- **Raghu Chivukula, M.D., Ph.D.**
  Assistant Professor of Medicine
  Massachusetts General Hospital

  “Elucidating the Molecular Basis of GGC Repeat Expansion Disorders”

  Key Words: GGC repeat expansion, Polyglycine, Neurodegeneration, tRNA splicing, Fragile X Tremor-Ataxia Syndrome (FXTAS), Neuronal Intranuclear Inclusion Disease (NIID)

Translation and aggregation of polyglycine are implicated in the pathogenesis of an emerging group of untreatable, adult-onset neurodegenerative GGC repeat expansion diseases. These diseases are characterized by intranuclear inclusions containing expanded polyglycine (polyGly)-containing proteins, but mechanisms underlying polyGly-mediated cellular dysfunction remain unclear. We recently showed that, through recruitment of endogenous glycine-rich intrinsically disordered domains (IDRs), polyGly aggregates functionally sequester specific protein complexes critical for the biogenesis of tRNAs, both in cultured cells and in human brain samples from affected patients. As Mendelian tRNA biogenesis defects cause infantile neurodegeneration, these findings suggest a unifying, but still opaque, common mechanism.

The overarching goals of this proposal are to investigate the molecular mechanisms and consequences of altered tRNA homeostasis caused by GGC repeat expansion diseases. To do so, we will pursue 2 related but experimentally independent aims: (1) Elucidate the molecular function of the tRNA ligase complex’s conserved glycine-rich IDR. We will investigate this question in parallel using biochemical (affinity proteomics and proximity biotinylation) and forward genetic (synthetic lethality) approaches. (2) Characterize the consequences of polyGly-mediated dysregulation on tRNA modification, abundance, and function. To do so, we will apply novel nanopore-based direct tRNA sequencing approaches as well as assess ribosome transit using ribosome profiling and engineered reporters of the integrated stress response.

- **Nora Kory, Ph.D.**
  Assistant Professor of Molecular Metabolism
  Harvard University

  “Mitochondrial transporters as sensors and responders to shifting environments”

  Key Words: Mitochondria, Mitochondrial Function, Solute carrier, Metabolism, Metabolic Compartmentalization, Mitochondrial Transport, Inborn Errors of Metabolism, Mitochondriopathies, Aging, Rare Diseases

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Mitochondrial transporters are critical gatekeepers of cellular nutrient supply and metabolism. Because of overlapping substrate specificities, crosstalk among metabolic pathways, and properties as membrane proteins, our understanding of key mitochondrial transporters remains limited. To overcome a major roadblock in the mechanistic study of mitochondrial transporters, my lab has developed a liquid chromatography–mass spectrometry–based transport assay for determining transporter function without a priori knowledge of substrates. Using this powerful and unbiased tool, we will screen for substrates of orphan or poorly characterized mitochondrial carriers with the goal of identifying the long-sought-after mitochondrial transporters for key metabolites and co-factors involved in neurotransmitter synthesis and degradation, iron-sulfur clusters, heme, and collagen synthesis (Aim 1). Using orthogonal approaches, including loss-of-function and overexpression studies in cells, organelle uptake assays, mitochondrial functional studies, and metabolite profiling, we will determine the role of identified mitochondrial transporters in metabolite compartmentalization and regulation (Aim 2). Identifying these transporters will form the basis for elucidating their transport mechanisms, identifying pharmacological modulators of mitochondrial function, unraveling key metabolic and signaling pathways, and uncovering previously unappreciated organelle-specific functions of metabolites. Ultimately, this work will provide important insights into the mechanisms underlying inborn errors of metabolism, hematological and neurological disorders, and age-related diseases.

- **Ryan Nett, Ph.D.**
  Assistant Professor of Molecular and Cellular Biology
  Harvard Medical School

  “Deciphering the Biosynthetic Chemistry of Plant-derived Medicines”

  Key Words: Plant natural products, Neuroactive, Biosynthesis, Enzyme chemistry, Plant metabolism, Metabolic engineering

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Plants produce an abundance of neuroactive compounds that serve as medicines for neurological disease. Despite the importance of plant-derived medicines, we understand just a fraction of the chemical principles that plants use to synthesize neuroactive molecules, which restricts our ability to source and discover new medicinal compounds. Interestingly, many neuroactive compounds from distantly-related plants derive from the common metabolic building block ‘piperidine’, which is critical for the neuroactive properties of both plant-derived compounds and synthetic pharmaceuticals. However, little is known about how plants incorporate this building block into neuroactive molecules. In this proposal, we seek to determine the fundamental biosynthetic logic used by plants to build and incorporate piperidine into structures of neuroactive molecules from several plant species. We will use metabolomics to understand the native chemistry of these plants, and then pair this with gene expression analyses to identify likely enzymatic genes involved in synthesizing piperidine-derived molecules. Using enzymatic logic and a plant-based expression system, we will then characterize candidate enzyme catalysts, with the goal of elucidating and engineering complete biosynthetic pathways for target neuroactive molecules. Ultimately, we expect this research to provide fundamental insight on how plants use a common building block to build diverse neuroactive medicines.

- **Sara Prescott, Ph.D.**
  Assistant Professor of Biology
  David H. Koch Institute for Integrative Cancer Research at MIT

  “The Rich, Unexplored Diversity of Airway Intrinsic Neurons”

  Key Words: Neurons, Lungs, Airways, Respiratory, Physiology, Parasympathetic

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From our first to last breath, breathing is under precise and dynamic neural control to ensure effective gas exchange. Within the respiratory tract, an elusive network of intrinsic neurons cooperates with local cells to regulate life-preserving functions of the airways such as airflow, congestion, and innate immunity. Despite their central role in airway physiology, the diversity and functional organization of airway-intrinsic neurons is still poorly understood, in part due to misconceptions about their abundance and technical challenges isolating neurons from solid organs. By leveraging advances in mouse genetics, we’ve overcome these limitations and developed strategies to systematically untangle their cellular/molecular heterogeneity, morphology, and contributions to local airway reflexes. Our work will demonstrate that airway-resident neurons are not simple relays for descending central inputs, as is the current model, but that they comprise an autonomous network that regulates airway reflexes independently of the brain. This work fills a long-standing gap in knowledge, implicates new circuits with central roles in lung disease, and opens fresh areas of respiratory and neuroscience research.

- **Hannah Yevick, Ph.D.**
  Assistant Professor of Physics
  Brandeis University

"Investigating the Role of Multinucleation in Achieving a Mechanically Robust Maternal Fetal Barrier."

Key Words: Cytoskeleton, Mechanics, Machine Learning, Multinucleation, Placenta

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The syncytiotrophoblast is a single multinucleated cell that lines the entire placenta. It acts as a chemical and mechanical barrier between fetus and mother. The syncytiotrophoblast is mechanically strained during placental morphogenesis. How this huge cellular layer resists rupturing under external stress, while lacking rigidifying cell-cell junctions, is unknown. We will control fusion in vitro to form syncytia of defined sizes. Quantitative image analysis will identify how the cytoskeletal connectivity inside the syncytiotrophoblast changes as it grows drastically in size. Force measurements, live imaging, quantitative microscopy and predictive machine learning will interpret how architectural changes in the cytoskeleton tune the multinucleated cell's mechanical properties and propensity to fuse with other cells. Together these results will elucidate the mechanical mechanisms that ensure the robustness of the maternal-fetal barrier, and deliver new avenues to interpret and treat placental defects.