University

"The Biophysical Basis of Neural Circuit Dynamics during Learning"

Key Words: Learning, Hippocampal formation, Neural circuit dynamics, Synaptic integration, Plasticity

---

The overarching goal of our research is to elucidate the fundamental principles of experience-dependent learning and memory formation. The brain has an extraordinary capacity to learn and to use past experiences to guide future behavior. When individuals learn, they create connections among features, e.g., the location of a restaurant and the food quality, to predict a future outcome. The hippocampal formation, a network of synaptically connected areas in the mammalian brain, is crucial for rapidly forming these associations and relaying the information about these feature associations to its downstream target regions. Here, we seek to understand how one of the major output regions of the hippocampal formation, the subiculum, contributes to this function. We hypothesize that the biophysical properties of individual neurons enable the subicular network to selectively route salient information to downstream connected regions. To test this theory at the synaptic, circuit, and behavioral levels, we will combine for the first time subicular whole-cell recordings, optogenetic perturbation of neural activity, and a spatial learning task. Our goal is to identify the cell-specific computations underlying subicular information processing and to determine the subiculum's impact on adaptive learned behaviors. Our findings will provide novel mechanistic insights into how basic cellular properties endow neurons in the poorly understood subiculum with the ability to produce circuit dynamics that govern learning. This work will also provide a starting point for investigating functional disruptions in neuropsychiatric disorders, in which the patients' ability to learn is impaired.

- **Naama Kanarek, Ph.D.**
  Assistant Professor
  Boston Children's Hospital

"The cellular response to folate deprivation;
sensing and signaling for survival in a folate-poor environment"

Key Words: Folate, Nutrient sensing, Metabolism, Signaling pathway, Cellular survival following nutrient depletion, Functional genomics

---

Folate is an enzymatic cofactor essential for all cell types. It is vital for the biosynthesis of RNA and DNA and is therefore required for gene expression and cell proliferation. The importance of folate is underlined by the consequences of folate deprivation, which include anemia and birth defects, and by the fact that it is a major target in cancer and autoimmune diseases therapy.
It is not known if and how the essential vitamin folate is sensed by cells. Does folate deprivation provoke a signaling response that modifies gene expression, protein function, or metabolic activity to enable survival, like other nutrient-sensing pathways? Our preliminary results indicate a distinct cellular response to folate deprivation including transcriptional and metabolic adjustment that enables survival in low folate. We propose to comprehensively explore the cellular response to folate deprivation. We will follow-up on our functional genomic screen that revealed genes that are essential for survival of folate-deprived cells and will elucidate their function in the context of the signal transduction events that orchestrate the response of cells to folate deprivation. Further, we will use unbiased methods to study the transcriptional response to low folate including regulators and target genes.

This work will contribute an unknown aspect of cellular nutrient-sensing: it will reveal the cellular response to folate deprivation and will provide a comprehensive understanding of the regulation of cellular functions in an environment of low folate, relevant to conditions ranging from fetal development to cancer.

- **Megan Orzalli, Ph.D.**
  Assistant Professor of Medicine
  University of Massachusetts Medical School

"The role of tonic interferon signaling in cutaneous antiviral defense."

Key Words: Organoids, Intrinsic immunity, HSV-1, Skin, Keratinocytes, Fibroblasts, Signaling pathways

---

Human skin is important immunological barrier against diverse viruses. Despite being an initial portal for viral entry, how barrier cells in the skin contribute to antiviral defense remains poorly understood. There is a gap in our understanding of how communication between multiple cell types in the skin shapes barrier defense. The inducible production and secretion of antiviral interferons (IFNs) is one way that cells protect against viruses. However, many viruses that infect the skin, including herpes simplex virus 1 (HSV-1), encode proteins the inhibit inducible IFN production. It remains unclear whether additional innate defense strategies exist to protect against immune-evasive viruses. Here, we will investigate the contribution of basal communication between skin keratinocytes and fibroblasts in antiviral defense. We recently discovered that fibroblasts cultured with keratinocytes to produce human skin organoids were highly resistant to HSV-1, suggesting that keratinocytes constitutively produce factor(s) that protect fibroblasts from infection. Preliminary studies implicate the tonic production of IFNs in mediating this protection, but the identify of this IFN and its role in cutaneous antiviral defense remain undefined. In Aim 1, we will identify the keratinocyte-derived factor(s) that promote resistance to HSV-1 in human skin organoids. In Aim 2, we will define the cellular components in skin organoid fibroblasts that promote resistance to HSV-1 infection. By completing these aims, we will gain fundamental mechanistic insights into antiviral defense strategies that are active in human skin and that provide protection against important human pathogens.

- **Berna Sozen, Ph.D.**
  Assistant Professor of Development and Stem Cell Biology
  Yale School of Medicine

"Dynamic Regulatory Mechanisms of Pattern Formation in Human Embryonic Development"

Key Words: Human Embryo, Stem Cells, Development, Single Cells, Bioengineering, Epigenome, Metabolism

Fundamental questions in stem cell and developmental biology revolve around understanding how cells differentiate from uncommitted progenitors into specialized tissues and organs. Nowhere is this more poorly understood than in the early human embryo which, for technical and ethical reasons, has historically remained experimentally intractable past the onset of gastrulation – the point when the foundational lineages of the human body are specified. In order to understand normal human development and the etiology of human developmental disorders, we must build a comprehensive dataset of the genomic and epigenetic regulatory processes that establish cellular differentiation trajectories during early embryonic life. This proposal aims to achieve precisely this. Capitalizing on our pioneering expertise in stem cell modeling, we propose a new experimental approach combined with cutting-edge bioengineering technologies to enable controllable, efficient and scalable modeling of human early development extending through gastrulation in vitro (Aim-1). Through single-cell-multi-omics, sophisticated computational analyses and gene editing approaches, we will decipher the molecular regulatory logic that governs lineage commitment (Aim-2) and define the role of reciprocal signals in embryonic patterning (Aim-3). Insights gained from this work will be vital for advancing developmental and reproductive health and will benefit cell engineering strategies for regenerative medicine.