RNA: From Biology to Drug Discovery 2025

Poster Abstracts

Presenter: Aaron A. Smargon, Ph.D.

Affiliation: University of California San Diego

Poster Title: Small Nuclear RNAs Enhance Endogenous Protein-Mediated RNA-Programmable Base Conversion on Mammalian Coding Transcripts

Abstract: Endogenous Uridine-rich small nuclear RNAs (U snRNAs) form RNA-protein complexes responsible for eukaryotic processing of pre-mRNA into mature mRNA. Previous studies have demonstrated the utility of guide-programmable U snRNAs in targeted exon inclusion and exclusion. We investigated whether snRNAs can also enhance conversion of RNA bases over state-of-the-art RNA targeting technologies in human cells. When compared to adenosine deaminase acting on RNA (ADAR)-recruiting circular RNAs, we find that guided A>I snRNAs consistently increase adenosine-to-inosine editing efficiency for genes with higher exon counts, perturb substantially fewer genes in the transcriptome, and localize more persistently to the nucleus where ADAR is expressed. A>I snRNAs can also more efficiently edit lncRNAs and pre-mRNA 3′splice sites to promote splicing changes. Finally, snRNA fusions to H/ACA box snoRNAs (U>ΨsnRNAs) increase targeted RNA pseudouridylation efficiency, facilitating improved CFTR RNA rescue from nonsense-mediated decay in a human bronchial epithelial cellular model of Cystic fibrosis. Altogether, our results advance the endogenous protein-mediated RNA base conversion toolbox and enhance minimally invasive RNA targeting technologies to treat genetic diseases.

Presenter: Ahram Ahn

Affiliation: University of Miami Miller School of Medicine

Poster Title: COX1 mRNA Folding and Translation in Yeast Mitochondria

Abstract: Mitochondria are essential organelles responsible for cellular energy production through oxidative phosphorylation (OXPHOS), a process that depends on the coordinated expression of genes from the nuclear and mitochondrial genomes. In yeast, mitochondrial translation involves nucleus-encoded translational activators that bind specific mitochondrial mRNA 5’ untranslated regions (UTRs) to regulate translation initiation or elongation. Yet, how these activators influence mRNA structure and translation remains unclear. We applied DMS-MaPseq to map the secondary structures of mitochondrial mRNAs, focusing on *COX1*, a mitochondrial DNA gene coding for a catalytic subunit of cytochrome c oxidase or OXPHOS complex IV. We investigated how the absence of key translational activators Pet309, Mss51, and the DEAD-box helicase Mss116, alters *COX1* mRNA structure. Each deletion resulted in distinct structural changes in *COX1* mRNA, particularly near the translation initiation site. While loss of Mss51 increased structural complexity within the COX1 coding region without significantly altering its 5’ UTR, Pet309 or Mss116 deletions markedly altered the 5’ UTR structure. These data indicate substantial roles of translational activators in modulating *COX1* mRNA structure, potentially impacting translation efficiency. Using mitoribosome profiling we further found that deleting any of these translational activators reduced mitoribosome binding to *COX1* mRNA. Notably, absence of Mss116 also impacted ribosome occupancy across other mitochondrial transcripts, consistent with its known broader roles in RNA splicing and mitoribosome biogenesis. Together, our findings reveal that mitochondrial translational activators shape mRNA structure to regulate translation, offering new insights into the complex control of mitochondrial gene expression in yeast.

Presenter: Alana O'Brien

Affiliation: University of Florida, Center for Neurogenetics

Poster Title: Tissue-specific Alternative splicing to Regulate Gene Therapy (TARGET) for X-linked myotubular myopathy

Abstract: AAV gene therapy can be highly effective for treatment of genetic neuromuscular diseases, but there is need for more precisely regulated cargo expression. Current myotropic capsids efficiently transduce skeletal muscle and heart, but in some cases, such as X-linked myotubular myopathy (XLMTM) or limb-girdle muscular dystrophy type 2A, high expression of therapeutic transgene (MTM1 orCAPN3, respectively) in heart can be toxic. To create safer gene therapies for XLMTM and other muscle diseases, we repurposed tissue-specific alternative exons to control transgene expression. Through computational search and in vivo screening of transgene cassettes, we developed a truncatedBIN1 exon 11 cassette with high inclusion in skeletal muscle and low inclusion in heart. This exon was engineered to end with an AUG, such that exon inclusion results in downstream protein expression, while exclusion eliminates it. To further optimize splicing, we generated 672 variants in which donor, acceptor, and RNA binding protein (RBP) binding site sequences varied within the splicing cassette and delivered this library to mice by MyoAAV. Two optimized variants were selected and separately administered to mice for comparison to the first-generation cassette. The optimized variants showed enhanced inclusion in muscle and decreased inclusion in heart, and increased protein expression in muscle relative to heart. Importantly, in cell-based systems, each optimized variant showed enhanced responsiveness to over-expression of muscle- or heart-enriched RBPs. In conclusion, we built a platform for high throughput screening of alternatively spliced AAV cassettes, yielding safer and more effective gene therapies for XLMTM and other muscle diseases.

Presenter: Amaya Fong

Affiliation: University of Florida

Poster Title: Differential pathology and susceptibility to MBNL loss across mouse muscles in a myotonic dystrophy model muscles in a myotonic dystrophy mode

Abstract: There are two subtypes of Myotonic Dystrophy (DM): DM1 caused by a CTG repeat expansion in the 3'UTR of the DMPK gene and DM2 caused by a CCTG repeat expansion in intron 1 of the CNBP gene. The leading DM pathogenic mechanism is RNA mediated toxicity whereby (C)CUG expansions lead to sequestration of the muscleblind-like (MBNL) family of RNA binding proteins. A key difference between DM1 and DM2 is the muscle groups affected with distal muscles more severely affected in DM1 and proximal muscles more affected in DM2. DM1 is also characterized by type I fiber atrophy, while type IIatrophy is more common in DM2. The cause of these disparities in affected muscles is unknown, and it is currently unclear if DM mouse models recapitulate these differences. To address these issues,we collected a series of muscles from Mbnl knockout mice and evaluated them for characteristic histologic and molecular features of DM pathology. Our results indicate that Mbnl loss discordantly affects muscles; however, it does not recapitulate the distal-proximal or proximal-distal gradient observed in either disease. Some muscles, such as the TA, better resemble histological features of DM, while muscles such as the EDL and diaphragm have fiber atrophy profile more like DM1 than DM2.Thesefindingsbeginto explain the affected muscle disparity in DM and have important implications for the muscle of choice when performing analyses in new mouse models as well as evaluating new therapeutic modalities and biomarkers.

Presenter: Amber Eliason

Affiliation: Dr.David Marciano lab at Florida International University, Center for Translational Science, Port St.Lucie FL

Poster Title: Effects of Nitro-Oxidative Stress on Endothelial Cells & ExtracellularVesicle Signaling

Abstract: Endothelial cells (EC) sense diverse stimuli in the circulation and release responsive signaling molecules including extracellular vesicles (EV) to coordinate the multicellular adaptations required to maintain vascular homeostasis. Pulmonary arterial hypertension (PAH) is a progressive vascular disease in which a loss of protective EV or gain of pathological EV signaling have been suggested to contribute to disease pathogenesis. A major challenge in investigating the contribution of aberrant endothelial EV signaling in PAH is the in vitro production of sufficient material for molecular characterization. Here we report the development of a parallel plate microfluidic system to culture EC under laminar shear stress (LSS), physiological conditions that stimulate a significant increase in EV release. Using this system, we compared EC cultured under physiological oxygen (5%pO2) and hyperoxia (21%pO2) which leads to a significant increase in cellular nitro-oxidative stress and altered gene expression. EV were isolated and characterized under these conditions, revealing differences in EV number and protein nitration. Multi-omic analyses of EV cargo identified several PAH associated genes (protein and/or mRNA), demonstrating the potential application of EV as circulating biomarkers of endothelial stress and somatic mutation. Future studies will focus on determining the functional consequence of EV on endothelial, smooth muscle, and immune cell phenotypes.

Presenter: Cameron Niazi

Poster Title: MBNL Multivalency Drives RNA Foci Formation & Tethers MBNL Targets to RNA Foci

Abstract: MBNL proteins drive RNA foci formation inDM1, however, the precise molecular mechanisms underpinning this phenomenon are poorly understood. MBNL proteins have two pairs of zinc fingers (ZnFs) capable of binding RNAs independently and a C-terminal homodimerization domain. We hypothesize that MBNL drives foci formation by crosslinking expanded CUG RNAs through independent binding by each ZnF pair, or by homodimerization. We also propose that MBNL target RNAs may be tethered to RNA foci through these mechanisms, potentially contributing to disease. We used a series of MBNL ZnF mutants with disrupted RNA binding and/or removed C-terminal domain and introduced them into mouse embryonic fibroblasts expressing 480 CUG repeats and lacking endogenous MBNL1/2and used RNA FISH to quantitate the RNA foci. To investigate if MBNL target RNAs are tethered to foci, we employed O-MAP proximity labeling to pulldown RNAs near the expanded DMPK mRNA in primary DM1 myoblasts, and patient frontal cortex, heart, and skeletal muscle. We observed a significant decrease in RNA foci in cells expressing ZnF mutants and truncated MBNL proteins. O-MAP identified RNAs enriched near foci, which was subsequently confirmed via RNA FISH. These results suggest MBNL does indeed drive RNA foci formation in DM1 through two distinct RNA crosslinking mechanisms: independent binding by each ZnF pair and homodimerization. O-MAP and RNA FISH confirmed enrichment of certain RNAs at RNA foci, including heart and muscle specific genes known to be dysregulated in DM1, and work is ongoing to explore how MBNL multivalent tethering may contribute to DM1 pathology.

Presenter: Elena Bisotto, Sean Paz, and Massimo Caputi

Affiliation: Department of Biomedical Science Charles E Schmidt College of Medicine Florida Atlantic University, Boca Raton, FL 33431

Poster Title: SRSF1’s domains functions in transcription and splicing.

Abstract: Serine/Arginine Splicing Factor 1 (SRSF1) is an RNA-binding protein (RBP) with roles in several cellular processes including splicing and transcription. SRSF1 has a modular structure composed of two RNA recognition motifs (RRMs) that interact with specific RNA sequences and are required for efficient RNA binding, in addition to a C-terminal RS (serine/arginine-rich) domain that is necessary for protein-protein interaction but not required for the RNA binding specificity of SRSF1. Analysis of RNA-seq data inHEK293 cells overexpressing SRSF1 revealed that the transcriptional and splicing functions of SRSF1 regulate two unique subsets of cellular genes. It is not understood how the SRSF1 protein domains contribute to its distinct splicing and transcriptional functions. We have investigated the role of the single SRSF1 domains in transcription and splicing regulation utilizing a series of deletion mutants. Since some of the deletion mutants failed to localize in the nucleus, we added a SV40 derived nuclear localization signal (NLS) to all the SRSF1 sequences utilized in the assay. The NLS tagged wild-type and deletion-mutants were transiently over-expressed in HEK293 cells. An analysis of over 20 genes, which alternative splicing is directly modulated by SRSF1 showed that the protein’s domain's role on alternative splicing is dependent on the gene being alternatively spliced. Similarly, we analyzed the expression of 12 genes, which expression is directly modulated by SRSF1 and shown that the protein domains have diverse effects on the expression of different genes.

Presenter: **Eva Nyvltova 1**, Paola Manara 2, Jonathan H. Schatz 2, Flavia Fontanesi 3, and Antoni Barrientos 1,2,4

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Poster Title: Mitochondrial ATP Homeostasis as a Critical Regulator of Organellar Translation

Abstract: **Role of the mL45 N-terminus in mitochondrial translation**

The human mitochondrial ribosome is a biological machine specialized in the synthesis of thirteen hydrophobic inner membrane proteins that are essential components of the oxidative phosphorylation complexes. Recent mitoribosome cryo-EM structures have revealed unique properties of the mammalian mitoribosome and have allowed proposing hypotheses regarding mitochondrial mechanisms of translation. Specifically, a structure of the human mitoribosome with OXA1L bound helped to propose how the co-translational membrane insertion of newly synthesized proteins occurs. The mechanism involves mL45, a mitoribosome protein located at the polypeptide exit tunnel. In the non-translating mitoribosome, the long N-terminal domain of mL45 intrudes into the exit tunnel, but during translation, mL45 undergoes folding within the tunnel, creating two specific constriction sites that limit nascent chain helix formation. Simultaneously, the retraction of the mL45 N-terminus displaces protein uL23m, facilitating the delivery of the newly synthesized polypeptide into inner mitochondrial membrane. To further investigate the role of the mL45 N-terminus, we engineered an *mL45*-KO cell line and reconstituted it with wild-type mL45 and variants featuring progressive N-terminal truncations, preserving the mitochondrial targeting sequence. Our results show that, as expected, mL45 is an essential mitoribosome subunit, with N-terminal truncations up to K71 having no impact on mitoribosomal function under standard conditions. However, truncations encompassing the uL23m contact point, extending to I84 result in 40% attenuation in mitochondrial protein synthesis compared to wild-type. Remarkably, truncation until P90 maintains mL45 steady state levels but

abolishes mitoribosomal function entirely. Thus, precise role of the mL45 N-terminus within the polypeptide exit tunnel remains unclear. Current investigations aim to assess whether mL45 truncations alter OXA1L recruitment by the mitoribosome. Additionally, we are also exploring whether the mL45 N-terminus becomes relevant under stress conditions or acts as a protective mechanism against specific antibiotics.

Presenter: Fei Qu

Affiliation: Florida International University

Poster Title: Kinetic and Structural Insights into m6ATP Incorporation in RNA by DNA Polymerases β and η

Abstract: RNA modifications are crucial in regulating gene expression, genome stability, and other cellular functions. Among them, N6-methyladenosine (m6A) is the most prevalent epitranscriptomic mark in mRNA, while 8-oxoGs and basic (AP) sites are the most common oxidative RNA base damage. m6A and oxidized RNA bases are associated with cancer and neurodegenerative disorders. However, the interplay between m6A and oxidative RNA damage remains unknown. We recently discovered that the m6A level on cellular RNA was increased by an oxidative damage agent, potassium bromate (KBrO3), in HEK293 and HeLa cells. Furthermore, we found that CRISPR knockout of DNA repair enzyme, AP endonuclease 1, significantly increased m6A in HEK293 cells. Using the strand break-mediated RNA modification profiling assay, we demonstrated that m6A on Pol β mRNA transcripts was shifted upon the AP site and 8-oxoG induced byKBrO3. This was correlated with an increased Pol β expression level revealed by RT-PCR and WB. We then demonstrated that the repair DNA polymerases incorporated m6ATP through DNA-templated RNA synthesis with similar efficiency as ATP incorporation using steady-state kinetics. We found that m6ATP adopted base stacking interaction with the template T to facilitate the incorporation. The results suggest that the incorporation of m6ATP by repair DNA polymerases can induce nonscheduled m6A to modulate m6A profiles on mRNA of DNA repair genes, which regulates their expression. Our studies provide novel insights into the interplay between epitranscriptomics and DNA repair. The results will open an avenue to develop novel targets for RNA-based disease prevention, treatment, and diagnosis.

Presenter: Jack Stahl

Affiliation: University of Miami Center for Therapeutic Innovation/The RNA Inc.

Poster Title: Combining oligonucleotides with different mechanisms of action synergistically upregulates SCN1A mRNA

Abstract: While there has been extensive work developing therapeutic strategies to inhibit gene expression with oligonucleotides, relatively little work has been done to explore novel strategies to upregulate gene expression for protein deficiency disorders, such as those caused by haploinsufficiency. Using antisense oligonucleotides (ASOs) or small interfering RNA (siRNA), several cellular processes can be modulated to achieve gene upregulation. However, it is not well known how different types of oligonucleotides targeting separate cellular processes may function in combination. Two separate ASO-based strategies to upregulate sodium voltage gated channel alpha subunit 1(SCN1A) for Dravet Syndrome have been described. We sought to determine if an ASO targeting a repressive non-coding RNA for SCN1A in combination with a splice modifying ASO for SCN1A may yield superior gene upregulation relative to either ASO alone. We designed novel chemically modified ASOs targeting a natural antisense transcript (NAT) of SCN1A and co-transfected our top hit with an ASO which promotes exclusion of a poison exon with an in-frame premature stop codon. We found that the NAT-targeting ASO more effectively upregulates SCN1A relative to the splice modifying ASO but co-transfecting the separate ASOs synergistically upregulates SCN1A to a significantly higher degree than individual ASOs. We also found the co-transfection group is less toxic than individual ASOs, suggesting that ASO-mediated toxicity is dependent on individual ASO concentration rather than degree of SCN1A upregulation. Our data suggest combining oligonucleotides targeting separate cellular processes to converge upon a biological effect is a strategy to enhance therapeutic efficacy while minimizing toxicity

Presenter: Jackson Carter

Affiliation: The Wertheim UF Scripps Institute

Poster Title: lncRNA ADEPTR loss-of-function elicits sex-specific behavioral and spine deficits

Abstract: Spatiotemporal control of gene expression within the cytoplasm is crucial for numerous biological processes, including activity-dependent structural modification of neurons at the synapse, the fundamental mechanism of long-term memory storage. Dysfunction of subcellular protein localization has implications in both neurodegenerative and neurodevelopmental disorders. Recently, many long non-coding RNAs (lncRNAs) have been shown to act within the cytoplasm to regulate the localized expression of genes at various stages via diverse mechanisms. Our lab has identified a specific lncRNA, Activity-Dependent Transported lncRNA (ADEPTR), whose abundance is enriched at neuronal synapses following various forms of excitatory stimulation. The presence of ADEPTR was also shown to be necessary for a transient increase in size of stimulated synapses following 2-photon glutamate uncaging in vitro. My research findings, alongside my collaborators, have shown that genetic perturbation of ADEPTR results in deficits to expression of key neurodevelopmental genes, neuronal morphology, and results in a mild anxiety phenotype (as evidenced by qPCR, extensive behavioral analyses, in vitro morphological analysis of primary hippocampal cultures, and morphological analysis of Golgi-stained CA1 pyramidal neurons in slice culture). Interestingly, the phenotype was more robust in p0-3 animals compared to p14 or p42 animals, and was also stronger in Females compared to Males.

Presenter: **Jeanpierre Fuente 1**, Prem Chapagain 1,3,4, and Yuan Liu 1,2,4

Affiliation: 1 Biochemistry Ph.D. Program, Florida International University 2 Department of Chemistry and Biochemistry, Florida International University 3 Department of Physics, Florida International University 4 Biomolecular Sciences Institute, Florida International University

Poster Title: Mechanisms for sugar puckering to govern RNA-guided DNA synthesis by repairing DNA polymerases

Abstract: DNA repair is essential for genome maintenance. Recent studies have shown that DNA repair can also be mediated by RNA-guided DNA repair. We have discovered an RNA-guided DNA base damage repair mediated by repairing DNA polymerases polβand pol η. We have further demonstrated that polβand pol η exhibit different efficiencies in RNA-guided DNA synthesis. However, the underlying mechanisms remain unclear. Using molecular dynamics simulation, we identified the new interaction of His285, Arg299, andArg283 with the RNA template in polβ. This prevented the formation of the Arg333-Arg283 clamp for interaction with the template G base-paired with the incoming nucleotide. This further disrupted the interaction between the RNA template and Lys27and Tyr36 on the pol b dRP lyase domain. In contrast, polh adopted similar dynamics on both the RNA-and DNA-templated substrates, with the thumb domain exhibiting a close-open conformational change. The enzyme employed Asn324, Lys323, Ser322, Gln38, and Lys293 located on β-strands and loops to interact with DNA and RNA templates. It used Ser257, Gly259, Leu261, and Leu262 from the thumb domain and Arg377, Arg383, and Cys384 from the little finger domain to interact with the primer of the substrates. The results suggest that pol η evolved an effective substrate interaction and catalysis on an RNA template through dynamic adaptation to the C3-endo sugar puckering. Our study revealed the unique mechanisms of RNA-guided DNA repair associated with human cancer and neurodegeneration, thereby facilitating the development of new targets for RNA-based disease treatment.

Presenter: Jenna Wingfield, PhD

Affiliation: The Wertheim UF Scripps Institute

Poster Title: Synaptically-targeted long non-coding RNA SLAMR promotes structural plasticity by increasing translation and CaMKII activity

Abstract: Long noncoding RNAs (lncRNAs) play crucial roles in maintaining cell homeostasis and function. However, it remains largely unknown whether and how neuronal activity impacts the transcriptional regulation of lncRNAs, or if this leads to synaptic changes and contributes to the formation of long-term memories. Here, we report the identification of a novel lncRNA, SLAMR, which becomes enriched in CA1-hippocampal neurons upon contextual fear conditioning but not in CA3 neurons. SLAMR is transported along dendrites via the molecular motor KIF5C and is recruited to the synapse upon stimulation. Loss-of-function of SLAMR reduces dendritic complexity and impairs activity-dependent changes in spine structural plasticity, and translation. Gain-of-function of SLAMR, in contrast, enhances dendritic complexity, spine density, and translation. Analyses of the SLAMR interactome reveal its association with CaMKIIα protein through a 220-nucleotide element also involved in SLAMR transport. A CaMKII reporter reveals a basal reduction in CaMKII activity with SLAMR loss-of-function. Furthermore, the selective loss of SLAMR function in CA1 disrupts the consolidation of fear memory in male mice, without affecting their acquisition, recall, or extinction, or spatial memory. Together, these results provide new molecular and functional insight into activity-dependent changes at the synapse and consolidation of contextual fear.

Presenter: Kattayani Sarkar

Affiliation: Fanucci Research Group, Chemistry Department, University of Florida

Poster Title: Tuning the Biocontinuous Cubic Mesophases of Monoolein using Chelated Lipids for Drug Delivery

Abstract: A wealth of information has been gleaned during the last few decades on self –assembled Lyotropic Liquid Crystals, or LLCs, formed when amphiphilic molecules encounter water. Incorporating chelated lipids into these LLCs represents a promising approach for designing molecular assemblies with potential applications in drug/protein delivery. Monoolein is an FDA approved amphiphile, and it shows various LLC phases like cubic, lamellar, and hexagonal at different temperatures and concentrations in the presence of water. Works of others showed that using different cosurfactant combinations with Monoolein, the water channel diameter of the cubic phases as well as phase transitions can be tuned. Building upon this, this study explores lipid mixture of glyceryl monooleate (MO) and other cosurfactants. Specifically, we show how the incorporation of chelated lipids influences lipid phase behavior, hydration properties, and relaxometric properties, thus evaluating their potential as drug delivery agents. The resultant lipidic mesophases were characterized through a range of techniques, including Nuclear Magnetic Resonance methods—1H NMR and NMR relaxometry—along with Small Angle X-ray Scattering (SAXS), Dynamic Light Scattering (DLS), zeta potential measurements, and Cryogenic Transmission Electron Microscopy (cryo-TEM).

Presenter: Kaushik Chanda, PhD.

Affiliation: Department of Neuroscience, The Herbert Wertheim UF Scripps Institute for Biomedical Innovation & Technology (Sathya Puthanveettil lab)

Poster Title: Activity mediated circular RNAs govern spine plasticity and mitochondrial dynamics

Abstract: Activity dependent structural changes in neurons underlies synaptic plasticity and long-term memory storage. Though we have identified several circRNAs int he nervous system, their role in structural plasticity remains elusive. In this study, we explore the role of circRNAs in initiating and maintaining activity dependent changes in neuronal architecture. Using Glycine induced chemical LTP in mouse primary neurons, followed by a circRNA array screen, we found bidirectional regulation of several circRNAs (N=6 each group, p value cutoO-1, FC cutoff 1) for the 3-hour set. Among these, we posited that circSamm50 (upregulated in 3-hour, FC= +12.85 w.r.t. 0 min), formed from the host gene Samm50 (a mitochondria outer membrane protein), might have a mitochondrial phenotype. Using Super resolution imaging (SIM) we found that a transient knockdown ofcircSamm50 by CRISPR Cas13 showed robust changes in mitochondrial morphology. Live cell mitochondrial imaging showed staggered movements with rapid, frequent pauses. Moreover, circSamm50 KD elicited significant changes in oxygen consumption rate, measured by Seahorse assay. Apart from impacting mitochondrial dynamics, circSamm50 KD resulted in decreased spine density, and altered single spine plasticity, assayed by Glutamate uncaging experiments. Bioinformatics prediction showed that miR-186-5p could interact with both linear and circSamm50, which were validated using Luciferase assays. circSamm50 was also predicted to interact with EIF4A3, an RNA helicase implicated in splicing, which we found to be upregulated by chemLTP. Interestingly, a transient knockdown of EIF4A3 resulted in decreased expression of circSamm50. Together, these results uncover novel circular RNAs modulated by plasticity, of which circSamm50 governs mitochondrial and spine dynamics.

Presenter: Madison Jones and Ulas Kaplan

Affiliation: The Herbert Wertheim UF Scripps Institute for Biomedical Innovation & Technology

Poster Title: Exploring the Molecular Landscape of Learning and Memory

Abstract: The complex morphology and high energy demand of neurons pose a unique challenge for regulating energy in distal regions, particularly in dendrites. This region contains the post-synapse which receives signals from another neuron and modulates synaptic strength. Recent findings indicate that dendritically localized, nuclear-encoded mitochondrial RNAs and micropeptides translated from their 5’ untranslated regions (5’ UTR) are upregulated during neuronal activation, suggesting a novel mechanism of local mitochondrial regulation. These RNAs harbor an eIF4G2-binding motif in their 5’UTRs, which facilitates their local translation in response to neuronal stimulation. Notably, a subset of these RNAs exhibits increased translation of their upstream open reading frame (uORFs) which in turn affects the downstream coding sequence, highlighting a potential regulatory role in synaptic function. However, the functional relevance of these RNAs, uORFs, and their translation products in maintaining mitochondrial dynamics and synaptic plasticity remains unclear. To address this gap, we employed live-cell imaging, super resolution microscopy, and functional assays revealing how distinct activation paradigms induce specific mitochondrial changes at the post-synapse. Our analyses identified key transcripts whose local translation and 5’UTRs modulate mitochondrial dynamics under neuronal activation. Furthermore, implementation of TurboID-based proximity labeling enables the isolation and characterization of dendritic mitochondria, offering insights into spatial and temporal integration of newly synthesized mitochondrial proteins. Collectively, these findings highlight that local RNA regulation is a pivotal mechanism by which neurons adapt mitochondrial function to meet the energy demands of synaptic transmission, shedding light on key processes that support learning and memory.

Presenter: Michele Brischigliaro

Affiliation: Department of Neurology, University of Miami Miller School of Medicine

Poster Title: mRNA-regulated expression of the bicistronic ATP8/6 transcript in health and disease

Abstract: Mitochondrial diseases, the most common genetic metabolic disorders, arise from mutations in either the nuclear or mitochondrial genomes. These conditions often manifest in childhood, affecting approximately 5 to 15 individuals per 100,000, and primarily impact the brain and muscles, leading to encephalomyopathies. Among these disorders, those caused by ATP6 and ATP8 mtDNA mutations are relatively frequent yet remain poorly understood, despite these genes encoding subunits of the well-characterized mitochondrial enzymeF1Fo-ATP synthase. ATP6 and ATP8 proteins originate from a single bicistronic transcript with overlapping reading frames, suggesting a unique translational control mechanism. Recent advancements in mitochondrial mRNA folding analysis using mitoDMS-MaPseq, a technique that combines DMS labeling, mutational profiling (MaP), and RNA sequencing (RNAseq) have implicated a programmed ribosome frameshifting (PRF) mechanism regulated by RNA structure to ensure balanced ATP8 and ATP6 synthesis. To further investigate this process, we are applying state-of-the-art mtDNA gene editing approaches to dissect the molecular basis of PRF. Additionally, we aim to determine how neuropathy-related mutations alter ATP8/6mRNA structure and contribute to disease pathogenesis using mitoDMS-MaPseq. By elucidating these disease-associated changes in mRNA folding, our study will provide novel insights into RNA structural regulation in human disorders. This research has the potential to lay the groundwork for innovative therapeutic strategies focused on modulating mRNA structure, opening new avenues for understanding and treating mitochondrial diseases.

Presenter: Mustapha Olatunji 1.,Yang, W. 4, and Liu, Y. 1,2,3

Affiliation: 1 Biochemistry Ph.D. Program, Florida International University 2 Department of Chemistry and Biochemistry, Florida International University 3 Biomolecular Sciences Institute, Florida International University 4 Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health

Poster Title: Oxidative RNA Damage Modulates DNA Synthesis and RNA-templated DNA Repair

Abstract: RNAs are multifunctional nucleic acid materials with diverse cellular functions. Recent studies have shown that RNA plays a crucial role in the repair of DNA double-strand breaks (DSB) through RNA-mediated DNA repair. Studies from our group recently revealed the mechanism for RNA-guided DNA base damage repair mediated by DNA repair polymerases through a unique nick translation. However, it is unknown how RNA damage may affect RNA-mediated DNA repair by DNA repair polymerases under oxidative stress since reactive oxygen species (ROS) can indiscriminately damage the DNA, RNA, and DNA/RNA hybrids in cells to cause cell dysfunction and genome instability. In this study, we revealed the molecular mechanism underlying the effect of oxidative RNA damage on RNA-guided DNA synthesis and base damage repair mediated by human repair DNA polymerases. We showed that the presence and increased frequency of oxidative RNA damage, 8-oxoG, on the RNA template altered the DNA synthesis efficiency and fidelity of DNA repair polymerases, pol β and pol η, during RNA-guided DNA repair. Using molecular dynamics simulation, we further revealed the conformational changes caused by the 8-oxoG on the substrate orientation and enzyme interaction. We demonstrated that oxidative RNA damage could alter RNA-guided DNA repair and introduce transition and transversion mutations. Our study revealed critical insights into the effect of oxidative RNA damage that could affect RNA-templated DNA synthesis processes like RNA-guided DNA DSB and base damage repair, telomere maintenance, transposon, and retroviral propagation.

Presenter: **NIDHI KALIA**, KIMBERLY SNELL, TONG HUANG, MICHAEL E. HARRIS

Affiliation: Department of Chemistry, University of Florida, Gainesville, FL
Poster Title: EXPLORING THE KINETIC MECHANISMS AND SPECIFICITY OF SARS-COV-2 ENDORIBONUCLEASE NSP15

Abstract: SARS-CoV-2, like all coronaviruses, relies on a set of specialized proteins to replicate and evade the immune system. One of these, the hexameric U-specific endoribonuclease Nsp15, plays a key role in breaking down viral RNA, helping the virus escape immune detection. Despite its importance, many details about how Nsp15 recognizes and cleaves RNA remain unclear. In our studies, we explored its catalytic mechanism and substrate specificity to better understand its function. We found that while divalent metal ions like Mn^{2+}can enhance Nsp15's activity, they are dispensable for catalysis. Biphasic kinetics of ssRNA substrates demonstrate that Mn2+stabilizes alternative enzyme states that have faster substrate cleavage on the enzyme. pH-rate profile supports an acid–base catalytic mechanism proceeding through an anionic transition state. Interestingly, the structure of the RNA itself plays a much bigger role in determining how efficiently Nsp15 cuts. While base pairing in dsRNA suppresses cleavage, the presence of a bulged U enhances catalytic efficiency by an order of magnitude. This suggests that Nsp15 is finely tuned to target specific RNA structures, rather than just sequences. These findings provide a quantitative framework for understanding Nsp15's enzymatic properties, uncovering mechanistic insights into its role in viral RNA processing and immune evasion. By elucidating determinants of specificity and catalytic efficiency, our work advances the understanding of coronavirus biology and informs the development of targeted antiviral strategies

Presenter: Olivia Milam

Affiliation: UF Department of Molecular Genetics and Microbiology, Center for NeuroGenetics

Poster Title: Ribosomal Expansion Segment Drives Translation Fidelity via N-terminal Processing of Ribosomal Proteins

Abstract: Eukaryotic ribosomes exhibit higher mRNA translation fidelity than prokaryotic ribosomes, partly due to eukaryote-specific rRNA insertions. Among these, expansion segment 27L (ES27L) on the 60S subunit enhances fidelity by anchoring methionine aminopeptidase (MetAP) at the nascent protein exit tunnel, accelerating co-translational N-terminal initiator methionine (iMet) processing. However, the mechanisms by which iMet processing influences translation fidelity remain unknown. Using yeast in vitro translation (IVT) systems, we found that inhibiting co-translational iMet processing does not impact ribosome decoding of ongoing peptide synthesis. Instead, our novel method to monitor iMet processing in vivo revealed that ribosomes purified from strains lacking MetAP ribosomal association (ES27L∆b1-4) or major yeast MetAP (∆map1) increases iMet retention on specific ribosomal proteins (RPs). Given the densely packed structure of ribosomes, iMet retention on RPs may distort ribosomal structure and impair its function. Indeed, reconstituted IVT systems containing iMet-retaining ribosomes from ES27L∆b1-4or∆map strains, combined with translation factors from wild-type strains, demonstrated that such ribosomes are error-prone. Our study elucidated the critical role of ES27L in adjusting ribosome association of universally conserved MetAP enzyme to fine-tune iMet processing of key RPs, thereby ensuring the structural integrity and functional accuracy of eukaryotic ribosomes.

Presenter: **Paul Fernandez 1**, Ryan P. Hildebrandt 1, Zhuangyue Li 1, Devi Prasad Boggupalli 1, Eric T. Wang 1

Affiliation: 1 Department of Molecular Genetics & Microbiology, Center for Neurogenetics, Genetics Institute, University of Florida, Gainesville, FL, USA

Poster Title: Elucidating the Kinesin & RNA Binding Protein Structure-Function Code

Abstract: Critical to cellular regulation of spatial gene expression is controlled RNA organization by specific subcellular trafficking. A key mechanism is via kinesin motor proteins, which specify cargoes using adapter proteins and can direct transport to the cellular periphery by traveling toward positive poles of microtubule networks. RNA binding proteins (RBPs) can function as these adapters; known pairs include APC withKIF1C, ZBP1 with KIF11, and nucleolin with KIF5A (Pichon et al., 2021; Song et al.,2015; Doron-Mandel et al., 2021). Previously, we demonstrated that MBNL1 and other RBPs with similar C3H zinc fingers, including Tis11d and Cpsf4, show preferences forKIF1Cand KIF1Bα but not KIF1Bβ tails, whereas ZC3H14, an RBP with a different type of zinc finger, saw no associations (Hildebrandt et al., 2023). These results indicate a broader code between RBPs and kinesins where specific RBP domains are suited to certain kinesin tails for transport. Here, we describe the platform and early results of our efforts to screen 40kinesin tails with 371 RBPs, many of which have been previously identified to play roles in RNA localization. The basic screen consists of centrosome recruitment assays in which BICD2-kinesin tail fusions are stably expressed in cells and travel to the centrosome via dynein motors. EGFP-fused RBPs are co-expressed by transfection, and their recruitment to the centrosome is quantitated by microscopy and a custom computational pipeline. Our results should elucidate a large dataset of kinesin and RBP interactions to gain an increased understanding of RNA transport in health and disease.

Presenter: Seungwoo Hong

Affiliation: University of Miami Miller School of Medicine

Poster Title: Regulation of non-canonical junction processing in human mitochondrial transcripts by FASTKD5

Abstract: Mitochondrial DNA (mtDNA) encodes 13 essential polypeptides organized into two long polycistronic transcripts that require endo-nucleolytic cleavage for expression. While most junctions conform to the “tRNA punctuation” model—where mitochondrial RNase P and RNase Z excise intervening tRNAs—several non-canonical sites (notably the 5′ ends of MT-CO1 and MT-CYB) lack flanking tRNAs and instead depend on the alternative processing machinery. FASTKD5 knockout (FASTKD5-KO) HEK-293T cells display a dramatic loss of non-canonical transcripts and their protein products, and unprocessed CO1 is inefficiently translated. To define how FASTKD5 discriminates these junctions, we applied mitoDMS-MaPseq in isolated mitochondria from wild-type (WT) and FASTKD5-KO cells. Although overall coverage of mature transcripts was unchanged, FASTKD5-KO mitochondria accumulated DMS-reactive reads specifically at the 5’-end of CO1, ND5–CYB junction, and ATP8/6–CO3 junction. Comparative linear arc-plots revealed that unprocessed CO1 precursors adopt unique folding patterns, including multiple hairpins ~13–15 nt upstream of cleavage sites, and sequence analysis uncovered a conserved CCA triplet immediately adjacent to each processing site. Together, these results show that FASTKD5 integrates local RNA secondary structures and sequence motifs to drive precise cleavage at diverse non-canonical junctions, revealing transcript-specific pathways in mitochondrial RNA maturation.

Presenter: Skyler Briggs

Affiliation: The Herbert Wertheim UF Scripps Institute for Biomedical Innovation & Technology

Poster Title: OAS3 Condensation Thresholds RNase L Activation

Abstract: Oligoadenylate synthetase (OAS) proteins are key components of the mammalian antiviral response. Upon binding viral dsRNA, OASs synthesize 2'-5'-oligo(A), which signals for the activation of the antiviral endoribonuclease, RNase L. Herein, we investigate the antiviral activities of human OAS isoforms during West Nile virus (WNV) infection. We show that OAS3 is required for initiating RNase L-mediated RNA decay in response to WNV, and its condensation on dsRNA at viral replication complexes initiates RNase L activation. OAS3 contains three distinct dsRNA-binding domains that enhance its ability to condense on dsRNA. The potential for OAS3 to condense on dsRNA is modulated by dsRNA loads and OAS3 expression level, which can be constitutively expressed and induced by type I interferons.

Presenter: Soleil Torres[1], Vaibhav Jain[1], Jiaqiang Zhu[2], Muhammed Naeem[3], Lauren Gay[1], Daniel Stribling[1,4], Melody Baddoo[3], Michael Harris[2], Erik Flemington[3], Scott Tibbetts[1], Rolf Renne[1,4,5]

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Poster Title: Functional and structural analysis of circ-vIRF4 in Kaposi's sarcoma-associated herpesvirus

Abstract: Circular RNAs (circRNA) are single-stranded RNAs that form a closed structure via backsplicing, are relatively stable and resistant to exonucleases, and implicated in human diseases. In Kaposi's sarcoma-associated herpesvirus (KSHV), the viral interferon regulatory factor 4 (vIRF4) locus expresses two circRNA isoforms with high expression in KSHV tumors, suggesting circ-vIRF4 may contribute to pathogenesis and/or tumorigenesis. To characterize the function of circ-vIRF4, we are utilizing genetic and structural studies. First, a KSHV mutant (Δcirc-vIRF4) lacking the splice donor site was generated in the BAC16 bacmid. RT-PCR of mutant-infected iSLK cells did not detect WT isoforms, but revealed a novel isoform that utilizes an alternative backsplice donor site. RNA-seq of the mutant during latency or reactivation demonstrated differential expression of host and viral genes. There were 322 and 1,187 differentially expressed genes (DEGs) in latent and lytic libraries, respectively, suggesting a role for circ-vIRF4 in gene expression regulation during both viral lifecycle phases via an unknown mechanism. Additionally, we are investigating the secondary structure of circ-vIRF4. Both isoforms are predicted to fold into highly structured molecules, and size exclusion chromatography indicates they exhibit low structural heterogeneity. To investigate specific structural elements, *in vivo* Selective 2′ Hydroxyl Acylation analyzed by Primer Extension Mutational Profiling (SHAPE-MaP) will be performed in infected BC3 cells. Preliminary results show circ-vIRF4 can be modified by 2-methylnicotinic acid imidazolide (NAI) and yield full-length PCR amplicons suitable for sequencing library preparation. With this approach, we aim to reveal the first high resolution structure of a human tumor virus-encoded circRNA.

Presenter: Sunfeel Akaphan

Sunfeel Akaphan[1,2,3], Indu Tripathi[1,3], Ashakiran Rochette[1,3], and Kotaro Fujii[1,2,3]

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Poster title: Molecular mechanisms and spatiotemporal dynamics of translation fidelity

Abstract: The regulation of gene expression at the level of mRNA translation is important for the specification of cell fate and the development of tissues. However, the mRNA translation machinery has the lowest fidelity compared to DNA replication and mRNA transcription. Our research focuses on identifying the factors that impact translation fidelity. Here, we began to look at the coding sequence of mRNAs. Leveraging the dual-luciferase gain-of-activity reporters to detect translation errors. We found that mRNA translation fidelity of reporter constructs correlates with the optimality of codon selection in the coding sequences in mouse embryonic stem cells (mESCs), suggesting that each transcript has differential translation fidelity defined by codon optimality. Next, we further hypothesized that the translation fidelity of each transcript might change between cell types. Preliminary data from our lab demonstrated that the brain has higher translation fidelity than mESCs and other tested organs. To test our hypothesis, I generated new mESC lines that each of our reporters is integrated into the genome to monitor temporal changes of translation errors. Leveraging these mESC lines, I developed in vitro neuron differentiation for the first time in the lab and revealed that neurons have higher translation fidelity than non-neuron cells. Together, my research provides the first insight into the regulation of translation fidelity between transcripts and reveals differential translation fidelity between cell types. This work will be the groundwork for understanding the spatiotemporal regulation of transcript-specific fidelity regulation.

Presenter: **Swapnil S. Joshi 1**, Loc T. Hyunh 1, Nidhi Kalia 1, Tianshou Liu 2, Anna M. Pyle 2, Michael E. Harris 1

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Poster Title: Discovery of Small Molecule Inhibitors of ribonuclease P from Staphylococcus aureus using assay based high throughput screening

Abstract: Antimicrobial resistance continues to present a formidable medical challenge and there is an urgent need to discover and develop new antibiotics. Highly structured RNAs play essential roles in numerous biological processes and many current antibiotics target rRNA, underscoring the potential for further discovery. Non-coding RNAs in bacteria offer a plethora of attractive but relatively unexplored targets. Bacterial RNase P contains a large (400nt) highly structured non-coding RNA (P RNA) that catalyzes an essential step in tRNA maturation. P RNA and the smaller protein unit (rnpA) are both essential genes in pathogenic bacteria by virtue of their role in supporting protein synthesis making RNase P an attractive target for developing new antimicrobials. Previous efforts to identify RNase P small molecule inhibitors have been unsuccessful or yielded leads with limited potential or targeted enzymes from non-pathogens. Using an established fluorescence polarization assay we screened three different small molecule libraries (total ~27000 compounds), including an RNA targeted collection, for inhibitors of S.aureus RNase P. We report the discovery of three novel small molecule inhibitors of RNase P from S.aureus with low micromolar potency(50µM-6µM). These compounds are specific to RNase P inhibition and do not form protein aggregates as a mechanism of inhibition. We also report two new assays to examine the specificity of the inhibitors towards RNase P inhibition. The small molecules preferentially inhibit the type B enzyme as compared to the ancestral type A enzyme. Furthermore, the small molecules also exhibit specificity between two different type B RNase P enzymes. An array of experimental tools available for studying RNase P assembly, substrate binding, and kinetics are now being employed to establish mechanism of action and identifying binding sites. Targeting essential non-coding RNAs represents a fertile area for RNA-based drug discovery, and our study of small molecule inhibitors of S. aureus RNase P represents an important step forward.

Presenter: Ukesh Karki[1], Kandarp Joshi[2,4], Prem Chapagain[1,4], Ranjan Perera[2,3]

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Poster Title: Medulloblastoma tumor single cell RNA-seq analysis reveals cell type specific differential editing patterns linked to prognosis and metastasis

Abstract: Adenosine-to-Inosine (A-to-I) RNA editing is a prevalent post-transcriptional modification. RNA editing is an essential process for normal physiological development in mammals and perturbations in editing events can lead to various defects including cancers. RNA editing is mediated by deaminase enzymes like ADARs. Medulloblastoma (MB) is one of the most common forms of pediatric brain tumor arising in the cerebellum. ADARs are dysregulated in MB patient tumors indicating a functional role of RNA editing. We reanalyzed single-cell RNA sequencing data from 28 MB patients to identify de novo RNA editing sites. Known polymorphisms were removed, and the resulting editing sites were merged with known RNA editing events from RADAR, DARNED and REDIportal. Editing frequencies were calculated for all editing sites as the ratio of G over A+G for each site. Differential editing and co-editing analysis was conducted between malignant and non-malignant cells, and functional roles were annotated. Survival analysis was performed using the survival R package. 388 differentially edited sites were identified, primarily associated with genes involved in catabolic processes. These sites presented a distinct cluster between malignant and nonmalignant cells. Weighted gene co-expression network analysis revealed turquoise module, whose sites mapped to genes with essential neurodevelopmental functions. Survival analysis revealed a significant association of editing frequency of seven genes with overall survival. Single-cell RNA sequencing study of medulloblastoma revealed elevated A-to-I RNA editing levels in malignant cells compared to non-malignant controls, underscoring the potential role of RNA editing in medulloblastoma pathogenesis and as a potential target for therapeutic intervention.

Presenter: Xiao Song

Affiliation: Northwestern University

Poster Title: A Single-Cell Atlas of Alternative Isoform Usage in Glioma-Infiltrating Macrophages

Abstract: Glioma is the most aggressive adult brain tumor, with limited therapeutic options and minimal success from immunotherapies. A major challenge is its immunosuppressive microenvironment, dominated by tumor-associated macrophages (TAMs), which include both tissue-resident microglia and monocyte-derived macrophages. Over the past decade, advances in single-cell RNA sequencing have expanded our understanding of the cellular heterogeneity in glioma TAMs and their roles in immunosuppression. However, most studies have focused on gene-level expression, overlooking isoform-level alterations—despite the fact that over 90% of human multi-exon genes undergo alternative splicing (AS) to generate multiple protein isoforms. To bridge this knowledge gap, we analyzed the isoform-level transcriptomic landscape in glioma TAMs using both short-read and long-read single-cell RNA sequencing. These analyses revealed distinct AS patterns between microglia and monocyte-derived macrophages, including an exon skipping (SE) event inCD74, a key regulator of MHC-II antigen presentation, as well as widespread alternative first exon usage (AFE) as a common regulatory mechanism in immune cells. In addition, an SE event inMS4A7correlated with clinical responses to anti-PD-1 therapy in both glioma and melanoma patients. These findings collectively underscore the importance of integrating both gene-centric and isoform-centric approaches to better understand the complexity of glioma immune microenvironment and to inform the development of more effective immunotherapeutic strategies.

Presenter: Yibo Zhao

Affiliation: Scripps Research graduate student

Poster Title: Compartment-specific translational mechanisms for synapse maintenance

Abstract: In neurons, modulation of compartmentalized translation is a key process underlying synaptic structural plasticity and long-term memory storage (LTM). However, whether compartment-specific and cell-specific translation play key roles in synapse formation, maintenance, and plasticity remains elusive. My study seeks to understand this gap in knowledge, utilizing the model *Aplysia californica*. Our recent investigations into dynamic changes in organelle transport in presynaptic sensory neurons discovered an enhanced bidirectional transport of mitochondria associated with synapse maintenance. This enhancement in transport is dependent upon translation and transcription. Building upon this finding, I hypothesize that compartment-specific regulations of translation contributed to this change in transport, and play an important role in synapse formation, maintenance, and plasticity. My puromycin labeling experiments have identified a global increase in translation in sensory neurons when it forms a functional synapse with target motor neuron L7. Analysis of immediate changes in sensory neurons suggests temporal enhancements in translation in axonal and somatic compartments within 24 hours after the formation of a functional synapse. These studies illuminate the role of translational regulation in modulating synapse function.

Presenter: Zhuangyue Li

Affiliation: University of Florida

Poster Title: Elucidating mechanisms of kinesin-dependent RNA localization in mouse muscle

Abstract: RNA localization and local translation at distal regions of differentiated cells is an important and ubiquitous phenomenon to maintain cell health and function. Although it has been extensively studied in neurons, it has been relatively understudied in muscle. We and others previously found that in cardiomyocytes and skeletal myofibers, mRNAs disperse along microtubules from perinuclear regions to populate the cytoplasm. However, there are 45 kinesin motors, and whether specific kinesins carry certain RNAs is not known. We have built a proximity labeling system that allows capture of RNAs in different regions of myofibers. By fusing TurboID to a KASH domain, we can selectively biotinylate perinuclear regions, and by fusing TurboID to telethonin, we can selectively biotinylate sarcomeres. We have used retro-orbital injection of the MyoAAV capsid to deliver these cargoes to mice, and subsequently provided biotin-supplemented water to the mice to perform in vivo proximity labeling. Tissue harvest and pull-down of biotinylated material can then be analyzed by various methods, including RNAseq. To determine whether specific kinesins preferentially transport specific mRNAs, we also co-injected dominant negative kinesin tails for three muscle-enriched kinesins (KIF1C, KIF1Bα, and KIF5B) and again performed proximity labeling in the perinuclear and sarcomeric regions. Analysis of RNAseq data from these experiments is ongoing and will reveal specificity between kinesin tails and mRNA cargoes in myofibers. Overall, this study will elucidate mechanisms of RNA localization in myofibers and cardiomyocytes, and improve our overall understanding of mechanisms of health and disease in cardiac and skeletal muscle.