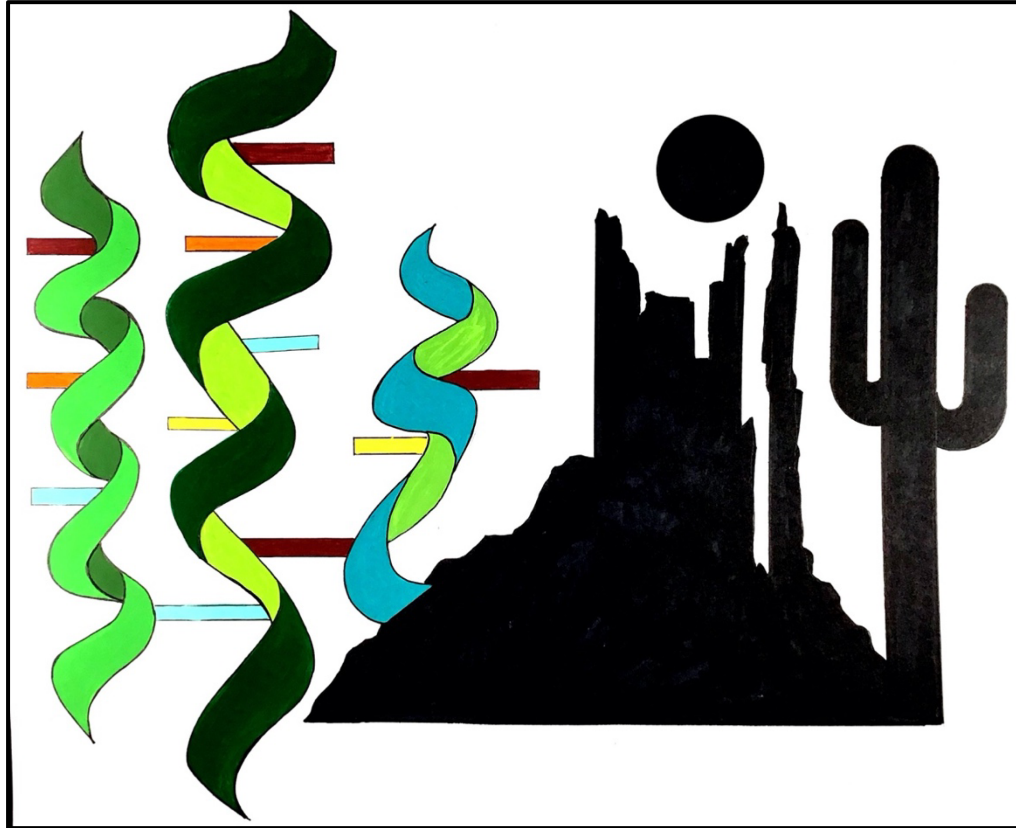
5th ARIZONA RNA SYMPOSIUM

March 14th, 2025 – 9:00 am – 4:00 pm

Biodesign Auditorium: 727 E Tyler St, Tempe, AZ 85281



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The RNA Society is a non-profit international scientific society with more than 1,800 members dedicated to fostering research and education in the field of RNA science. The Society hosts a peer-reviewed scientific journal, RNA, and an annual scientific conference, along with sponsoring other RNA-related scientific conferences through direct financial support and student travel grants. Mostly, The Society is a community of scientists who are passionate about better understanding the fascinating world of RNA biology. (https://www.rnasociety.org)

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Visitors can park in any ASU lot or parking structure. The closest parking structures to the Biodesign Institute are the Tyler Parking Structure (north of Biodesign at McAllister Avenue and Tyler Street) and the Rural Road Parking Structure (south of Biodesign, at Rural Road between Terrace and Lemon). Visitors can enter and exit through any entrance at either garage. For current parking rates, visit the ASU parking website. Visa, MasterCard and cash are accepted.

The visitor entrance is located on the northeast corner of the Biodesign B building, which faces Tyler Street.

To get to our facility via Valley Metro Light Rail, get off at the University Drive/Rural Road Stop and head southwest one-half block to our front door. Tyler Street is a dead end and does not provide vehicle access to Rural Road.

Biodesign Auditorium - Biodesign Building B; 727 E Tyler St, Tempe, AZ 85281

Welcome Remarks

We are delighted to welcome you to the **5th Arizona RNA Symposium**. This event brings together leading experts and emerging researchers in RNA biology to share groundbreaking discoveries and foster collaborations. We thank our sponsors, speakers, and attendees for making this symposium a success.

Organizers:
Shalini Sharma (University of Arizona) & Marco Mangone (Arizona State University)

Symposium website: http://arizonarnasalon.org/

Symposium Schedule

Morning Session

8:30 – 9:00 AM
Registration and Breakfast

9:00 – 9:15 AM
Welcome Remarks

9:15 – 9:30 AM
Oral Presentation 1: Structural Determinants and Biochemical Characterization of U1-SL3 Selective Binding by RNA Helicase UAP56
Ryan Yellamaty, Jason Wong, Shalini Sharma (University of Arizona)

9:30 – 9:45 AM
Oral Presentation 2: Nuclear export of TDP-43 is facilitated by ADAR2-mediated RNA editing
Dominic Julian, Stephen Moore, Ileana Lorenzini, Michael McMillan, Eric Alsop, Sam Macklin-Isquierdo, Erik Lehmkuhl, Petr Kalab, Lindsey Hayes, Daniela Zarnescu, Kendall Van-Keuren Jensen, Sami Barmada, Rita Sattler (Barrow Neurological Institute)

9:45 – 10:00 AM
Oral Presentation 3: A Degenerate Telomerase RNA Directs Telomeric DNA Synthesis in Lepidopteran Insects
Yu-Shu Chou, Dhenugen Logeswaran, Chi-Nga Chow, Phoebe L. Dunn, Joshua D. Podlevsky, Tianxiang Liu, Khadiza Akhter, Julian J.-L. Chen (Arizona State University)

10:00 – 10:15 AM
Oral Presentation 4: Integrating Left-Handed Z-DNA into DNA Nanostructures
Aleksandra Petrova, Liangxiao Chen, Cong Li, Olivia Holman, Ryan Truong, Anuvi Batra, Hao Yan, Di Liu (Arizona State University)

10:15 – 10:35 AM
Lexogen Presentation – **Brenna Lobb**

10:35 – 10:55 AM
Coffee Break

11:00 AM – 12:00 PM
Keynote Presentation 1: *“Nonsense-mediated mRNA Decay and Human Disease”*

Lynne Maquat
Director, Center for RNA Biology
J. Lowell Orbison Endowed Chair and Professor
Department of Biochemistry and Biophysics
University of Rochester Medical Center

12:00 – 12:10 PM
Group Photo. Gather at the registration desk

12:10 – 1:00 PM
Lunch Break

Afternoon Session

1:00 – 2:00 PM
Poster Session & Networking

2:00 – 3:00 PM
Keynote Presentation 2: *“FitRNAs for Duty: Free Introns of tRNAs as Complementarity-dependent Regulators of Gene Expression”*

Anita Hopper
Professor, Department of Molecular Genetics
College of Arts and Science
Ohio State University

3:00 – 3:45 PM
Mentoring Session with Keynote Speakers – *Ask them anything!*

3:45 – 4:00 PM
Awards & Closing Remarks

Keynote Presentation 1:

Dr. Lynne Maquat

J. Lowell Orbison Endowed Chair and Professor of Biochemistry & Biophysics, Pediatrics, and Oncology
Founding Director of the Center for RNA Biology
Founding Chair of Graduate Women in Science
University of Rochester
Rochester, NY

Nonsense-mediated mRNA Decay and Human Disease

Dr. Maquat earned her PhD in Biochemistry from the University of Wisconsin-Madison and completed postdoctoral work at the McArdle Laboratory for Cancer Research. She has held positions at Roswell Park Cancer Institute and the University of Rochester Medical Center. Her research focuses on mRNA decay mechanisms, including her discovery of nonsense-mediated mRNA decay (NMD) in 1981, the exon-junction complex (EJC), and Staufen-mediated mRNA decay. She has also defined a novel microRNA degradation mechanism and explores therapeutics for diseases with hyperactivated NMD, such as Fragile X Syndrome. Maquat is a member of the National Academy of Sciences, the National Academy of Medicine, and a recipient of major awards, including the Canada Gairdner International Award, the Wolf Prize in Medicine, and the Gruber Genetics Prize. She also advocates for women in science and serves on the Council of Scientific Advisors for the International Centre for Genetic Engineering and Biotechnology.

Keynote Presentation 2:

Dr. Anita Hopper

Professor
Department of Molecular Genetics
The Ohio State University
Columbus, OH

FitRNAs for Duty: Free introns of tRNAs as Complementarity-dependent Regulators of Gene Expression

Dr. Anita K. Hopper earned her Ph.D. in Cell Biology from the University of Illinois, Urbana, and completed postdoctoral research at the University of Washington. Dr. Hopper is a pioneer in RNA biology, with major contributions to understanding RNA processing and the subcellular distribution of RNAs and proteins. Her research focuses on the intracellular trafficking of tRNAs. While most RNAs are synthesized in the nucleus and function in the cytoplasm, many proteins follow the opposite path. Using genetic, biochemical, and cell biological approaches, her team discovered parallel nuclear export pathways and quality control mechanisms involving translation machinery. They also revealed that tRNAs undergo retrograde movement, returning from the cytoplasm to the nucleus under stress conditions, a process conserved in metazoans. This unexpected discovery highlights how tRNA trafficking regulates protein synthesis during nutrient stress and serves as a quality control mechanism for maintaining cellular homeostasis. Dr. Hopper has held leadership roles in the scientific community, including serving as President of the RNA Society (2002-2003) and Secretary of the Genetics Society of America (2003-2006). She is a Fellow of the American Academy of Microbiology, the American Association for the Advancement of Science, and the American Academy of Arts & Sciences. Among her numerous accolades, she is a recipient of the RNA Society's Lifetime Achievement Awards in Service (2009) and Science (2015).

Abstracts

1. **Structural Determinants and Biochemical Characterization of U1-SL3 Selective Binding by RNA Helicase UAP56**
2. **Nuclear export of TDP-43 is facilitated by ADAR2-mediated RNA editing**
3. **A Degenerate Telomerase RNA Directs Telomeric DNA Synthesis in Lepidopteran Insects**
4. **Integrating Left-Handed Z-DNA into DNA Nanostructures**
5. **Spatial Multi-omics in Situ Analysis of RNA-Protein Interactions in Alzheimer's Brains**
6. **Is Cap2 Superior to Cap1 in mRNA Translation? Facile Synthesis of Cap2 mRNAs**
7. **Controlled Release of Antigen in Self-adjuvanting Tumor Vaccine**
8. **Highly Sensitive and Multiplexed RNA Isoform Imaging in Human FFPE Tissues**
9. **The Interplay of Helicases, G-quadruplexes, and R-loops in Transcription-Replication Conflicts and Genome Stability**
10. **The Role of CD36 in Erythroid Differentiation of Human Hematopoietic Stem and Progenitor Cells**
11. **Erythropoietin-dependent Acquisition of CD71hiCD105hi Phenotype within CD235a− Early Erythroid Progenitors**
12. **NEDD4 and ESCRT regulate endolysosomal clearance of the RNA-binding protein TDP-43**
13. **RNA-binding proteins G3BP1 and G3BP2 promote cell cycle re-entry and cell survival during recovery from DNA replication stress**
14. **A comprehensive analysis of 3'UTRs in Caenorhabditis elegans**
15. **Strand Secrets: Uncovering Tissue-Specific Patterns behind microRNA Strand Selection Using the HiTmiSS Assay**
16. **Characterizing Cellular Clearance Mechanisms of TDP-43 and ALS-Associated Isoforms**
17. **Nanoarchitectural engineering of DNA and RNA for biological discovery**
18. **Coarse-grained modeling of RNA for biomedical applications**
19. **Structured mRNA Origami for Delivery of Gene Therapies**

1: Structural Determinants and Biochemical Characterization of U1-SL3 Selective Binding by RNA Helicase UAP56

Ryan Yellamaty[1,2], Jason Wong[2] and Shalini Sharma[2]; [1]School of Life Science, Arizona State University; [2]University of Arizona College of Medicine – Phoenix

UAP56 is a DExD-box RNA helicase with essential roles in pre-mRNA splicing and nucleo-cytoplasmic export of mature mRNA. Biochemically, UAP56 has been characterized as an RNA-dependent ATPase and helicase without sequence specificity. Recent structural studies have elucidated its key role in recruitment of export factors to mRNP complexes. While UAP56 has been shown to be required for A complex formation, its precise function in the early stages of spliceosome assembly remains unclear. Previously, we found that UAP56 plays an important role in facilitating the cross-intron contact between pre-mRNA bound U1 and U2 snRNPs. It selectively binds to U1 snRNA stem-loop 3 (U1-SL3) and promotes the interaction between U1 snRNA stem-loop 4 (U1-SL4) and the U2 protein SF3A1 in an ATP-dependent manner. In this study, we investigated the molecular basis of the selective interaction between UAP56 and U1-SL3 and how this binding may influence enzymatic activity. Our results show that U1-SL3 but not U1-SL4 selectively induces the ATPase and helicase activities of UAP56, with U1-SL3 being unwound at a significantly faster rate compared to U1-SL4. RNA binding analysis indicates that the stem sequence, cytosine bulge, and base pairing at the lower stem of U1-SL3 are crucial for selective recognition by UAP56. Furthermore, analysis of truncated UAP56 proteins identified two critical regions—spanning amino acids 34–44 in the N-terminus and 415–428 in the C-terminus—that contribute to this selective binding. These findings demonstrate that UAP56's enzymatic activity is selectively activated by U1-SL3, with recognition driven by both structural elements in U1-SL3 and distinct regions within UAP56. This mode of U1-SL3 recognition differs from UAP56's interaction with single-stranded RNA, where binding primarily relies on contacts with the phosphodiester backbone. Thus, our study provides new insights into the molecular interactions that govern splice site pairing and the commitment to intron removal.

2: Nuclear export of TDP-43 is facilitated by ADAR2-mediated RNA editing

Dominic Julian* (1,2), Stephen Moore (1), Ileana Lorenzini (1), Michael McMillan (3), Eric Alsop (4), Sam Macklin-Isquierdo (5), Erik Lehmkuhl (5), Petr Kalab (6), Lindsey Hayes (6), Daniela Zarnescu (7), Kendall Van-Keuren Jensen (4), Sami Barmada (3), Rita Sattler (1)

(1) Barrow Neurological Institute, Phoenix, AZ, USA; (2) University of Arizona College of Medicine – Phoenix, Phoenix, AZ, USA; (3) University of Michigan, Ann Arbor, MI, USA; (4) Translational Genomics Research Institute, Phoenix, AZ, USA; (5) University of Arizona, Tucson, AZ, USA; (6) Johns Hopkins University School of Medicine, Baltimore, MD, USA; (7) Penn State College of Medicine, Hershey, PA, USA

TAR DNA binding protein – 43 (TDP-43) is a critical RNA binding protein involved in multiple steps of RNA processing, including transcription, splicing, RNA transport, stability, localization, and translation. TDP-43 is known to accumulate and form prion-like solid aggregates in the cytoplasm of cells. This behavior of TDP-43 has been well established as a pathological hallmark of a neurodegenerative disease spectrum encompassing amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD) and has been described in Alzheimer’s disease and related dementias. TDP-43 pathology has been hypothesized to contribute to disease pathogenesis through either nuclear depletion, leading to the loss of function, and/or cytoplasmic aggregation, leading to a toxic gain of function. Despite extensive research, mechanisms that initiate this pathology under disease conditions remain elusive. Recent studies in our laboratory described aberrant adenosine-to-inosine (A-I) RNA editing in multiple brain regions of C9orf72 ALS/FTD, where we detected bidirectional changes of A-I editing. Since then, we have generated preliminary data suggesting that TDP-43 nuclear export can be regulated via Adenosine Deaminase Acting on double stranded RNA (ADAR)-mediated A-I RNA editing. We show that increased A-I RNA editing activity in mammalian cell lines induces TDP-43 translocation to the cytoplasm, mimicking what is observed in disease. In contrast, the presence of catalytically inactive ADAR2 or TDP- 43 carrying mutations within its RNA binding domains does not alter the nuclear localization of TDP-43. We further established that inosine-containing UI RNA oligomers can bind to TDP-43 in vitro, acting as a TDP-43 UG RNA binding motif mimic. These findings demonstrate that aberrant increases in A-I editing induces TDP-43 nuclear export through an RNA-dependent mechanism.

3: A degenerate telomerase RNA directs telomeric DNA synthesis in lepidopteran insects

Yu-Shu Chou a , Dhenugen Logeswaran a , Chi-Nga Chow a , Phoebe L. Dunn b , Joshua D. Podlevsky a , 1 , Tianxiang Liu a , Khadiza Akhter a , and Julian J.-L. Chen a

a School of Molecular Sciences, Arizona State University, Tempe, AZ 85281 b School of Life Sciences, Arizona State University, Tempe, AZ 85281 1 Molecular and Microbiology Department, Sandia National Laboratories, Albuquerque, NM 87185.

Most species solve the end replication problem by developing telomerase, a ribonucleoprotein complex. Telomerase adds telomere repeats to chromosome ends, preserving genome stability and cellular function by preventing DNA damage repair mechanisms and cell quiescence. Although telomerase has been extensively studied across eukaryotic kingdoms, the existence of insect telomerase remained unclear for decades because of the difficulty in identifying telomerase RNA (TR). In this study, we used a combination of bioinformatics and biochemical methods to uncover the presence and function of insect telomerase. We purified lepidopteran Spodoptera frugiperda telomerase holoenzyme and identify the 135-nucleotide S. frugiperda telomerase RNA (sfTR). The sfTR gene is the smallest TR, transcribed by RNA polymerase II and featuring an m7G cap without additional hypermethylation, in contrast to other animal. This is associated with the loss of the H/ACA domain. Moreinterestingly, the sfTR has only pseudoknot-template domain and lack the indispensable stem-loop junction important for telomerase activity in other species. However, the functionality of S. frugiperda telomerase reverse transcriptase (sfTERT) and sfTR is validated again after reconstituting telomerase in vivo, we successfully sequenced the synthesis of telomeric repeats. This discovery highlights the diversity of telomerase and underscores the need for further research to understand the compensatory mechanisms that enable insects to maintain telomere function despite the absence of certain canonical telomerase motifs in sfTERT and sfTR.

4: Integrating Left-Handed Z-DNA into DNA Nanostructures

Aleksandra Petrova, Liangxiao Chen, Cong Li, Olivia Holman, Ryan Truong, Anuvi Batra, Hao Yan, and Di Liu

Biodesign Institute, Arizona State University

The left-handed Z-nucleic acid conformation has attracted significant interest for its unique structural and biological properties since its discovery in the 1970s. While B-DNA underpins DNA nanotechnology, Z-DNA offers distinct topological advantages but remains underutilized due to its reliance on high salt concentrations and CG-rich sequence constraints. To overcome these limitations, we employed topological constraint principles and ribose modifications to enable stable Z-DNA formation in 1D linear arrays, 2D honeycomb lattices, discrete DNA rings, and 3D tetrahedra. We also attempted Z-RNA incorporation into DNA rings as part of ongoing efforts to expand the design space of left-handed nucleic acids in nanotechnology. Given the emerging role of Z-nucleic acids in immune regulation—particularly through interactions between Z-DNA-binding proteins such as ADAR1 and ZBP1 and viral Z-RNA—this work advances structural DNA nanotechnology while providing new avenues to explore their roles in biological regulation.

5: Spatial multi-omics in situ analysis of RNA-Protein interactions in Alzheimer's brains

Nishinki Thakshana De Silva Muthumuni, and Jia Guo (PhD)

Biodesign Institute & School of Molecular Sciences, Arizona State University, Tempe, AZ 85287, USA; mndesilv@asu.edu, jiaguo@asu.edu

Alzheimer's Disease (AD) is the most prevalent neurodegenerative disorder, primarily affecting individuals aged 65 and older. Due to the lack of effective disease-modifying treatments and its profound societal impact, studying AD at the molecular level is essential for developing targeted therapies. Emerging evidence suggests that RNA-protein interactions, which are critical for cellular function and neuroprotection, are dysregulated in AD. RNA-binding proteins (RBPs) play a key role in maintaining neuronal integrity, and their malfunction has been implicated in transcriptomic and proteomic dysregulation observed in AD brains. To investigate these alterations, we developed a novel spatial multi-omics approach combined with click chemistry to analyze the spatial distribution of RNA-protein interactions in AD brains. Formalin-fixed paraffin-embedded (FFPE) hippocampal tissues from healthy and AD donors were examined using Tyramide Signal Amplification (TSA). PPIB was selected as the target RNA, while hnRNP K, a nuclear RNA-binding protein known to interact with PPIB, was used as the protein marker. The experimental workflow included Hybridization Chain Reaction-In Situ Hybridization (HCR-ISH) for RNA detection, immunofluorescence-based protein labeling, click chemistry-mediated proximity ligation, and signal cleavage using reducing agents. Fluorescence imaging was performed before and after cleavage to assess interaction-dependent signal loss. In healthy tissues, nuclear PPIB signals remained stable after cleavage, while cytoplasmic signals showed a significant reduction, indicating RNA-protein interactions primarily occurring in the nucleus. In contrast, AD tissues exhibited a marked decrease in nuclear PPIB fluorescence post-cleavage, suggesting disrupted RNA-protein interactions. Negative controls using histone H3 confirmed these findings by demonstrating the absence of a click reaction in the nucleus, indicating that mere nuclear localization is insufficient for interaction. Cleavage efficiency was quantified from 250 randomly selected PPIB signal spots in the nucleus and cytoplasm, and statistical analysis (t-test) confirmed significant differences between healthy and AD tissues. These observations align with previous findings, reinforcing the reliability and reproducibility of the data. Future studies will expand the analysis to distinct cellular populations using specific markers, investigate variations across different stages of AD, and explore sex-based differences. Additionally, other brain regions, such as the cerebral cortex, will be examined to provide a comprehensive and spatially resolved understanding of RNA-protein interactions in AD progression.

6: Is Cap2 Superior to Cap1 in mRNA Translation? Facile Synthesis of Cap2 mRNAs

Cong Li‡, Xinyi Tu‡, Aleksandra Petrova, Lanshen Zhao, Hao Yan, Di Liu*

Center for Molecular Design and Biomimetics, The Biodesign Institute, Arizona State University, Tempe, Arizona 85281, United States

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The 5’ cap of eukaryotic mRNA is crucial for stability, translation initiation, and innate immune evasion. Eukaryotic mRNAs are typically capped as cap0 (m7GpppN), cap1 (m7GpppNm), or cap2 (m7GpppNmNm), with cap1 and cap2 featuring 2'-O-methylation on the first or the first two nucleotides, respectively. Innate immune sensors, such as RIG-I and IFIT proteins, preferentially recognize uncapped or improperly capped RNAs. Although cap2 mRNAs have been shown to provide the strongest protection against innate immune recognition, studies comparing the translation efficiency of cap2 versus cap1 mRNAs have yielded contradictory results, likely due to variations in synthesis efficiency. Here, we describe a simple, reliable, and scalable method for synthesizing cap2 mRNAs. Our approach utilizes a priming trinucleotide, ppAmAmG (where “Am” denotes 2'-O-methyladenosine), which is efficiently installed at the 5' end of mRNA co-transcriptionally, followed by the addition of m7G using the vaccinia capping enzyme, achieving nearly 100% capping efficiency. Dual luciferase assays—which provide enhanced quantification accuracy—and confocal imaging of fluorescent proteins in HeLa cells reveal that cap1 and cap2 mRNAs exhibit comparable translation efficiency under our experimental conditions. While further testing is underway, we reasonably expect cap2 mRNAs to outperform cap1 in immune cells or in vivo, where, unlike in HeLa cells, a fully functional innate immune system more rigorously detects improperly capped RNAs. Our synthesis method and comprehensive evaluation of cap2 mRNAs lay a solid foundation for their application in vaccines, gene therapy, and other biotechnological pursuits.

7: Controlled Release of Antigen in Self-adjuvanting Tumor Vaccine

Xinyi Tu, Aleksandra Petrova, Di Liu, Nicholas Stephanopoulos, Hao Yan

Center for Molecular Design and Biomimetics, The Biodesign Institute, Arizona State University, Tempe, Arizona 85281, United States

Cancer immunotherapy has revolutionized the treatment landscape, with immune checkpoint inhibitors (ICIs) becoming a standard therapy for many cancers. However, many patients remain non-responsive to ICIs, necessitating alternative therapeutic strategies such as therapeutic cancer vaccines. Peptide-based cancer vaccines, due to their high specificity, low risk of autoimmunity, and cost-effectiveness, have emerged as a promising approach. Despite their potential, several challenges remain, including limited immunogenicity, short antigen presentation duration, and the complexity of manufacturing long peptides. To address these challenges, we propose a novel nanovaccine platform that leverages the advantages of peptide-based vaccines while overcoming their inherent limitations. Our approach involves the use of a self-assembled RNA origami (ROG) platform to deliver antigen peptides in a controlled and efficient manner. By incorporating a cleavable dipeptide linker, Valine-Citrulline (Val-Cit), between the antigen peptide and the nanovaccine coating, we aim to enhance antigen cross-presentation and induce a stronger and more durable immune response.

8: Highly sensitive and multiplexed RNA isoform imaging in human FFPE tissues

Senal D. Liyanage (School of Molecular Sciences, Arizona State University) Yu-Sheng Wang, PhD (School of Molecular Sciences, Arizona State University) Jia Guo, PhD (School of Molecular Sciences, Arizona State University)

Alternative splicing generates diverse RNA isoforms that play crucial roles in cellular function and disease, yet their spatial distribution and regulation in complex tissues remain poorly understood. Existing in situ transcriptomic methods lack the sensitivity and multiplexing capability necessary to visualize RNA isoforms with high resolution in formalin-fixed paraffin-embedded (FFPE) tissues. To address this limitation, we developed a highly multiplexed fluorescence in situ hybridization (FISH) approach that enables direct imaging of individual RNA isoforms within intact tissues. Our method utilizes target-specific oligonucleotide probe pairs combined with branched DNA signal amplification and enzymatic tyramide deposition to enhance sensitivity. This strategy allows for the detection of exonic sequences as short as 150 nucleotides with high specificity, overcoming the limitations of conventional FISH techniques that require extensive probe sets for signal detection. We validated our approach in human FFPE cerebral cortex samples, successfully detecting endogenous UBC mRNA with minimal background noise. By incorporating iterative cycles of imaging, cleavage, and probe stripping, we further extend its multiplexing capability, enabling simultaneous detection of multiple RNA isoforms within single cells. This platform provides a powerful tool for studying alternative splicing events in situ. It has significant implications for understanding cellular heterogeneity, tissue organization, and disease-associated alterations at the transcriptomic level.

9: The Interplay of Helicases, G-quadruplexes, and R-loops in Transcription-Replication Conflicts and Genome Stability

Ajibola D. Adelakun1,2,4, Haining Zhu3, Jacob C. Schwartz1,4
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2 Department of Pharmaceutical Sciences, University of Arizona College of Pharmacy, Tucson, AZ
3 Department of Pharmacology & Toxicology, University of Arizona College of Pharmacy, Tucson, Az
4 University of Arizona Cancer Center, University of Arizona, Tucson AZ

G-quadruplexes and R-loops represent two kinds of non-canonical nucleic acid structures involved in many cellular processes, including transcription and genome stability. G-quadruplexes, formed from guanine-rich nucleotide sequences, pose significant challenges during DNA replication and transcription. R-loops, which consist of RNA:DNA hybrids, further complicate these processes by potentially hindering replication fork progression. Here, we aim to highlight the roles of various helicases such as BACH1, DHX9 and DHX36, in unwinding these structures and assessing how they can resolve conflicts that arise when R-loops and G-quadruplexes coexist. Employing the Ewing sarcoma (A673) cell line fused with the Auxin induced degron/transport inhibitor response 1 (A673 AID/TIR1), we conducted a screen of 30 G-quadruplex targeting compounds to assess the sensitivity and efficacy of the cell line in response to these drugs. Of all 30 compounds, we found that cells were sensitive to 4 compounds, with an increase in sensitivity observed in cells without the fusion protein (EWS-FLI1). We carried out a dose response with all 4 compounds to further evaluate the sensitivity and efficacy of these compounds by quantifying their effect on cell survival or proliferation across varying concentrations. We also carried out a comparative dose response experiment using a different cell-line, Human Mesenchymal (hMSC) stem cells, with or without the fusion protein, EWS-FLI1, and it was observed that the cells were also sensitive to the compounds. We further transfected these helicases into the A673 AID/TIR1 cells and to check for the expression BACH1, DHX9 and DHX36 proteins, we carried out a western blot. We predict that overexpression of these helicases will reverse the activity and sensitivity of the 4 compounds. Through this approach, we hope to gain insight into the mechanism underlying resolution, its impact on transcription stress, and genome stability in trying to better understand its implications in cancer biology and genetic disorders. Our results could point toward new therapeutic approaches by targeting these interactions to advance genomic stability.

10: The Role of CD36 in Erythroid Differentiation of Human Hematopoietic Stem and Progenitor Cells

Christina Lyons and Shalini Sharma
Department of Basic Medical Sciences, College of Medicine- Phoenix, University of Arizona, Phoenix, AZ

Acute myeloid leukemia (AML) is a severe myeloid malignancy and the most common type of acute leukemia in adults, with over 20,000 cases diagnosed each year in the US. It is associated with poor prognosis, most commonly low red blood cell (RBC) anemia. CD36, also known as fatty acid translocase (FAT), is a multifunctional transmembrane glycoprotein expressed in a variety of cell types. It is overexpressed in many cancers and has been implicated in hematologic malignancy progression, including transformation to AML, as well as metastasis, chemoresistance, and relapse. CD36 is considered a promising therapeutic target and small molecule inhibitors and antibodies are in development as a new approach to enhance the effects of chemotherapy. While CD36-targeted therapies are in pre-clinical development, the impact of CD36 in normal hematopoiesis is not well characterized. CD36 is an established erythroid differentiation marker that arises in unipotent erythroid lineage committed progenitors, colony forming unit-erythroid (CFU-E), however, its exact role in erythropoiesis remains to be determined. There is a significant unmet need to further characterize the expression of CD36 in hematopoietic cells and elucidate the role CD36 plays in erythroid differentiation. Based on our preliminary results, we hypothesize that interfering with CD36 function impedes normal erythropoiesis and can cause anemia in patients. Expression profiles and splice patterns of CD36 were evaluated in human leukemia cell lines and human umbilical cord blood (UCB)-derived mononuclear cells (MNCs) using flow cytometry, RT-qPCR, western blotting, and PCR. The 5' rapid amplification of cDNA ends (5'-RACE) technique was used to determine the precise transcription start site (TSS) of CD36 and PCR was used to investigate specific splice variants of CD36 that are present in hematopoietic cells. UCB-derived CD34+ cells were used as a model for ex vivo differentiation of hematopoietic stem and progenitor cells (HSPCs) to the erythroid lineage. Immunophenotyping using published flow cytometry strategies was used to analyze the impact of treatment with anti-CD36 antibodies (FA6-152) on differentiation stages along the erythroid continuum. Immunophenotypic analysis, western blotting, and RT-qPCR data showed that CD36 mRNA levels could be detected when protein levels were not detected, suggesting that mRNA transcripts are present even in cells that do not exhibit surface protein expression. Assessment of alternative first exons in UCB-MNCs and AML cell lines suggests exon 1c is the transcription start site in hematopoietic cells. FA6-152 treatment of human UCB- derived CD34+ HSPCs undergoing ex vivo differentiation did not have a significant effect on cell survival, but significantly decreased the expression of CD36 and the mature erythroid marker, CD235a (glycophorin A). Expression of CD34 and erythroid-associated markers, CD71 (transferrin receptor) and CD105 (endoglin), were not affected with FA6-152 treatment. Differences in CD36 mRNA and protein levels could be attributed to post-transcriptional modification or regulation of CD36 mRNA. Upstream alternative first exons, exon 1a and 1b, were not detected in the human leukemia cell lines or UCB-derived MNCs, which suggests CD36 may be regulated by tissue-specific gene transcription. The distribution of early erythroid populations was not disrupted by FA6-152 treatment, but additional analysis is required to assess the impact of treatment on terminal erythroid differentiation and RBC maturation. Further investigation is required to determine how FA6-152 treatment affects erythroid differentiation and whether CD36 inhibition contributes to anemia.

11: Erythropoietin-dependent Acquisition of CD71hiCD105hi Phenotype within CD235a– Early Erythroid Progenitors

Natascha Schippel[1], Jing Wei[1], Xiaokuang Ma[1], Jinhua Chi[2], Haiwei Gu[2], Mrinalini Kala[3], Shenfeng Qiu[1], Peter Stoilov[4], and Shalini Sharma[1]

[1]Department of Basic Medical Sciences, College of Medicine-Phoenix, University of Arizona, Phoenix, Arizona, USA; [2]Arizona Metabolomics Laboratory, College of Health Solutions, Arizona State University, Phoenix, Arizona, USA; [3]Flow Cytometry Core, College of Medicine-Phoenix, University of Arizona, Phoenix, Arizona, USA; [4]Department of Biochemistry, School of Medicine, West Virginia University, Morgantown, West Virginia, USA

Significance: Anemia resulting from altered red blood cell (RBC) production is a common symptom in many pathophysiologies, including myelodysplastic syndromes and acute myeloid leukemia. RBC transfusion and erythropoiesis stimulating agents (ESAs)—primarily erythropoietin (Epo)—are major treatment options for anemia, however, they are associated with organ damage and ESA resistance.

Background: In adults, erythropoiesis occurs in the bone marrow (BM) from hematopoietic stem cells through four stages: erythroid progenitor development, early erythropoiesis (EE), terminal erythroid differentiation (TED), and maturation. Epo is necessary for initiation of TED and survival and differentiation of the unipotent EE progenitors, burst forming unit-erythroid (BFU-E) and colony forming unit-erythroid (CFU-E). Our goal is to unravel the Epo-associated mechanisms that underlie EE transitions, thereby aiding identification of downstream transcriptional and mRNA-processing events that can be modulated for therapy.

Methods: Multiparametric flow cytometry and single cell RNA-sequencing (scRNA-seq) was applied for the assessment of population dynamics and transcriptional changes within human BM-derived CD34+ hematopoietic stem and progenitor cells cultured with and without (+/-) Epo.

Results: Based on a panel of previously-established markers, we have developed an immunophenotyping strategy that resolved heterogeneous populations and identified the Epo-dependent transition within EE. Our analysis stratified five EE subtypes using expression of CD34, CD71, and CD105: early-BFU-E (CD34+CD71loCD105lo), late-BFU-E (CD34+CD71hiCD105lo), early-CFU-E (CD34-CD71loCD105lo), mid-CFU-E (CD34-CD71hiCD105lo), and late-CFU-E (CD34-CD71hiCD105hi). Assessment of EE populations in +/- Epo cultures revealed that Epo is required for transition from mid- to late-CFU-Es, i.e. acquisition of the CD71hiCD105hi phenotype. Furthermore, scRNA-seq analysis from +/-Epo cultures confirmed the differentiation arrest in cells preceeding high coexpression of CD71 and CD105. This enabled identification of Epo-incduced changes in transcriptional events, including not only those associated with the previously-established STAT5, PI3K, and MAPK pathways, but also those that are have not previously been implicated in Epo-dependent EE differentiation. This list includes many RNA binding proteins, such as CELF2, MSI2, and SRPK2.

Discussion: Thus, by identifying the Epo-dependent EE population and changes in transcriptional regulation—including that of key alternative splicing factors—regulated by Epo, this study provides new insights into erythroid differentiation and a framework for investigating the role of mRNA processing in erythroid lineage commitment and differentiation.

12: NEDD4 and ESCRT regulate endolysosomal clearance of the RNA-binding protein TDP-43

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A pathological hallmark in >97% of all ALS cases is the cytoplasmic mislocalization and aggregation of a nuclear RNA binding protein, TDP-43, in motor neurons. Driving clearance of cytoplasmic TDP-43 reduces toxicity in various ALS models, though how TDP-43 clearance is regulated remains controversial. Understanding TDP-43 proteostasis is key for understanding toxicity associated with TDP-43 proteinopathy, and upregulating clearance of toxic TDP-43 species could be an avenue of therapeutic potential. My lab is interested in studying endolysosomal clearance of TDP-43, a pathway that functions independent of macroautophagy and the ubiquitin proteasome system (UPS). Using an unbiased, genome-wide, TDP-43 dot blot screen in S. cerevisiae (budding yeast), we identified genes related to ESCRT-dependent endolysosomal trafficking and the E3 ubiquitin ligase, Rsp5(yeast)/NEDD4(human), as key facilitators of TDP-43 endolysosomal clearance. In follow-up experiments, ESCRT mutants in yeast and HEK293A cells exhibited increased TDP-43 protein stability, cytoplasmic mislocalization, and aggregation. Inactivation of Rsp5/NEDD4 function increased TDP-43 toxicity, cytoplasmic aggregation, and protein levels, whereas RSP5/NEDD4 overexpression reversed these phenotypes. NEDD4 overexpression also increases TDP-43 K63-linked ubiquitination. Finally, moderate TDP-43 overexpression resulted in formation of “giant” multivesicular bodies (MVBs), suggesting impacts of increased TDP-43 on endolysosomal trafficking. NEDD4 knockdown reduced the number of MVBs containing TDP-43 suggesting NEDD4 is important for TDP-43 internalization into MVBs. Thus, we hypothesize that TDP-43 ubiquitination by Rsp5/NEDD4 facilitates interaction with ESCRT proteins and internalization within MVBs. Future studies will focus on understanding TDP-43 endolysosomal degradation in greater mechanistic detail as well as uncovering how TDP-43 levels, localization and physical state determine its degradation pathway. Broader impacts of our study include potential identification of novel ALS therapeutic targets, and a greater understanding of proteostasis regulation.

13: RNA-binding proteins G3BP1 and G3BP2 promote cell cycle re-entry and cell survival during recovery from DNA replication stress

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The RNA-binding proteins G3BP1 and G3BP2 are mainly known for their roles in the assembly of cytoplasmic stress granules (SGs), an RNA-protein biomolecular condensate that forms in response to many stresses. However, the role of G3BP proteins outside of stress granule assembly remains largely unclear. We have discovered novel roles for G3BP1 and 2 in the response to and recovery from DNA replication stress. Following treatment with the DNA replication stressor hydroxyurea (HU), we found that U2-OS cells lacking G3BP1 and 2 (G3BP1/2ΔΔ) are delayed in their re-entry into the cell cycle and exhibit altered DNA damage response (DDRs) and increased cell death. First, using flow cytometry for DNA content (propidium iodide) and S-phase activity (EdU pulse-labeling), we have found that, following release from arrest, G3BP1/2ΔΔ cells are significantly delayed in their re-entry into S-phase and cell cycling relative to WT cells. Second, G3BP1/2ΔΔ cells display increased phospho-S139-γ-H2AX and RAD51 staining at release and during recovery from S-phase arrest, in addition to increased cell death. These results indicate that G3BP1 and 2 facilitate cell cycle re-entry and cell survival following DNA replication stress, possibly by influencing whether cells undergo a favorable or unfavorable response. Finally, G3BP1/2ΔΔ cells display decreased expression and altered nuclear-cytoplasmic localization of p53, indicating a role for G3BP1 and 2 in regulating p53 activity during recovery from DNA replication stress. Future experiments include determining 1) which function/domain(s) of G3BP1 and/or 2 are required for a favorable response to DNA replication stress, 2) the extent to which p53 plays a role in G3BP1 and 2-mediated S-phase recovery, and 3) the individual functions of G3BP1 and G3BP2 in S-phase recovery.

14: A comprehensive analysis of 3'UTRs in Caenorhabditis *elegans*

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3'Untranslated Regions (3'UTRs) are essential portions of genes located between the STOP codon and the poly(A) tail in eukaryotic mRNAs which house sequences that serve as targets for various regulatory molecules. The lengths of these regions are defined during a process known as cleavage and polyadenylation, which is performed by a large multi-subunit complex known as the RNA cleavage and polyadenylation complex (CPC) that interacts with several sequence elements located within 3'UTRs. Disturbances in this process are linked to the emergence and advancement of many diseases, including neurodegenerative disorders, diabetes, and cancer. However, despite their importance, 3'UTRs remain poorly characterized in eukaryotes. We used a multi-pronged approach to extract and curate 3’UTR data from 11,533 publicly available datasets, corresponding to the entire collection of *C. elegans* transcriptomes stored in the NCBI repository from 2009 to 2023, and present its complete 3'UTRome dataset sequenced at single-base resolution. This updated *C. elegans* 3'UTRome is the most comprehensive resource in any metazoan, covering 97.4% of the 20,362 experimentally validated protein-coding genes with refined and updated 3'UTR boundaries for 23,489 3'UTR isoforms. In the future, the updated *C. elegans* 3'UTRome will serve as a powerful resource for further investigations into 3'UTR formation, function, and regulation in eukaryotes. Our analysis of this novel dataset identified and characterized sequence elements involved in pre-mRNA 3'end processing and updated miRNA target predictions. We also performed several genetic experiments that further characterized many of the 3'UTR elements involved in pre-mRNA 3'end processing. We are currently using an adapted version of the STAMP assay developed by the Yeo lab to identify the mRNA targets and binding sites of the Cleavage Factor I (CFI) in *C. elegans*, which is a poorly characterized subcomplex of the CPC that acts as an important enhancer and regulator of RNA cleavage and polyadenylation. The results of these experiments will greatly improve the scientific community’s understanding of the important roles 3’UTR sequence elements have in RNA cleavage and polyadenylation.

15: Strand Secrets: Uncovering Tissue-Specific Patterns behind microRNA Strand Selection Using the HiTmiSS Assay

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MicroRNAs (miRNAs) are 16-24 nucleotide non-coding RNAs that mature to form semi-complementary duplexes. One strand of each duplex is loaded onto an Argonaute-like protein to regulate gene expression by targeting semi-complementary elements in the 3’ untranslated regions (3’UTRs) of mRNAs. This process is highly conserved across metazoans, yet much about strand selection remains unknown. The identity of the 5' nucleotide and thermostability of the duplex play some role, yet a significant amount of miRNA strand selection cannot be predicted using these metrics, suggesting additional patterns at play. Here, we used a novel in-house High-Throughput miRNA Strand Selection (HiTmiSS) assay to track miRNA strand usage in all 190 *C. elegans* miRNAs throughout all six developmental stages. To deconvolve our results at the tissue level we optimized our HiTmiSS assay to detect miRNA strand selection in a tissue-specific manner. We first profiled the intestine and found patterns of miRNA expression that differed from the whole worm, suggesting the strand selection decision is regulated at the level of individual tissues. To further investigate this phenomenon, we modified our lab’s dual color reporter plasmid construct to develop a two-color fluorescent reporter strain of *C. elegans*. These *C. elegans* strains express a green and a red fluorescent protein each under the control of a different strand of the miRNA *let-7* and allowed us to capture live images of strand selection in the intestine for the first time. In conclusion, our research identified novel, important, and conserved patterns of miRNA strand selection throughout *C. elegans* development, correlating with previously observed developmental phenotypes. Specific to the intestine, our data both reinforced the tissue specificity of strand selection and revealed a novel phenotype upon depletion of both strands of *let-7* in the intestine. These findings highlighted novel structural principles underlying strand selection which can be applied to higher metazoans.

16: Characterizing Cellular Clearance Mechanisms of TDP-43 and ALS-Associated Isoforms

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease in which motor neuron atrophy results in paralysis and respiratory failure. In >95% of ALS patients, a key pathological hallmark is cytoplasmic mislocalization and aggregation within motor neurons of a nuclear RNA-binding protein, TDP-43. Pathological N- or C-terminal truncated TDP-43 isoforms are associated with ALS, and reducing their levels, and that of full-length cytoplasmic TDP-43, improves cell viability However, the mechanisms by which cells degrade full length, N and C-terminally truncated TDP-43 remain controversial. Recently, we identified an autophagy-independent, endolysosomal clearance pathway that degrades full-length TDP-43. Here, using a yeast TDP-43 model coupled to a novel genome-wide dot blot screen, we identified adaptors of the E3 ubiquitin ligase Rsp5/NEDD4, and membrane-invaginating ESCRT factors, as key facilitators of endolysosomal clearance. TDP-43 colocalized and physically interacted with Rsp5/NEDD4 and ESCRT proteins, and genetically inhibiting NEDD4 or ESCRT increased TDP-43 cytoplasmic accumulation, aggregation, and stability. In future work, using our knowledge of full-length TDP-43 degradation, and a refined dot-blot screening approach, we will elucidate how N and C-terminally truncated TDP-43 isoforms are degraded. Collectively, our work yields new insight into TDP-43 degradation, which may yield new ALS therapeutic targets and basic insight into endolysosomal means of cytoplasmic protein degradation.

17: Nanoarchitectural engineering of DNA and RNA for biological discovery

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Nucleic acids are highly programmable molecules: they have well-defined helical geometries and enable predictable recognition via base pairings (or complementarity). DNA/RNA nanotechnology, taking advantage of both aspects of the programmability of nucleic acids, enables the construction of sophisticated nanoscale architectures. My group is interested in employing the rational design principles developed in the field of DNA/RNA nanotechnology for solving fundamental and urgent problems in biological and pharmaceutical research. Specifically, we will (i) identify potential drugs targeting type IA topoisomerase, which hold the promise of broad-spectrum antibiotics; (ii) determine high-resolution RNA structures (such as bacterial regulatory RNAs or structured motifs from viral RNAs), which will enable the development of RNA-targeting drugs; and (iii) design improved mRNA vaccines or medicines for disease prevention or treatment.

18: Coarse-grained modeling of RNA for biomedical applications

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mRNA therapeutics have recently gained popularity but still face challenges with stability and inefficient delivery. We propose structuring the mRNA sequence within ssRNA (single stranded RNA) origami to form a compact nanoparticle to address these limitations. Within a single strand, mRNA and its “scaffolding” sequence are thermally annealed to fold into a defined shape. Using our lab’s oxRNA coarse-grained model in conjunction with our oxView interactive tool, we can run simulations to achieve optimal folding and design nanoparticles. A fast and reliable simulation and experimental pipeline to test designs *in silico* and validate *in vitro* is key for the development of mRNA therapeutics.

19: Structured mRNA Origami for Delivery of Gene Therapies

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Programmable structuring of mRNA has the potential to allow tailoring of nanoparticle stability, cell-type specificity, and innate immune activation. Thus far, highly structured mRNA nanoparticles have not yet been demonstrated to be translation-viable in vivo or in cell-free environments. Our current study aims to: 1) Develop mRNA origami nanoparticles encoding reporter genes with different structuring strands and 5' UTRs. 2) Assess the translational efficiency of gene-encoding mRNA origami NPs and define the ribosomal landscape surrounding synthetic crossover motifs and highly structured mRNA regions. 3) Assess 5' UTRs associated with cellular RNA helicases to facilitate intracellular origami unfolding. Methods : Structured eGFP-origami nanoparticles were assessed for their ability to fold correctly via gel EMSA and AFM prior to transfection into HEK293T. Nanoparticles were also assayed for reporter activity after incubation at 4C over the course of 2 months. Nanoparticles were developed with varying levels of dsRNA structuring and 5' UTRs derived from highly structured genes in viral and mammalian hosts to facilitate unwinding. Ongoing work on this project includes full translational mapping of nanoparticles in HEK293T cells with MALDI and western blots using N-terminally tagged nanostructures.

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