



RNA: From Biology to Drug Discovery 2025

Talk Abstracts

Claes Wahlestedt, MD, Ph.D.

RNA Targeted Gene Upregulation

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Therapeutic oligonucleotides can be utilized not only to suppress gene expression but also to achieve gene upregulation through targeting of RNAs controlling various components of the gene expression machinery. Notably, perturbation of endogenous non-coding natural antisense transcripts (NATs; a sub-class of long noncoding RNAs) (1) by AntagoNAT oligonucleotides, *in vitro* or *in vivo*, most typically reveals discordant inhibitory regulation and results in locus specific up-regulation of protein-coding gene expression. AntagoNATs are oligonucleotides that antagonize NATs either by blocking or cleaving them (2).

In the past, we identified AntagoNATs for upregulation of some 100 human protein coding genes of medical interest. For example, one such project is based on targeting a NAT controlling the expression of a brain sodium channel, SCN1A, with an AntagoNAT designed to restore haploinsufficient SCN1A protein expression in Dravet disease, a severe form of childhood epilepsy.

This lecture will describe a few novel aspects of gene upregulation technologies (3) and assess synergies of combinatorial strategies with oligonucleotides having distinct RNA targets and mechanisms of action relating to gene expression.

1. Katayama *et al.* Antisense transcription in the mammalian transcriptome. *Science* 309:1564-6 (2005).
2. Wahlestedt C. Targeting noncoding RNA to therapeutically up-regulate gene expression. *Nature Reviews Drug Discovery* 12:433-46 (2013).
3. Khorkova *et al.* Amplifying gene expression with RNA-targeted therapeutics. *Nature Reviews Drug Discovery* 22, 539–561 (2023).



Elias Sayour, MD, Ph.D.

RNA Damage Mimicry Restores Immune Surveillance

Neurosurgery and Pediatrics, University of Florida

We will discuss novel mRNA formulations that challenge existing paradigms such as need for packaging into nanoparticle cores, and need for delivery systems that localize to immune cells. Through multilamellar formulation and intravenous administration of mRNA particles, immune cells traffic to sites of localization (instead of particles to immune cells). This allows for creation of non-specific mRNA approaches that can be leveraged as universal tools to sensitize response to immunotherapy and personalized approaches to overcome evolutionary cancer biology.

Ezgi Hacisuleyman, Ph.D.

Decoding Local RNA-Mediated Mitochondrial Regulation in Synapses

The Herbert Wertheim University of Florida Scripps Institute for Biomedical Innovation and Technology, Jupiter, FL, USA

Neurons rely on precise spatial and temporal RNA regulation to support their asymmetric architecture and ability to respond rapidly to dynamic stimuli. At synapses—far from the soma—RNA localization and localized translation are critical for activity-dependent remodeling and plasticity. Synaptic plasticity, in turn, depends on local protein synthesis and the intricate coordination between neuronal activity and mitochondrial function. Yet, the post-transcriptional and translational mechanisms that support these local energetic and plasticity-related demands remain incompletely understood. To address this, we developed innovative proximity labeling platforms combined with transcriptome, translome, and proteome analyses. These approaches revealed depolarization-induced transcripts involved in synaptic plasticity and uncovered novel translational mechanisms, including the roles of upstream open reading frames (uORFs) and the noncanonical initiation factor eIF4G2 in dendritic translation. A subset of these RNAs plays key roles in mitochondrial regulation.

Using super-resolution imaging and reporter assays, we show that synaptic stimulation alters mitochondrial shape and triggers local mitochondrial accumulation and activity, along with the activity-dependent translation of these RNAs and uORFs. Notably, we demonstrate that uORFs are translated into small peptides, opening new avenues for



exploring local gene regulation in neurons. Our findings reveal how synaptic energy demands are met through localized mitochondrial gene regulation and expand our understanding of the spatial organization of gene expression underlying synaptic plasticity.

James Burke, Ph.D.

Condensation of dsRNA Sensors at Viral Replication Complexes Initiates Antiviral Signaling Cascades

The Herbert Wertheim University of Florida Scripps Institute for Biomedical Innovation and Technology, Jupiter, FL, USA

Protein kinase R (PKR) is a critical component of the innate immune response to viruses. Herein, we use super-resolution microscopy, proximity ligation assays, and immunogold transmission electron microscopy to examine the process of PKR activation in response to pathogenic coronaviruses, including MERS-CoV and SARS-CoV-2. We demonstrate that PKR activation initiates on viral dsRNA associated with viral replication complexes within double membrane vesicles. Activated PKR then assembles into condensates termed dsRNA-induced oligomerized PKR (DROPs), which disassociate from viral dsRNA/DMVs and dissolve, releasing activated PKR in the cytosol where it phosphorylates eIF2a to initiate global translation arrest and stress granule assembly. Our data support that the nsp15 and NS4a proteins encoded by MERS-CoV synergistically limit PKR activation by reducing dsRNA loads within viral replication complexes and inhibiting PKR condensation, respectively. PKR-DROPs assemble in response to several positive-strand RNA viruses. These findings define the subcellular localization and molecular processes of PKR activation.



Matt Disney, Ph.D.

RNA – Easy to Spell, Hard to Drug

The Herbert Wertheim University of Florida Scripps Institute for Biomedical Innovation and Technology, Jupiter, FL, USA

One major scientific challenge is to understand biological pathways and to exploit the targets within them for therapeutic development. Coding and non-coding RNAs both directly cause disease, whether by mutation or aberrant expression. Akin to proteins, RNA structure often dictates its function in health or dysfunction in disease. RNA, however, is generally not considered a target for small molecule chemical probes and lead medicines, despite its immense potential. The focus of our research program is to uncover fundamental principles that govern the molecular recognition of RNA structures by small molecules to enable design of chemical probes that targeting disease relevant RNA structures to perturb and study their function.

In this talk, I will describe using evolutionary principles to identify molecular recognition patterns between small molecules and RNA structures by studying the binding of RNA fold libraries to small molecule libraries. The resultant, privileged interactions are computationally mined across the human transcriptome to define cellular RNAs with targetable structure. Such an approach has afforded bioactive interactions that have uncovered new biology, where the small molecules bind to functional structures within a target RNA. Recently, we have devised a strategy to imbue biologically silent RNA-small molecule interactions with cellular activity. In particular, chimeras comprising an inactive small molecule and ribonuclease recruiter trigger targeted degradation of disease-causing RNAs. These degraders affect the biology of RNA in specific ways in cells and in mouse models of various diseases and can rationally reprogram protein-targeted medicines for RNA.

Matthew Pipkin, Ph.D.

Transcriptional dynamics that establish protective memory CD8 T cells

The Herbert Wertheim University of Florida Scripps Institute for Biomedical Innovation and Technology, Jupiter, FL, USA

Individual naive CD8 T cells are quiescent precursors which can differentiate into diverse effector and memory CD8 T cell populations that provide adaptive immunity following their initial activation in response to intracellular infections, malignancy or vaccination. However, the early remodeling of chromatin structure and transcriptional control that establishes diversity between these mature CD8 T cell states and instills “memory” in their long-term protective capacities remain ill-defined. We used single cell RNA sequencing and RNA velocity inference to define how initial lineage trajectories establish phenotypic diversity from naive CD8 T cells responding to viral infection. In conjunction, large-scale RNA interference-based screens were used to identify transcription factors and chromatin regulatory factors that function early during naive CD8 T cell priming to establish chromatin structure that defines formation of long-term protective memory CD8 T cells.

Michalina Janiszewska, Ph.D.

Oncofetal RNA binding protein IMP2 – from cancer to structural biology

The Herbert Wertheim University of Florida Scripps Institute for Biomedical Innovation and Technology, Jupiter, FL, USA

IMP2 is an mRNA binding protein involved in cell proliferation, motility and metabolism by controlling transcript stability, localization and translation. It is highly expressed during embryonal development and can provide multifactorial advantage to cancer cells able to re-express this protein. Previously, we have shown that IMP2 is essential for survival of cancer stem-like population in adult glioblastoma, the most aggressive brain tumor. These stem-like cells are responsible for sustaining cellular diversity, driving resistance to chemo- and radiotherapy. Targeting IMP2 could thus provide a therapeutic avenue to limit the evolution of the highly heterogeneous brain tumors.

Thus far only two weak inhibitors of IMP2 were reported. Therefore, in collaboration with Forli lab at Scripps Research, we performed an *in silico* screening for tool compounds covalently binding to new pockets in IMP2. Our experimental validation of the



compounds revealed 2/21 tested molecules to impact thermal stability of IMP2 in cell lysate. Interestingly, the binding did not occur on predicted lysine. We hypothesized that formation of an oligomeric state might be the culprit. Using biophysical and structural methods including mass photometry, hydrogen-deuterium exchange coupled to mass spectrometry (HDX-MS), and small angle x-ray scattering (SAXS), we found that full-length IMP2 forms multiple oligomeric states but predominantly adopts a dimeric conformation. Upon RNA binding, IMP2 forms a pseudo-symmetric dimer different from its apo/RNA-free state. Moreover, formation of IMP2 oligomeric species, which includes dimers and higher-order oligomers, is sensitive to ionic strength and RNA binding. Our findings provide the first insight into the structural properties of full-length IMP2, which may lead to novel opportunities for disrupting its function.

Rolf Renne, Ph.D.

Kaposi's Sarcoma-Associated Herpesvirus-Encoded lncRNA Induces Aberrant Host Splicing by Sequestering Splicing Factors During Lytic Replication

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Kaposi's sarcoma-associated herpesvirus (KSHV) encodes multiple noncoding RNAs which contribute to viral latency and persistence, host gene regulation, and immune evasion. The Antisense-to-Latency Transcript (ALT) is a 12.4 kb long noncoding RNA located on the opposite strand of the major latency-associated region. ALT is a nuclear lncRNA that is expressed at low levels during latency but highly upregulated during lytic replication. Using RNA antisense purification (RAP) under PAR-CLIP condition and mass spectrometry, we identified 51 human and 3 KSHV proteins that directly interact with ALT, 48 of which are splicing factors (SFRs), including core spliceosome components, U2AF2, PTBP1/2, and SRSF1/3. Predicted binding sites for splicing



factors on ALT overlapped with PAR-CLIP induced T to C transitions on the RAP assay purified ALT RNA further validating direct ALT/SFR interactions. Single molecule fluorescent *in situ* hybridization combined with IFA revealed that ALT/SFR interactions form intranuclear domains of different size and shape during lytic replication. To probe for functional consequences of ALT/SFR interactions, RNAseq analysis was performed and revealed thousands of host gene splicing changes including a large number of exon skipping events in cells of lymphoid and epithelial origin. Many of these exon skipping events are predicted to induce nonsense mediated decay. In addition, perturbation of ALT by either transient knockdown or ectopic expression in the absence of viral infection altered splicing events. Finally, we demonstrated that knock-down of ALT strongly inhibits viral reactivation and virion production. Hence, ALT-dependent sequestering of splicing factors, interferes with host cellular gene expression. Our results uncover a novel host shut-off mechanism to shift gene expression from the host to the virus and to antagonize essential cellular processes including potential host immune defenses during reactivation.

Sathya Puthanveetil, Ph.D.

Unlocking lncRNA functions at the synapse

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Long non-coding RNAs (lncRNAs) have emerged as key regulators of long-term memory (LTM) formation, yet their roles beyond the nucleus remain poorly understood. While nuclear-enriched lncRNAs are well known for orchestrating transcriptional and epigenetic landscapes, our recent work reveals that specific cytoplasmic lncRNAs are actively transported from the soma to dendrites in neurons. Once localized, these lncRNAs act as dynamic scaffolds, assembling multi-molecular complexes that include coding RNAs, other non-coding RNAs, and regulatory proteins. In this presentation, I will highlight the molecular mechanisms driving the expression, localization, and dendritic transport of these lncRNAs, as well as their real-time dynamics in response to neuronal activity. I will also discuss their role in modulating synaptic plasticity and the implications of these findings for understanding RNA-based regulation of memory. These insights not only expand our understanding of lncRNA biology in neurons but may also open new avenues for RNA-targeted strategies in cognitive and neurodegenerative disorders.



Yanjun Li, Ph.D.

AI-driven RNA-Small Molecule Interaction Prediction

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Accurate prediction of RNA-ligand interaction is crucial for RNA-targeted drug discovery by complementing experimental screens. However, current prediction approaches often exhibit unsatisfactory scoring accuracy. Additionally, commonly used docking-based methods are highly time-consuming, leading to prohibitive computational costs when applied to ultra-large chemical libraries. In this talk, I will introduce a novel sequence-based RNA-ligand interaction prediction approach, SmartBind, which leverages advances in contrastive learning and pre-trained RNA foundation models to accurately and efficiently predict RNA-small molecule interactions. Our approach aligns the representations of binding RNA sequences and small molecules in a shared feature space while contrasting nonbinding molecules, without explicitly relying on limited binding affinity data. By bypassing the need for RNA structure, pocket information, and docking process, our model predicts binding based on the distance between learned representations of RNA and small molecules. Meanwhile, our versatile SmartBind model can also predict RNA-ligand binding sites for a given RNA and active binders, providing deeper interaction insights. Extensive experiments demonstrate that SmartBind significantly outperforms current state-of-the-art deep learning-based methods and conventional docking-based methods across various benchmark datasets and case studies, while substantially reducing computational cost compared to docking-based methods. These promising results highlight the potential of SmartBind in RNA-targeted small molecule drug discovery, offering an effective and efficient virtual screening strategy, especially for RNAs lacking high-quality 3D structural information.