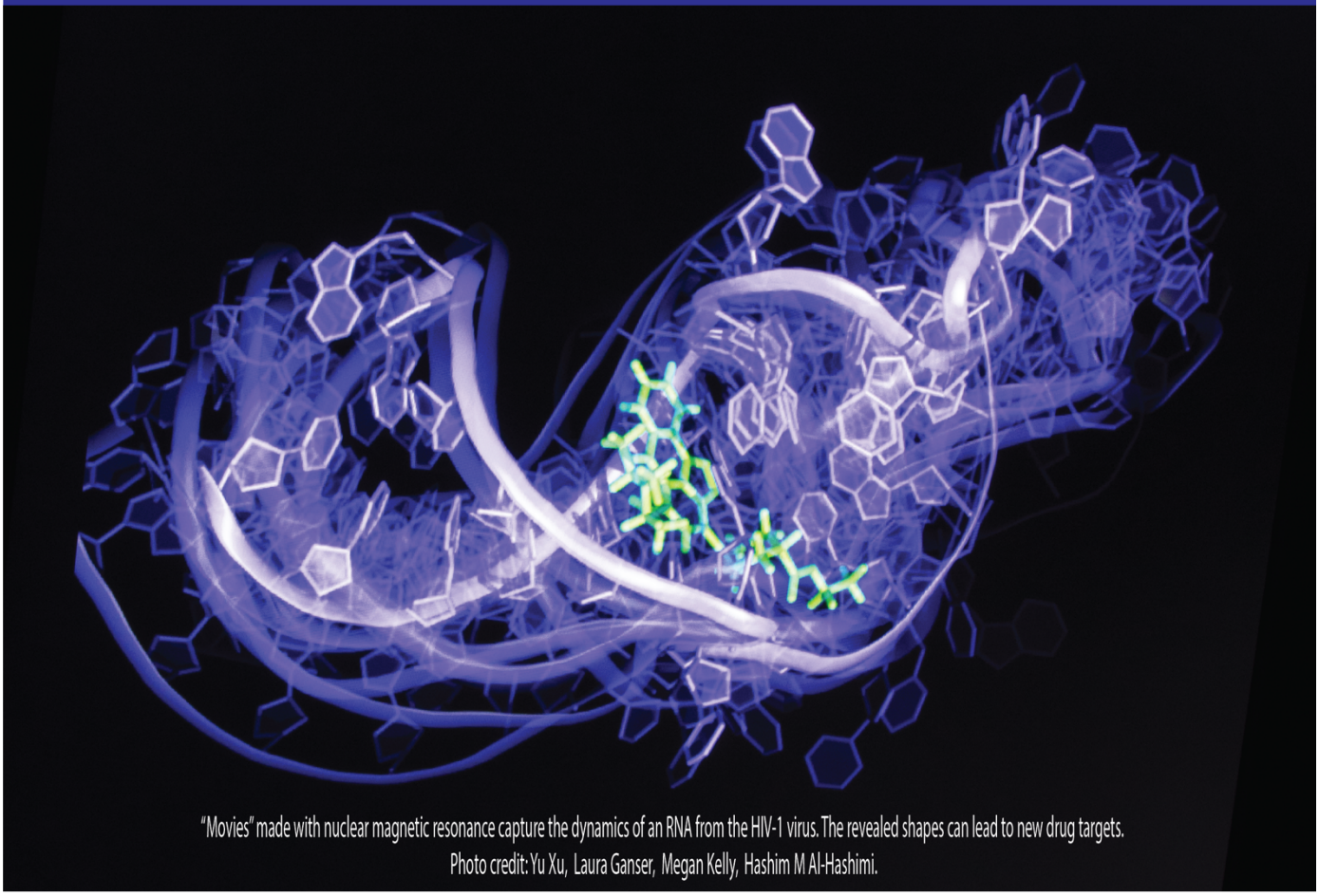
2019 Symposium on RNA Biology XIII: Tool and Target

October 17-18th, 2019
Trent Semans Center
Duke University



"Movies" made with nuclear magnetic resonance capture the dynamics of an RNA from the HIV-1 virus. The revealed shapes can lead to new drug targets.
Photo credit: Yu Xu, Laura Ganser, Megan Kelly, Hashim M Al-Hashimi.

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Co-Chairs of the Organizing Committee:

Stacy Horner, Ph.D.	(Duke)
Robin Stanley, Ph.D.	(NIEHS)

Members of Organizing Committee:

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Kate Meyer, Ph.D.	(Duke)
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Meeting Agenda

Thursday, October 17th, 2019

Great Hall of the Trent Semans Center, Duke University

12:00 – 1:00 PM	Registration (Great Hall)
1:00 – 2:35 PM	**SESSION I: TRANSLATION FIDELITY** *Chair: Amanda Hargrove, Duke University*
1:00 PM	Opening Remarks, Stacy Horner, Duke University
1:10 PM	**Keynote Lecture:** Rachel Green, Johns Hopkins University "Colliding Ribosomes as an integrator of Cytoplasmic Stress Responses"
1:50 PM	Yu-Hua Lo, NIEHS "Unraveling the mechanism of substrate processing by the AAA-ATPase Rix7"
2:05 PM	Hani Zaher, Washington University "RNA damage, the ribosome and quality control"
2:35 PM	Break
3:00 – 5:00 PM	**SESSION II: RNA PROCESSING** *Chair: Jimena Giudice, UNC Chapel Hill*
3:00 PM	Rui Zhao, University of Colorado **"**A unified mechanism for intron definition, exon definition, and back-splicing"
3:30 PM	Adam Black, UNC Chapel Hill "Clathrin heavy chain splice forms differentially affect striated muscle physiology"
3:45 PM	Nicholas Conrad, University of Texas Southwestern "Mechanisms regulating S-adenosylmethionine homeostasis through intron detention"
4:15 PM	Marcos Morgan, NIEHS "Role of RNA uridylation in germ line differentiation"
4:45 PM	Jianguo Huang, Duke University "Long non-coding RNA NEAT1 promotes sarcoma metastasis by regulating RNA splicing pathways"
5:00 – 6:30 PM	**POSTER SESSION & HAPPY HOUR**
5:00 PM	Even Numbered Posters
5:45 PM	Odd Numbered Posters
6:30 - 9:00 PM	**SYMPOSIUM BANQUET AND NC RNA SOCIETY AWARDS PRESENTATION**

Friday, October 18th, 2019

Great Hall of the Trent Semans Center, Duke University

8:00 – 8:30 AM	Breakfast (Great Hall)

8:30 – 10:15 AM **SESSION III: RNA EPIGENETICS**
Chair: Mauro Calabrese, UNC Chapel Hill

8:30 AM Kate Meyer, Duke University
"Detecting m^6A and its role in gene expression"

9:00 AM Ke Zhang, Wake Forest University
"Lsd1 and Lsd2 lysine demethylases regulate one another and play parallel roles with other histone modifying enzymes in *Schizosaccharomyces pombe"*

9:30 AM Christopher Holley, Duke University
"Modification of mRNA by snoRNA-guided 2'-O-methylation"

10:00 AM Coffee Break

10:30-12:10 PM **SESSION IV: RIBOSOMES**
Chair: Robin Stanley, NIEHS

10:30 AM **Keynote Lecture:** Susan Baserga, Yale University
"The RNA polymerase II transcription factor, Paired Box 9 (PAX9), regulates ribosome biogenesis and craniofacial development."

11:10 AM Gustavo Silva, Duke University
"Ribosome control by K63 ubiquitin during oxidative stress"

11:40 PM Christine Dunham, Emory University
"RNA-mediated mechanisms of translation control"

12:10 – 1:15 PM LUNCH

1:15 – 3:10 PM **SESSION V: RNA STRUCTURE AND GENE EXPRESSION**
Chair: Qi Zhang, UNC Chapel Hill

1:15 PM **Ribometrix Keynote Lecture:** Phillip Bevilacqua, Penn State University
"RNA structure-function relationships under *in vivo* and *in vivo-like* conditions: Impacts on RNA catalysis, folding, and transcriptome-wide response to stress"

1:55 PM Joseph Rodriguez, NIEHS
"Environmental regulation of the estrogen response in single cells"

2:25 PM Eda Yildirim, Duke University
*"*Regulation of chromatin architecture and transcription by nucleoporin proteins"

2:55 PM Angela Yu, Cornell/Northwestern University
"Computationally reconstructing cotranscriptional RNA folding pathways from experimental data"

3:10 PM Break

3:30 – 5:00 PM **SESSION VI: RNA PROTEIN INTERACTIONS**
Chair: Traci Hall, NIEHS

3:30 PM Daniel Dominguez, UNC Chapel Hill
"Features of RNA binding motifs"

4:00 PM	Anthony Mustoe, Baylor College of Medicine “Visualizing RNA structural ensembles by single molecule chemical probing”
4:15 PM	Hong Wang, NCSU “Cohesin SA1 and SA2 are RNA binding proteins”
4:30 PM	Awards Presentation
5:00 PM	Meeting Adjourns

Poster #	Name	Institution
1	Aaztli Coria	UNC Chapel Hill
2	Aline Umuhire Juru	Duke University
3	Anais Monroy-Eklund	UNC Chapel Hill
4	Araceli Valverde Estepa	University of Illinois at Chicago
5	Ashleigh Rawls	Duke University
6	Atul Kaushik Rangadurai	Duke University
7	B. Fadi Marayati	Wake Forest University
8	Breanne Hatfield	UNC Chapel Hill
9	Brooke D'Arcy	Duke University
10	Cassandra Hayne	NIEHS
11	Chase Weidmann	UNC Chapel Hill
12	Chia-chieh Chu	Duke University
13	Christina Moss	University of South Florida
14	Christopher Day	NIEHS
15	Eduardo Torres	UNC Chapel Hill
16	Elizabeth Jolley	Pennsylvania State University
17	Emily B. Harrison	UNC Chapel Hill
18	Emily McFadden	Duke University
19	Emily Satterwhite	East Carolina University
20	Emma Hinkle	UNC-CH
21	Gabrielle Gentile	UNC Chapel Hill
22	Gary Cantor	UNC Chapel Hill
23	Giacomo Padroni	Duke University
24	Haein Kim	Duke University
25	Hala Abou Assi	Duke University
26	Hannah Cafaro	UNC Chapel Hill
27	Hannah Neiswender	Medical College of Georgia
28	Hannah Wiedner	UNC Chapel Hill
29	Harmony Salzler	UNC Chapel Hill
30	Hsiang-Ting Ho	Duke University
31	Ivan Jimenez Ruiz	UNC Chapel Hill
32	Jackson B. Trotman	UNC Chapel Hill
33	Jacob Gordon	NIEHS
34	Jared T Baisden	UNC Chapel Hill
35	Jayashree Kumar	UNC Chapel Hill
36	Jennifer Simpson	UNC Greensboro
37	Jeongeun Hyun	Duke University
38	Jodie Fleming	North Carolina Central University
39	Jon Kastan	Duke University
40	Jordan Koehn	UNC Chapel Hill
41	Katherine Elizabeth Berman	Northwestern University
42	Kathleen McCann	NIEHS

43	Kevin Tsai	Duke University
44	Luyi Cheng	Northwestern University
45	Maria Skamagki	Memorial Sloan Kettering
46	Mariah Hoye	Duke University
47	Mark Boerneke	UNC Chapel Hill
48	Mathieu Flamand	Duke University
49	Matt Sacco	Duke University
50	Matthew Tegowski	Duke University
51	Megan Browning	Utah
52	Megan Kelly	Duke University
53	Meredith Zeller	UNC Chapel Hill
54	Michael McFadden	Duke University
55	Michelle Potter-Birriel	UNC Chapel Hill
56	Mohammed Dorgham	East Carolina University
57	Molly Evans	Northwestern University
58	Monica Pillon	NIEHS
59	Muge Kuyumcu-Martinez	University of Texas
60	Nicole Rivera-Espinal	UNC Chapel Hill
61	Rachel Cherney	UNC Chapel Hill
62	Renee Tamming	Duke University
63	Rita Meganck	UNC Chapel Hill
64	Roger P. Alexander	Pacific Northwest Research Institute
65	Salma Azam	UNC Chapel Hill
66	Samuel W Olson	UNC Chapel Hill
67	Sarah Wicks	Duke University
68	Scott Allen	Duke University
69	Seung Ho Choi	Duke University
70	Silvia B Ramos	UNC Chapel Hill
71	Soumyadip Sahu	NIEHS
72	Swetha Devi	UNC Chapel Hill
73	Tom Christy	UNC Chapel Hill
74	Victor Ruthig	Duke University
75	Yinzhou Zhu	Duke University
76	Zahra Kabiri	Duke University
77	Zhengguo Cai	Duke University

Oral Presentation Abstracts (In Order of Presentation)

Colliding Ribosomes as an integrator of Cytoplasmic Stress Responses

Rachel Green, Johns Hopkins University

Unraveling the mechanism of substrate processing by the AAA-ATPase Rix7

Yu-Hua Lo[1], Mack Sobhany[1], Allen L. Hsu[2], Brittany L. Ford[2], Juno M. Krahn[2], Mario J. Borgnia[2], and Robin E. Stanley[1]

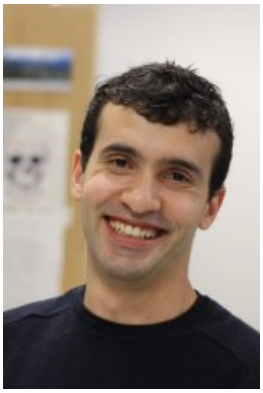
[1]Signal Transduction Laboratory, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services
[2]Genome Integrity and Structural Biology Laboratory, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services

Rix7 is an essential type II AAA-ATPase required for the formation of the large ribosomal subunit. Rix7 has been proposed to utilize the power of ATP hydrolysis to drive the removal of assembly factors from pre-60S particles, but the mechanism of release is unknown. Rix7's mammalian homolog, NVL2 has been linked to cancer and mental illness disorders, highlighting the need to understand the molecular mechanisms of this essential machine. Here we report the cryo-EM reconstruction of the tandem AAA domains of Rix7 which form an asymmetric stacked homohexameric ring. Five of the six Rix7 protomers grip the substrate through conserved pore loops that line the central channel formed by the two ATPase rings, revealing Rix7's role as a molecular unfoldase. This arrangement suggests a processive hand-over-hand mechanism of substrate unwinding. The complex structure establishes that type II AAA-ATPases lacking the aromatic-hydrophobic motif within the first AAA domain can engage a substrate throughout the entire central channel. The structure also reveals that Rix7 contains unique post-α7 insertions within both AAA domains important for Rix7 function in vivo. Taken together, our results suggest that Rix7 using the unfolding activity to pull on substrates and drive the release of assembly factors from the immature 60S particles.

RNA damage, the ribosome and quality control

Liewei L. Yan and **Hani S. Zaher**

Department of Biology
Washington University in St. Louis.



Maintaining the integrity of nucleic acids is an essential feature of all organisms. Unwanted modification of DNA, if left unrepaired, is deleterious to cellular homeostasis and could have far-reaching consequences that include genomic instability and accumulation of mutations. Similarly, accumulation of damaged RNA has been correlated with various neurodegenerative diseases. Given their inherent reactivity, nucleic acids are susceptible to damage from both endogenous and exogenous reactive oxygen species (ROS) as well as alkylation agents. We have recently begun to address how some of these modifications on mRNA impact the function of the ribosome and in particular the decoding process. We find that most modifications severely change the speed and accuracy of translation. These observations revealed that oxidation of RNA is most likely to stall the ribosome and necessitate the presence of quality-control processes to handle damaged mRNA. To this end, we have uncovered a connection between the process of no-go decay (NGD), which degrades mRNAs that stall translation, and chemical insults. In the absence of key NGD factors in yeast, the levels of damaged mRNA significantly increase and cells are rendered sensitive to oxidizing and alkylation agents. Furthermore, these agents activate ribosome quality control (RQC) of nascent peptides. Deletion of the E3 ligase responsible for the ubiquitination of the nascent peptides resulted in the accumulation of protein aggregates in the presence of oxidizing and alkylating agents. These observations suggest that chemical damage stalls translation, activating NGD and RQC. Interestingly, the addition of nucleic-acids-damaging agents was also found to result in K63-linked ubiquitination of ribosomal proteins. This signaling-mode of ubiquitination precedes DNA-damage marks, indicating that cells respond to RNA damage due to stalled ribosomes rapidly. Finally, in mammalian cell culture, mRNAs harboring a single oxidized base in their open reading frame are degraded more quickly relative to ones with this modification in the UTR. Collectively our data highlights the burden of chemically-damaged mRNA on cellular homeostasis and suggests that organisms evolved ribosome-based mRNA-surveillance processes to rapidly degrade it.

A unified mechanism for intron definition, exon definition, and back-splicing

Xueni Li[1], Shiheng Liu[2], Lingdi Zhang[1], Aaron Issaian[1], Ryan C. Hill[1], Sara Espinosa[1], Shasha Shi[1], Yanxiang Cui[3], Kalli Kappel[3], Rhiju Das[3], Kirk C. Hansen[1], Z. Hong Zhou[2], **Rui Zhao**[1]

1. Department of Biochemistry and Molecular Genetics, University of Colorado Denver Anschutz Medical Campus, Aurora, CO
2. Department of Microbiology, Immunology, and Molecular Genetics, UCLA, Los Angeles, CA
3. Biophysics Program, Stanford University, Stanford, CA

The molecular mechanisms of exon definition and back-splicing are fundamental unanswered questions in pre-mRNA splicing. We recently determined the cryo-EM structures of the yeast spliceosomal E complex assembled on introns, providing a view of the earliest event in the splicing cycle that commits pre-mRNAs to splicing. The E complex architecture suggests that the same spliceosome can assemble across an exon, and that it either remodels to span an intron for canonical linear splicing (typically on short exons) or catalyses back-splicing to generate circular RNA (on long exons). The model is supported by our experiments, which show that an E complex assembled on the middle exon of yeast EFM5 or HMRA1 can be chased into circular RNA when the exon is sufficiently long. This simple model unifies intron definition, exon definition, and back-splicing through the same spliceosome in all eukaryotes and should inspire experiments in many other systems to understand the mechanism and regulation of these processes.

Clathrin heavy chain splice forms differentially affect striated muscle physiology

Black AJ[1]*, Blue RE[1]*, Cafaro HE[1], Wiedner HJ[1,2], Hinkle ER[1,2], Engels NM[1], Giudice J[1,2,3]

[1] Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill

[2] Curriculum in Genetics and Molecular Biology (GMB), University of North Carolina at Chapel Hill;

[3] McAllister Heart Institute, University of North Carolina at Chapel Hill, *equal contribution

We recently reported that exon 31 of the *clathrin heavy chain (Cltc*) gene is included during striated muscle development and is skipped in other tissues. CLTC is the main driver of clathrin-mediated endocytosis and regulates skeletal muscle structure. CRISPR mice expressing only the Cltc splice form lacking exon 31 (*Cltc*-ho) exhibit higher body and muscle mass compared to mice expressing both isoforms (*Cltc*-wt). We hypothesize that splicing regulation of CLTC contributes to striated muscle physiology and aim to: 1) determine how Cltc isoforms are regulated, 2) identify RNA-binding proteins governing Cltc splicing, 3) identify molecular targets contributing to muscle differences between *Cltc*-wt and *Cltc*-ho mice, and 4) characterize cardiac physiology of *Cltc*-wt and *Cltc*-ho mice in baseline conditions and under pressure-overload.
We first show that *Cltc* transcripts containing exon 31 are more stable than those lacking exon 31. Second, polypyrimidine tract-binding protein-1 and quaking, oppositely regulate Cltc splicing. Third, skeletal muscles of *Cltc*-ho mice exhibit larger cross-sectional area and reduced myostatin expression and AMPK phosphorylation. Fourth, echocardiography indicated normal baseline cardiac functions in *Cltc*-wt and *Cltc*-ho mice. However, under pressure-overload, *Cltc*- wt mice exhibit cardiac dysfunction and hypertrophy while *Cltc*-ho mice are protected. *Cltc*-wt mice exhibit increased cardiomyocyte cross-sectional areas, disorganized plasma membranes, increased AMPK phosphorylation, and reduced cardiac expression of connexin43. These effects were not observed in *Cltc*-ho mice. Echocardiography after pressure-overload further supported cardiac dysfunction and hypertrophy in *Cltc*-wt but not *Cltc*-ho mice. Combined, these data suggest that Cltc splicing regulation contributes to skeletal muscle and cardiac physiology.

Mechanisms regulating S-adenosylmethionine homeostasis through intron detention

Anna M. Scarborough[1], Olga V. Hunter[1], Kuanqing Liu[2], Ashwani Kumar[3], Chao Xing[3], Ben P. Tu[2], and **Nicholas K. Conrad[1]**

[1]Department of Microbiology, [2]Department of Biochemistry, [3]McDermott Center for Bioinformatics, University of Texas Southwestern Medical Center, Dallas TX

S-adenosylmethionine (SAM) is the essential methyl donor for nearly all cellular methylation events. SAM levels are critical for regulating a variety of cellular functions, but the factors that regulate intracellular SAM concentrations remain incompletely understood. The N^6-adenosine methyltransferase METTL16 regulates intron detention of MAT2A, the only SAM synthetase expressed in most cells. Our working model proposes that under SAM-replete conditions, METTL16 binds and methylates a vertebrate-conserved hairpin (hp1) in the MAT2A 3´ UTR. Efficient methylation by METTL16 correlates with detention of the last intron of MAT2A and nuclear degradation of the transcript. Under SAM-limiting conditions, METTL16 dwell-time on MAT2A hp1 increases, presumably due to slower enzymatic turnover. The resulting increased occupancy of METTL16 promotes splicing of the detained intron to produce a functional MAT2A mRNA. However, the mechanism of METTL16 induction of MAT2A splicing remains unknown. To identify factors that regulate MAT2A splicing, we generated a reporter gene that fuses GFP with the MAT2A detained intron and 3´ UTR, integrated this into the genome of HCT116 cells, and isolated isolate a clone in which SAM starvation induces GFP. We used this line to perform unbiased genome-wide CRISPR screens to identify factors required for induction of MAT2A upon SAM depletion. We identified the factor NUDT21 (CFI_m25), a part of the cleavage and polyadenylation complex, to be necessary for induction of MAT2A. NUDT21 lacks known splicing domains, but complexes with CPSF6 (CFI m68) and CPSF7 (CFI m59), which contain RS domains. Upon knock-down of NUDT21, or co-depletion of CPSF6 and CPSF7, MAT2A fails to induce splicing under SAM limiting conditions, mimicking the phenotype of METTL16 knock-down. Our work suggests METTL16's role in SAM homeostasis requires NUDT21 and implies a novel role for the cleavage and polyadenylation factor NUDT21 in SAM metabolism.

Role of RNA uridylation in germ line differentiation

Marcos Morgan

Reproductive and Developmental Biology Laboratory
National Institute of Environmental Health Sciences

Post-transcriptional RNA modifications are known to play a critical role in cellular differentiation. However, the physiological relevance of many RNA modifications still remains unknown. Here, I will present the importance of 3' terminal uridylation during mouse germ line development, and other differentiation processes. To study the function of uridylation in vivo, I used conditional mutagenesis approaches where I removed TUT4 and TUT7 (TUT4/7), the two main terminal uridylyl-transferases, during early embryogenesis and the late stages of male and female gametogenesis. TUT4/7 can add non-templated 3' Us to a number of RNA species such as miRNAs, piRNA and mRNA in many different tissues. However, TUT4/7 activity is only essential during specific stages of cellular differentiation. This work contributes to our better understanding of the role of RNA modifications, in particular, uridylation, in mammalian physiology.

Long non-coding RNA NEAT1 promotes sarcoma metastasis by regulating RNA splicing pathways

Jianguo Huang, Eric Xu, Mohit Sachdeva, Timothy Robinson, Yan Ma, Lixia Luo, Nerissa Williams, Lisa Cervia, Fan Yuan, Xiaodi Qin, Dadong Zhang,Kouros Owzar,Nalan Gokgoz, Andrew Seto,Tomoyo Okada, Samuel Singer,Irene Andrulis, Jay Wunder, Alexander Lazar, Brian Rubin, David G. Kirsch

Department of Radiation Oncology
Department of Pharmacology and Cancer Biology
Duke University

40% of soft tissue sarcoma (STS) patients develop fatal lung metastasis with a median survival of 15 months. The mechanisms driving lung metastasis in sarcoma patients are poorly understood. To investigate sarcoma metastasis, we used a genetically engineered mouse model (GEMM) of primary STS with conditional mutations in Kras and Trp53(KP) where 40% of mice develop lung metastasis. This KP sarcoma model recapitulates human patients with Undifferentiated Pleomorphic Sarcoma (UPS), one of the most common subtypes of STS diagnosed in adults. RNA sequencing and Real time PCR determined that the expression of the long non-coding RNA (lncRNA) Neat1is increased in lung metastases compared to paired primary mouse sarcomas. Furthermore, NEAT1RNA in situ hybridization on tissue microarrays of human primary UPS and lung metastases determined that the expression of NEAT1is upregulated in lung metastases. Next, loss of Neat1significantly reduced lung metastasis in vivo. We then performed an RNA pull down assay with mass spectrometry and identified severalNeat1interactingproteins, including Khsrp, which regulate RNA splicing pathways. In addition, upregulation of KHSRP and NEAT1are significantly correlated with poor prognosis in sarcoma patients .Moreover, RNA splicing pathways were also shown to be downregulated in lung metastases compared to paired KP primary mouse sarcomas. Finally, knockout of Khsrp significantly reduced lung metastasis in vivo. Overall, these results suggest that upregulation of Neat1promotes sarcomametastasis through regulating RNA splicing pathways and NEAT1is a potential target to prevent or treat lung metastasis in sarcoma patients.

Detecting m^{6}A and its role in gene expression

Kate D. Meyer

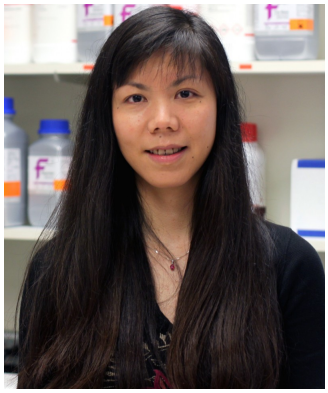
Department of Biochemistry, Duke University School of Medicine, Durham, NC 27710

m^{6}A is a highly abundant base modification which is present in thousands of cellular mRNAs. m^{6}A contributes to nearly every aspect of mRNA regulation, including splicing, stability, localization, and translation. Furthermore, emerging links between m^{6}A regulation and cancer have revealed the importance of this modification for human disease. To date, most studies exploring m^{6}A distribution transcriptome-wide have relied on antibody-based approaches for global m^{6}A mapping. While such methods have enabled unprecedented insights into the location and function of m^{6}A within cells, they suffer from several limitations, including the requirement for large amounts of input material, poor measures of m^{6}A stoichiometry, and cross-reactivity of m^{6}A antibodies with other RNA modifications. Here, we present DART-seq (deamination adjacent to RNA modification targets), a new method for global m^{6}A profiling which overcomes many of the limitations of antibody-based approaches. DART-seq can identify m^{6}A sites transcriptome-wide using as little as 10 nanograms of total RNA and provides information on m^{6}A abundance at individual sites. Furthermore, DART-seq can identify m^{6}A dynamics in cells and reveal new insights into m^{6}A distribution using long-read m^{6}A profiling. This approach will enable future studies of m^{6}A regulation in limited samples and will potentially enable m^{6}A profiling in single cells.

Lsd1 and Lsd2 lysine demethylases regulate one another and play parallel roles with other histone modifying enzymes in *Schizosaccharomyces pombe*

James F. Tucker[1], Bahjat F. Marayati[1], Jason Hou[1] and **Ke Zhang**[1]

[1] Department of Biology and Center for Molecular Communication and Signaling, Wake Forest University, North Carolina, U.S.A



Epigenetic gene silencing plays a critical role in regulating gene expression and contributes to organismal development and cell fate acquisition in eukaryotes. In fission yeast, *Schizosaccharomyces pombe*, heterochromatin-associated gene silencing is mediated by transcriptional gene silencing factors such as histone modifying enzymes and post-transcriptional silencing mechanisms including RNAi machinery and/or pathways eliminating RNAs and transcription termination factors. Our current work is focused on elucidating the functions of two conserved lysine demethylases, Lsd1 and Lsd2, which serve complex roles in the control of gene expression. The null mutations of Lsd1 and Lsd2 result in either severe growth defects or lethality while catalytic inactivation causes minimal defects, indicating that Lsd1 and Lsd2 serve essential functions beyond their known histone demethylase activity. However, a lack of available mutants with intermediate defects of these proteins has prevented exploration into their non-enzymatic roles. In addition, how Lsd1 and Lsd2 serve divergent functions in fission yeast has not been explored. We have generated a series of novel mutants of Lsd1 and Lsd2 and show that Lsd1 and Lsd2 repress heterochromatic transcripts through a mechanism that is partially independent of their known catalytic activity. We present evidence that Lsd1 and Lsd2 regulate one another and serve parallel roles with other histone modifiers.

Modification of mRNA by snoRNA-guided 2'-O-methylation

Brittany A. Elliott[1,4], Hsiang-Ting Ho[1,4], Srivathsan V. Ranganathan[2], Sweta Vangaveti[2], Olga Ilkayeva[3], Hala Abou Assi[1], Alex K. Choi[1], Paul F. Agris[1] & **Christopher L. Holley** [1]

[1]Department of Medicine, Duke University Medical Center, Durham, NC 27705, USA. [2] The RNA Institute, State University of New York, Albany, NY 12222, USA. [3]Duke Molecular Physiology Institute, Duke University, Durham, NC 27701, USA. [4] These authors contributed equally: Brittany A. Elliott, HsiangTing Ho.

Epitranscriptomic modifications of mRNA are important regulators of gene expression. While internal 2′-O-methylation (Nm) has been discovered on mRNA, questions remain about its origin and function in cells and organisms. Here, we show that internal Nm modification can be guided by small nucleolar RNAs (snoRNAs), and that these Nm sites can regulate mRNA and protein expression. Specifically, two box C/D snoRNAs (SNORDs) and the 2′-Omethyltransferase fibrillarin lead to Nm modification in the protein-coding region of peroxidasin (Pxdn). The presence of Nm modification increases Pxdn mRNA expression but inhibits its translation, regulating PXDN protein expression and enzyme activity both in vitro and in vivo. Our findings support a model in which snoRNA-guided Nm modifications of mRNA can regulate physiologic gene expression by altering mRNA levels and tuning protein translation.

The RNA polymerase II transcription factor, Paired Box 9 (PAX9), regulates ribosome biogenesis and craniofacial development.

Katherine I Farley-Barnes[1,2,3], Engin Deniz[4], Maya M Overton[1], MK Khokha[2,4], **Susan J Baserga**[1,2,3*]

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[2] Department of Genetics, Yale University School of Medicine, New Haven, Connecticut, United States of America
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[4] Pediatric Genomics Discovery Program, Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut, United States of America

Dysregulation of ribosome production can lead to a number of developmental disorders called ribosomopathies. Despite the ubiquitous requirement for these cellular machines used protein synthesis, ribosomopathies manifest tissue specifically, with many affecting the development of the face. Here we reveal a connection between craniofacial development and making ribosomes through the protein Paired Box 9 (PAX9). PAX9 functions as an RNA Polymerase II transcription factor to regulate the levels of proteins required for craniofacial and tooth development in humans. We now expand this function of PAX9 by demonstrating that PAX9 acts outside of the cell nucleolus to regulate the levels of proteins critical for building the small subunit of the ribosome. This function of PAX9 is conserved to the organism *Xenopus tropicalis*, an established model for human ribosomopathies. Depletion of *pax9* leads to craniofacial defects due to abnormalities in neural crest development, a result consistent with that found for depletion of other ribosome biogenesis factors. This work highlights an unexpected layer of how the making of ribosomes is regulated in human cells and during embryonic development.

Ribosome control by K63 ubiquitin during oxidative stress.

Gustavo Silva

Department of Biology, Duke University

As part of the defense mechanism, eukaryotic cells accumulate a large amount of ubiquitinated proteins during exposure to oxidative stress. Oxidative stress damages a variety of biomolecules and is the underlying cause of several human diseases. Although ubiquitin is the canonical signal for protein degradation by the proteasome, new signaling functions uncovered demonstrate that cells can form a variety of functionally distinct ubiquitin chain types. While investigating these chains, we observed that polyubiquitin chains linked by lysine 63 (K63) heavily accumulate in response to stress. We developed a proteomics method that showed that ribosomes are the main target of K63 ubiquitin, and the resolution of this method allowed us to identify several sites of ubiquitination in the head of the 40S ribosome. We further showed that ubiquitination of monosomes and polysomes is promoted by the ubiquitin enzymes Rad6 (E2) and Bre1 (E3) and is removed by the deubiquitinating enzyme Ubp2, which is reversibly inhibited by H_2O_2. Cells lacking K63 ubiquitin are sensitive to stress and to translation inhibitors, and structural biology and functional assays are providing the information required to understand how K63 ubiquitin impacts ribosome structure, thus aiding the essential reprogramming of translation during stress.

RNA-mediated mechanisms of translation control

Christine M. Dunham, Eric D. Hoffer, Ha An Nguyen, Sunita S., Samuel Hong, Tatsuya Maehigashi

Department of Biochemistry
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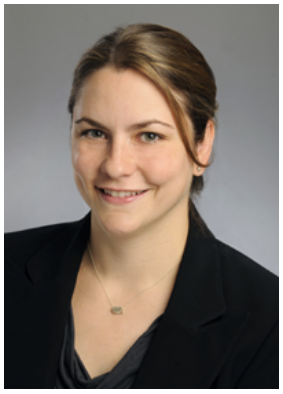

Gene expression relies on the interplay of dozens of proteins and large nucleic acid molecules that assemble into complex macromolecular machines such as the ribosome. These machines are regulated by posttranscriptional and posttranslational modifications. Numerous tRNA modifications, for example, are known to influence the fidelity of mRNA decoding by the ribosome. In the absence of this translational fidelity, nonsense protein products are expressed or premature termination occurs resulting in toxic protein products that overwhelm the cellular proteolysis machinery resulting in cell death. Posttranscriptional modifications of tRNAs have been known for decades but most studies have focused on how anticodon modifications expand the genetic code by allowing non Watson-Crick base-pairing at the third position of the codon-anticodon interaction on the ribosome. However, most tRNAs are also extensively modified outside their anticodon stem-loop. These modifications affect other aspects of translational fidelity such as the ability of the ribosome to maintain the three-nucleotide codon of the mRNA as it moves through the ribosome. The absolute requirement for precise correlation between the mRNA frame and the correct protein sequence to be expressed underlies an fundamental question in molecular biology: what regulates the mRNA reading frame? To address this question, we study two examples of defined biological systems that *subvert* the three-nucleotide mRNA reading frame resulting in high levels of programmed frameshifting. Our biochemical and structural results reveal that tRNA distortion and conformational changes of the small ribosomal subunit are induced by frameshift-prone tRNAs and highly structured mRNAs. These programmed gene expression events cause changes in the dynamic motions of the ribosome required for productive translation. This dysregulation causes the ribosome to lose its grip on the mRNA, allowing the tRNA to shift into a new reading frame. Together these studies reveal how the ribosome can be programmed for to encode for alternative mRNA frames and in addition suggest how dysregulation of the mRNA frame may occur by the disruption of key interactions between tRNAs, mRNA, and translation factors with the ribosome.

RNA structure-function relationships under *in vivo* and *in vivo-like* conditions: Impacts on RNA catalysis, folding, and transcriptome-wide response to stress

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RNA structure is intimately connected to function. The structure of RNA can differ *in vivo* versus *in vitro* owing to temperature, crowding, pH, and Mg^{2+} concentration. My lab has developed ways to mimic *in vivo* conditions using weakly-chelated Mg^{2+} ions, polyamines, crowders, and metabolites.[1,2] I will discuss how such *in vivo-like* conditions influence RNA chemical stability, thermal stability, and catalytic activity.[1,3] In addition, our lab in collaboration with the Assmann lab at Penn State developed Structure-seq, a method that combines chemical probing of RNA structure with high-throughput sequencing, thus allowing characterization of RNA secondary structure genome-wide and *in vivo*.[4] I will discuss applications of Structure-seq to organisms across all three domains of life, with a focus on response to thermal stress.[5] Efforts to develop a suite of chemical probes that can be incorporated into Structure-seq that query the base-pairing status of all four nucleotides *in vivo* will be presented as well.[6,7] This work involves all members of my lab and is in close collaboration with the Assmann, Babitzke, and Ferry labs at Penn State.

[1] Yamagami, R., Bingaman, J. L., Frankel, E. A., and Bevilacqua, P. C. (2018) Cellular conditions of weakly chelated magnesium ions strongly promote RNA stability and catalysis, *Nat. Commun. 9*, 2149.
[2] Yamagami, R., Huang, R., and Bevilacqua, P. C. (in press) Cellular concentrations of nucleotide diphosphate-chelated magnesium ions accelerate catalysis by RNA and DNA enzymes, *Biochemistry*.
[3] Messina, K. J., and Bevilacqua, P. C. (2018) Cellular small molecules contribute to twister ribozyme catalysis, *J. Am. Chem. Soc. 140*, 10578-10582.
[4] Ding, Y., Tang, Y., Kwok, C. K., Zhang, Y., Bevilacqua, P. C., and Assmann, S. M. (2014) *In vivo* genome-wide profiling of RNA secondary structure reveals novel regulatory features, *Nature 505*, 696-700.
[5] Su, Z., Tang, Y., Ritchey, L. E., Tack, D. C., Zhu, M., Bevilacqua, P. C., and Assmann, S. M. (2018) Genome-wide RNA structurome reprogramming by acute heat shock globally regulates mRNA abundance, *Proc. Natl. Acad. Sci. U S A 115*, 12170-12175.
[6] Mitchell, D., 3rd, Renda, A. J., Douds, C. A., Babitzke, P., Assmann, S. M., and Bevilacqua, P. C. (2019) In vivo RNA structural probing of uracil and guanine base-pairing by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), *RNA 25*, 147-157.
[7] Mitchell, D., 3rd, Ritchey, L. E., Park, H., Babitzke, P., Assmann, S. M., and Bevilacqua, P. C. (2018) Glyoxals as *in vivo* RNA structural probes of guanine base-pairing, *RNA 24*, 114-124.

Environmental regulation of the estrogen response in single cells

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Gene regulation is complex, involving the coordination of hundreds of proteins, including chromatin remodelers, enhancer RNA and chromosome looping. The estrogen response has served as a model of gene regulation due in part to its rapid and extensive induction. Single molecule RNA imaging in living cells has shown that this process is dynamic and heterogenous in time leading to differences in cell responsiveness and expression in the cell population. This variability in expression at the tissue and single cell level increases with age, implicating environmental epigenetic regulation. Environmental factors such as endocrine disruptor chemicals are found in our food and can disrupt the normal estrogen response. However, how single cells respond to endocrine disruptor chemicals is unclear. To investigate the interplay between the environment and the estrogen response we are using single-molecule imaging of the estrogen receptor and transcription in living cells. This work will reveal mechanistic insights into how endocrine disrupting chemicals perturb estrogen receptor mediated transcription.

Regulation of chromatin architecture and transcription by nucleoporin proteins

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Efficient coupling of transcription with mRNA export through the Nuclear Pore Complexes (NPCs) is essential for control of gene expression. The NPCs are formed of large multi-protein channels consisting of ~30 nucleoporin proteins. Emerging evidence suggests that in addition to their well-established role in nucleocytoplasmic trafficking at the NPCs, a subset of nucleoporin proteins including Nucleoporin 153 (NUP153) interact directly with chromatin and these interactions impact transcription of genes that associate with development. One of the outstanding questions involves delineating the underlying mechanisms of nucleoporin-mediated gene expression control at the chromatin level and how these processes are functionally linked to mammalian development. To provide mechanistic insights, we focused on NUP153, which was shown to be critical for maintenance of pluripotency in mouse embryonic stem cells (ESCs). We use a combination of biochemical, cell biological and genome-wide assays and two experimental systems- HeLa cells and mouse ESCs. Using HeLa cells enables us to take advantage of the well-characterized immediate early genes (IEGs) at which transcription can be triggered rapidly and transiently upon stimulation of cells with Epidermal Growth Factor (EGF) and investigate transcription dependent mechanisms of NUP153. The use of ESCs enables us to explore the role of NUP153 in gene regulation during early development. Our studies provide new and critical mechanistic insights that causally link NUP153 to Pol II-pausing mediated gene regulation and distribution of chromatin architectural proteins, placing NUP153 as a critical regulator of chromatin landscape and transcription during stem cell development.

Computationally reconstructing cotranscriptional RNA folding pathways from experimental data

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The series of RNA folding events that occur during transcription can influence the functional roles of cellular RNAs. However, few methods generate high-resolution models of this ubiquitous process that is important for gene expression and macromolecular assembly. Here we present a method, Reconstructing RNA Dynamics from Data (R2D2), to uncover details of cotranscriptional folding by predicting RNA secondary and tertiary structures from cotranscriptional SHAPE-Seq data. We applied R2D2 to the Escherichia coli Signal Recognition Particle (SRP) RNA and demonstrate that it navigates through cotranscriptional structures, which in turn require rearrangements to reach the functional fold. Folding pathway predictions and all-atom molecular dynamics simulations suggest that these rearrangements can proceed through a toehold-mediated strand displacement mechanism, which can be disrupted and rescued with point mutations. Our results demonstrate complex folding processes during RNA synthesis, and small sequence variations can drastically affect cotranscriptional folding. We also showed computationally that a natural precursor sequence present at the 5'endof the SRP RNA does not interfere with this folding mechanism. This precursor sequence was thought to be cleaved post-transcriptionally by RNase P, an endonuclease known primarily for its role in tRNA 5'-maturation. However, we found that a truncated precursor SRP RNA containing some canonical RNase P recognition determinants is indeed cleaved in vitro by recombinant E. coli RNase P, suggesting that precursor SRP RNA undergoes 5'-processingcotranscriptionally. Overall, R2D2 provides a powerful strategy for utilizing experimental data to gain deeper insights into cotranscriptional RNA folding and processing and its biological impact.

Features of RNA binding motifs

Daniel Dominguez, UNC Chapel Hill

Visualizing RNA structural ensembles by single molecule chemical probing

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The function of many RNAs is intimately linked to a capacity to fold into and dynamically switch between multiple structures. However, resolving the structural ensembles that are populated by dynamic RNAs remains an outstanding challenge, particularly in vivo. We present a new strategy that integrates machine learning and single molecule chemical probing experiments to directly visualize RNA structural ensembles in cells. Experiments performed on the V. vulnificus add adenine riboswitch system validate the power of our approach, allowing us to directly and quantitatively visualize conformational switching in response to adenine ligand binding. We then apply our approach to identify a large-scale structural switch in the human 7SKnon-coding RNA that plays a key role in regulating transcription by sequestering the positive transcription elongation factor (P-TEFb) complex. These structural data enabled us to design antisense oligonucleotides (ASOs) that specifically induce 7SK structural switching and modulate transcription in cells, representing a potential novel therapeutic for HIV and cancer. Combined, our data demonstrate the enormous potential of single molecule chemical probing for characterizing and targeting complex RNA structural ensembles in the native cellular environment.

Cohesin SA1 and SA2 are RNA binding proteins

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The cohesin complex plays important roles in sister chromatid cohesion, DNA replication, repair and recombination. In vertebrates, the core cohesin complex consists of a tripartite ring assembled from SMC1, SMC3, and RAD21 (also known as SCC1), and the stromal antigen subunit (SA) SA1 (STAG1) or SA2 (STAG2). Importantly, cohesin SA2 has been identified as 1 of 12 genes mutated in four or more cancer types. Specifically, the most recurrent mutation in Ewing sarcoma is on SA2, occurring in 15% of tumors. Despite the importance of cohesin SA1 and SA2, their biophysical properties were largely unknown. Recently, we reported that cohesin SA1 and SA2 are ss and dsDNA binding proteins. Furthermore, our recent finding of the elevated R-loop level in Ewing sarcoma cells raise the important question as to whether cohesin SA1 and SA2 are RNA binding proteins. R-loops are three-stranded nucleic acid structures consisting of an RNA-DNA hybrid and a displaced ssDNA loop. Using single-molecule imaging techniques including atomic force microscopy (AFM), fluorescence microscopy imaging, and bulk fluorescence anisotropy, we discovered that cohesin SA1 and SA2 bind to RNA containing nucleic substrates, including ssRNA, dsRNA, dsRNA with overhang, RNA/DNA hybrid, and R- loops. Furthermore, SA1 and SA2 are capable of binding to long ssRNA transcripts. These findings shed new light onto the mechanisms underlying the diverse cellular functions of SA1 and SA2 inside cells.

Poster Presentations

Poster 1

Pack it up, pack it in!
Protein-Directed RNA Structural Changes Mediate Rotavirus Segment Assembly

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The rotavirus genome is composed of 11 segments of double-stranded RNA. Each segment of the genome is monocistronic, making each protein product integral for the ability of the virus to propagate and infect host cells. To evade the host immune response, the segmented genome is ejected into the host cell as ssRNA. The virus is then replicated and repackaged in viral factories called viroplasms. In the viroplasm the ssRNA has to participate in segment gymnastics to ensure that each nascent virion has a single copy of each genomic segment packaged.

We hypothesize that RNA folding, inducing a mix of inter and intramolecular interactions, between the segments ensures correct genomic packaging. Here we present a secondary structure model of the ssRNA genome of the rotavirus obtained using SHAPE. This in vitro model of the rotavirus genome was modeled in presence and absence of the viral RNA chaperone, NSP2. The presence or absence of this RNA folding chaperone influences the emergence different structural motifs in the rotavirus genome. These data support our hypothesis of RNA structure mediating segment packaging.

Poster 2

Imine-based dynamic combinatorial chemistry for selection of small molecules targeting HIV RNAs

Aline Umuhire-Juru, Adina Jan, Fikemi Faleye, Amanda E. Hargrove
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Non-protein coding RNA transcripts have been increasingly recognized as potential drug targets owing to their important roles in cellular processes. Since peptide-based and RNA-based therapeutics often exhibit poor in vivo delivery and pharmacokinetics, small molecules offer an excellent alternative for targeting RNA. However, despite several emerging examples of small molecules targeting RNA selectively in the cell, only a small subset of disease-relevant RNAs has been explored as drug targets. Targeting a wider range of RNAs has been hampered in part by current limitations in three-dimensional structure characterization of complex RNAs. Therefore, there is a need to develop generalized techniques for RNA ligand discovery that do not rely on structure-based design.
We are developing an imine-based dynamic combinatorial chemistry (DCC) technique to expedite ligand discovery for diverse RNAs. In DCC, a target biomolecule is incubated with a thermodynamically-controlled dynamic library of small molecules, allowing the target to select its highest affinity binders. To date, we have conducted comparative studies of amine reactivity towards imine formation and have begun validation studies on a known RNA-binding scaffold. An aldehyde scaffold is incubated with aromatic amines to select ligands for three HIV RNA constructs with diverse structures. Preliminary studies show amplification of highest-affinity binders over non-binders in the presence of RNA. In the future, we will use this methodology to identify the first ligands for a number of complex HIV RNA targets. Ultimately, this work will provide a platform for ligand discovery for large RNAs, particularly those that have yet to be structurally characterized.

Poster 3

The Role of Structure in Conserved Long Non-coding RNA MALAT1

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Alain Laederach, Department of Biology, University of North Carolina, Chapel Hill, NC,27599

LncRNAs are ubiquitous, but their function is generally unknown. Their functions are hypothesized to be linked to their secondary structure in the cell, but it is difficult to explore this in most lncRNAs as they are rapidly evolving and lowly expressed. MALAT1, however, is a lncRNA that is highly expressed, well conserved, with known function, including splicing, transcriptional regulation, and acting as a miRNA sponge. Using in vivo structure probing techniques (SHAPE-MaP and DMS-MaP), we probed MALAT1 structure in multiple cell types.
Having established consistency of structure in multiple cell types, we turned our attention to examining MALAT1 structure between species. We identified a candidate cell line derived from Green Monkeys which have an approximate 97.3% sequence similarity to human MALAT1. We have successfully probed regions of MALAT1 in the Green Monkey cell line. Additionally, our group has established a full-length model of in vitro MALAT1 in a human cell line. We are currently developing methods to successfully probe the structure of the full-length MALAT1 transcript in both species. These data will provide insight to which RNA structures are maintained and which are lost over this short evolutionary distance.

Poster 4

Impaired cell migration and structural defects in myeloid cells overexpressing miRNA-30b and miR-142-3p

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Macrophages (MΦ) and dendritic cells (DC) play a fundamental role in shaping immune response by sensing plethora of Pattern Associated Molecular Patterns (PAMPs), their phagocytosis and antigen presentation to T lymphocytes. These important biological processes require an efficient cell movement and an intact cellular morphology. Role of microRNAs (miRs) in this regard, however, is not well understood. In the present study, we identified both miR-30b and miR-142-3p affecting the migration and structural morphology in MΦ and DCs. Transient overexpression of miR-30b and miR-142-3p attenuates migration and reveals unique morphological deformities under electron microscopy indicating that these miRNAs impact structural feature of APCs. In addition, miR-142-3p overexpression in MΦ impaired phagocytosis of FITC conjugates latex beads using live microscopy imaging. Interestingly, live cell imaging revealed marked changes in the actin polymerization status and polarity. To identify miR-142-3p regulated pathways, we performed global transcriptome analysis in miR-142-3p or control mimic transfected DC. Several genes that were differentially altered by miR-142-3p were associated with pathways related to cell movement, cell adhesion, cytoskeletal rearrangement, etc., were revealed a significant subset of downregulated genes were predicted to harbor miR-142-3p binding sites strongly suggesting that direct post-transcriptional impact of the miRNA on multiple transcripts. In summary, our results show that miR-30b and miR-142-3p acts as negative regulators of myeloid cell cytoskeletal homeostasis and morphology.
Key words: MicroRNA, cell migration, cytoskeletal network, myeloid cells.

Poster 5

MicroRNA Biomarkers associated with Chronic Pain and Pain Resolution

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NIH/NIDCR R56 DE025296-01 “Proteins, MicroRNAs and Genes Associated with TMD and Overlapping Conditions” awarded to AG Nackley and G Slade and NIH/NINDS R03 NS106166 “Defining the role of adipocyte Adrb3 in chronic pain” awarded to A Nackley.

Chronic overlapping pain conditions (COPCs), like temporomandibular disorder (TMD), irritable bowel syndrome (IBS), and migraine headaches are poorly managed affecting more than 15% of the population. These conditions often co-occur, suggesting a shared etiology. Identifying biomarkers that regulate the onset and maintenance of COPCs will advance treatment strategies for improved pain management. MicroRNAs have proven to be useful biomarkers in predicting patient outcomes and treatment response in other chronic disease states. This study aims to identify microRNA biomarkers associated with TMD and other COPCs. Circulating microRNA expression was measured in blood samples collected from 200 TMD cases and 292 healthy controls using small RNA seq. Differential expression of microRNA was calculated using linear modeling. The expression of 17 miRNAs was differentially expressed between healthy controls and TMD cases. Of these miRNAs, four were correlated with case status at a highly significant level, $p < 0.001$ miRNAs 766-3p, 197-3p,1226-3p, and 3609. We defined these miRNAs as the core of our TMD miRNA-mRNA network and analyzed mRNA targets of these miRNAs. Changes in microRNA expression over time were also measured in a subset of TMD resolvers (N=14) and non-resolvers (N=8). When comparing expression between TMD resolvers versus non-resolvers, we found that circulating levels of miR 181a-5p were upregulated in the TMD resolver group, while levels of miR 150-5p are upregulated in the TMD non-resolver group. The results of mRNA-miRNA analysis suggest that regulation or dysregulation of immunity is a dominant factor for TMD and its’ resolution.

Poster 6

Why are Hoogsteen base pairs energetically disfavored in A-RNA compared to B-DNA?

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A(*syn*)-U/T and G(*syn*)-C^+ Hoogsteen (HG) base pairs (bps) are energetically more disfavored relative to Watson–Crick (WC) bps in A-RNA as compared to B-DNA by >1 kcal/mol for reasons that are not fully understood. Here, we used NMR spectroscopy, optical melting experiments, molecular dynamics simulations and modified nucleotides to identify factors that contribute to this destabilization of HG bps in A-RNA. Removing the 2′-hydroxyl at single purine nucleotides in A-RNA duplexes did not stabilize HG bps relative to WC. In contrast, loosening the A-form geometry using a bulge in A-RNA reduced the energy cost of forming HG bps at the flanking sites to B-DNA levels. A structural and thermodynamic analysis of purine-purine HG mismatches reveals that compared to B-DNA, the A-form geometry disfavors *syn* purines by 1.5–4 kcal/mol due to sugar-backbone rearrangements needed to sterically accommodate the *syn* base. Based on MD simulations, an additional penalty of 3–4 kcal/mol applies for purine-pyrimidine HG bps due to the higher energetic cost associated with moving the bases to form hydrogen bonds in A-RNA versus B-DNA. These results provide insights into a fundamental difference between A-RNA and B-DNA duplexes with important implications for how they respond to damage and post-transcriptional modifications.

Poster 7

Loss of Elongation-Like Factor 1 Spontaneously Induces Diverse, RNase H-Related Suppressor Mutations in *Schizosaccharomyces pombe*.

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A healthy individual may carry a detrimental genetic trait that is masked by another genetic mutation. Such suppressive genetic interactions, in which a mutant allele either partially or completely restores the fitness defect of a particular mutant, tend to occur between genes that have a confined functional connection. Here we investigate a self-recovery phenotype in *Schizosaccharomyces pombe*, mediated by suppressive genetic interactions that can be amplified during cell culture. Cells without Elf1, an AAA+ family ATPase, have severe growth defects initially, but quickly recover growth rates near to those of wild-type strains by acquiring suppressor mutations. *elf1Δ* cells accumulate RNAs within the nucleus and display effects of genome instability such as sensitivity to DNA damage, increased incidence of lagging chromosomes, and mini-chromosome loss. Notably, the rate of phenotypic recovery was further enhanced in *elf1Δ* cells when RNase H activities were abolished and significantly reduced upon overexpression of RNase H1, suggesting that loss of Elf1-related genome instability can be resolved by RNase H activities, likely through eliminating the potentially mutagenic DNA-RNA hybrids caused by RNA nuclear accumulation. Using whole genome sequencing, we mapped a few consistent suppressors of *elf1Δ* including mutated Cue2, Rpl2702, and SPBPJ4664.02, suggesting previously unknown functional connections between Elf1 and these proteins. Our findings describe a mechanism by which cells bearing mutations that cause fitness defects and genome instability may accelerate the fitness recovery of their population through quickly acquiring suppressors. We propose that this mechanism may be universally applicable to all microorganisms in large-population cultures.

Poster 8

Targeting the oncogenic long non-coding RNA SLNCR1 by blocking its sequence-specific binding to the androgen receptor

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Long non-coding RNAs (lncRNAs) regulate several biological processes, and some lncRNAs are aberrantly expressed in cancer cells. Disease-specific lncRNAs are ideal candidate therapeutic targets. However, the mechanisms by which lncRNAs promote disease are not well understood, limiting the development of lncRNA-based cancer therapeutics. An interaction between the androgen receptor (AR) and the lncRNA SLNCR1, which is exclusively expressed in melanomas, promotes melanoma invasion. Using in-cell SHAPE-MaP structure determination we show that AR binds to a region of unstructured RNA in SLNCR1. Additional biochemical assays show that the N-terminus of AR binds to SLNCR1 in a sequence specific manner. Delivery of antisense oligonucleotides designed to block the interaction between SLNCR1 and AR reduces SLNCR1-mediated melanoma invasion in a cell invasion assay. Integrating information on RNA structure and RNA-protein interactions thus enabled successful inhibition of a lncRNA-protein interaction in human cancer cells. We expect similar approaches to be critical for future development of lncRNA-targeted therapies.

Poster 9

Elucidating the role of RNA localization and local translation in Radial Glial Cell endfeet

Brooke D'Arcy, Duke University

Although subcellular local gene regulation has been well studied and found to be instrumental in neurons, studies in Radial Glial Cells (RGCs) are in their infancy. Along these lines, our lab has discovered that RNA is actively transported in RGCs to distal compartments, called endfeet, where it undergoes local translation. RGCs are essential for brain development due to their role as progenitors producing neurons and glia. The elongated basal processes of the RGCs stretch from the ventricle to the basement membrane forming a scaffold to guide migrating neurons. The integrity of this scaffold is dependent on the endfeet which attach to the basement membrane, stabilize the basal process, and provide a barrier which prevents over migration of neurons. Improper neuron migration due to endfoot detachment has been shown to result in neurological deficits. Our lab has previously developed *in vivo* methods for studying RNA localization in RGCs which have shown that a subset of RNAs are localized to the endfeet where local translation can occur. We recently found that Tensin 3 (TNS3) is present in endfeet at the RNA level and is significantly enriched in endfeet compared to the cell body at the protein level. TNS3 is a focal adhesion protein which connects the extracellular matrix to the cytoskeleton by binding both β _integrins and actin. Previous studies suggest roles for TNS3 in basement membrane attachment, RhoA signaling, and actin dynamics all which are important for endfoot functionality. Here we show that TNS3 mRNA localization is dependent on a cis-element in the 3'UTR. Also, TNS3 is locally translated in endfeet independent of the cell body. Following knockdown of TNS3 in RGCs *in vivo*, we have observed interruptions in basement membrane integrity. These studies begin to inform an understanding of how TNS3 protein becomes enriched in endfeet and its local function there. Additionally, our *in vivo* paradigm will provide new insights into RNA localization and local translation in the developing cortex.

Poster 10

Uniting Cleavage: Reconstitution of the Human tRNA Splicing Endonuclease Complex reveals that the core heterotetrameric TSEN complex is sufficient for pre-tRNA cleavage.

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The tRNA splicing endonuclease (TSEN) complex is an essential protein complex responsible for removing introns from intron-containing tRNAs in eukaryotes and archaea. The eukaryotic TSEN is comprised of four core subunits (TSEN54, TSEN2, TSEN34, and TSEN15); however, the human complex additionally associates with the polynucleotide kinase, CLP1. Mutations in genes encoding all four TSEN subunits, as well as CLP1, are known to cause certain neurodegenerative disorders, collectively known as pontocerebellar hypoplasia (PCH). Despite this disease connection, CLP1's role in tRNA splicing remains a mystery. The human TSEN complex has never been recombinantly reconstituted, so to begin better understanding the molecular basis of PCH caused by CLP1 and TSEN mutations, we reconstituted the human TSEN complex. We utilized our in vitro reconstitution to confirm that the active sites of TSEN2 and TSEN34 are conserved from yeast to humans. Further, we combined *in vivo* RNAi knockdowns of the *Drosophila* CLP1 homolog, cbc, in S2 cells with to suggest a putative regulatory role of CLP1 in tRNA maturation.

Poster 11

RNP-MaP: Mapping protein interaction networks on any RNA in living cells

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RNA-protein complexes (RNPs), which are tied together by interactions between RNA and RNA-binding proteins (RBPs), are critical in many biological regulatory networks. RBPs rarely act alone, but understanding how multiple RBPs coordinate on an RNA is currently difficult without extensive prior knowledge of the constituents of an RNP. We have developed a strategy, called RNP mapping by mutational profiling (RNP-MaP), to comprehensively identify protein-binding sites and to characterize protein interaction networks on an RNA in a single, straightforward experiment. RNP-MaP combines live-cell chemical probing, selective for RNA-protein interactions, with a simple sequencing readout to locate protein interaction sites within any RNA and with single nucleotide resolution. Moreover, RNP-MaP enables detection of simultaneous binding events by multiple proteins within single RNA molecules and reveals where proteins cooperate to form functional interaction networks. We have used RNP-MaP to interrogate non-coding RNAs ranging in length from 100 to 20,000 nucleotides. RNP-MaP accurately identifies all major protein binding sites on the U1 small nuclear RNA and highlights interactions between proteins that form the RNP. RNP-MaP also reveals the overlapping RNP networks in two structurally-related, but sequence-divergent RNAs: RNase P and RMRP. Finally, we applied RNP-MaP to explore how RNP networks interface with conserved sequences within the XIST long non-coding RNA that are

critical for X chromosome dosage compensation, and we identify functional motifs in XIST that regulate RNA stability and localization. RNP-MaP will be widely useful for discovery and mechanistic analysis of protein interaction networks across any RNA of interest.

Poster 12

Uncovering Dynamic Ensemble of HIV-1 Rev Response Element RNA as New Small Molecule Targets

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Antiretroviral combination therapies suppressing HIV-1 replication have transformed the fatal illness of AIDS into a long-term controllable disease. However, emergence of drug-resistant HIV-1 strains has raised the risks of transmission of the drug-resistant strains among individuals, and development of drugs targeting new replication steps is still needed. HIV-1 Rev response element (RRE) is a highly conserved viral *cis*-acting RNA element which plays an essential role in promoting incompletely-spliced viral RNA export through cooperative Rev binding. Because of its central role in HIV replication, RRE stem IIB is an attractive drug target for the development of *anti*-HIV therapeutics. Here, we used NMR spectroscopy to characterize the various conformational states that are sampled by stem IIB. The results indicate that the stem IIB adopts an average ground-state (GS) conformation with high flexibility. In addition, relaxation dispersion NMR data reveal the existence of two non-native excited state (ES) conformations that disrupt the Rev-binding site as inactive forms, and the mutants stabilizing ESs decrease ~ 50% Rev-RRE activity in the cell-based assays. Future work will construct atomic resolution ensembles for both the GS and ESs and perform computational docking against the dynamic ensembles to identify new small molecules that can inhibit the Rev-RRE interaction for *anti*-HIV therapeutics.

Poster 13

CDLINC: Novel Alternative Splicing of a LincRNA in Lung Cancer

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Long intergenic non-coding RNAs (lincRNAs) share many features with protein coding genes such as capping, splicing, and polyadenylation. Alternative splicing of lincRNAs may play a role in cancer progression. We identified an uncharacterized lincRNA located on chromosome 19 which we have renamed CDKN2A Dependent Long Intergenic Non-Coding (CDLINC). CDLINC has 11 known alternatively spliced transcripts with 3 main isoforms: CDLINCa, CDLINCb, and CDLINCc. Cells normally express CDLINCa, but not all express CDLINCb and CDLINCc. Loss of CDKN2A expression correlates with loss

of CDLINCb and CDLINCc expression in both non-small cell lung cancer (NSCLC) and triple negative breast cancer (TNBC). Further investigation has confirmed that this regulation of b and c isoform expression is p53 independent. Localization studies determined CDLINCa is present mainly in the nucleus while the CDKN2A dependent isoforms CDLINCb and CDLINCc are found primarily in the cytoplasm. Our findings suggesting a novel CDKN2A linked role for these alternatively spliced isoforms.

Poster 14

Heterogenous activation of estrogen responsive genes upon exposure to endocrine disrupting chemicals

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Gene regulation is complex, involving the coordination of hundreds of proteins, including chromatin remodelers, as well as enhancer promoter looping. Single cell live imaging has shown that this process is dynamic, and heterogenous in time leading to differences in cell responsiveness and expression in the cell population. Moreover, this variability in expression at the tissue and single cell level increases with age, implicating environmental epigenetic regulation. Environmental factors such as endocrine disruptor chemicals are found in our food and can disrupt the normal estrogen response. However, the single cell response to endocrine disruptor chemicals has not been investigated. To investigate the interplay between the environment and the estrogen response we are using single-molecule imaging of the estrogen receptor and transcription in living cells. This work will reveal mechanistic insights into how endocrine disrupting chemicals perturb estrogen receptor mediated transcription.

Poster 15

Regulation of membrane trafficking genes by alternative splicing

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Alternative splicing is a regulatory mechanism by which pre-mRNAs transcripts are processed to include or exclude exons. Changes in splicing patterns are important in heart and skeletal muscle development, as they can lead to the expression of proteins required for tissue identity acquisition and maintenance. During postnatal heart development, trafficking genes experience shifts in levels of alternative splicing. Through previous studies, it has been shown that epigenetic marks that alter chromatin state can influence patterns of alternative splicing. Here, we aim to determine the effects that chromatin state has on postnatal alternative splicing. We utilized C2C12 muscle cells, a well-established in vitro model of muscle cell differentiation (myogenesis), that recapitulates splicing transitions seen during heart and skeletal muscle development. In these cells, we modulated chromatin structure using the histone deacetylase inhibitor Trichostatin A (TSA), and evaluated splicing patterns of trafficking genes using reverse transcription PCR and quantification of the percent of inclusion of alternative exons. We found that there was significant skipping of exons in many of the trafficking genes. After we observed that chromatin structure could impact splicing patterns, we proceeded to measure the levels of various histone marks over the course of differentiation by western blot studies. Overall, we observed that permissive epigenetic marks increased during muscle cell differentiation (i.e. H3K36me3, H3ac). In conclusion, alterations in the chromatin state seemingly effect alternative splicing in muscle cell differentiation. Additionally, endogenous chromatin marks that are differentially expressed during differentiation potentially play a role in alternative splicing regulation.

Poster 16

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Bacterial RNA structure is known to both passively participate in and actively control gene regulation. These riboregulators respond to a variety of environmental and cellular stimuli, one of which is temperature. Structure-seq2, an *in vivo* genome-wide structure probing method, was used to determine temperature-dependent RNA structure changes in the model bacterium, *Bacillus subtilis*. Cell cultures were grown at a low temperature (23°C), a basal temperature (30°C), an intermediate temperature (37°C), and a heat-activated temperature (42°C) until mid-exponential phase. Three biological replicates for each temperature were then treated with dimethyl sulfate (DMS), methylating *in vivo* single-stranded A- and C- residues. After in-house library preparation, sequencing results show approximately 756 million reads spread across the 24 libraries. Biological replicates were compared to each other to ensure consistency, and downstream analyses were performed using StructureFold2. Our results capture the dynamic response of the transcriptome to temperature and highlight important areas for further study.

Poster 17

A microRNA/circle RNA axis promotes metastasis through cerebellar degeneration related protein 1

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Lung squamous carcinoma (LUSC) is a highly metastatic disease with a poor prognosis. Using an integrated screening approach, we found that miR-671-5p reduced LUSC metastasis by inhibiting a circular RNA (circRNA), CDR1as. Although the putative function of circRNAs is through miRNA sponging, we found miR-671-5p more potently silences an axis of CDR1as and its anti-sense transcript, cerebellar degeneration related protein 1 (CDR1). Silencing of CDR1as or CDR1 significantly inhibited LUSC metastases, and CDR1 was sufficient to promote migration and metastases. CDR1, which directly interacted with adaptor protein 1 (AP1) complex subunits and COPI proteins, no longer promoted migration upon blockade of Golgi trafficking. Epistasis experiments confirmed that the COPI subunit COPA is downstream of CDR1-mediated migration. Therapeutic inhibition of the CDR1as/CDR1 axis with miR-671-5p mimics reduced metastasis in vivo. To our knowledge, this is the first report demonstrating a role for CDR1 in promoting metastasis and Golgi trafficking. Our findings reveal a miRNA/circRNA axis that regulates LUSC metastases through an enigmatic protein, CDR1.

Poster 18

Analysis of Long-noncoding RNA Structure-Function Relationship in Prostate Cancer

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The noncoding RNA (ncRNA) revolution has revealed myriad RNA species that play critical roles in all forms of life, including crucial developmental biology and disease progression. For example, the lncRNA Metastasis Associated Lung Adenocarcinoma Transcript-1 (MALAT1; ~6.5 kb) and Second Chromosome Locus Associated with Prostate-1 (SChLAP1; ~1.5 kb) are basally expressed in normal prostate tissue but are dysregulated in prostate cancer. MALAT1 acts in trans at nuclear speckles during post-transcriptional processing while SChLAP1 acts in cis to influence oncogenic gene expression. As lncRNA represent an underexplored therapeutic avenue in general, this work aims to investigate the role of lncRNA structure and dynamics in driving prostate cancer metastasis.
Current work is two-fold: 1) Understanding the role of the MALAT1 3'-end triple helix dynamics in global transcript stability and 2) Conducting the first biophysical analyses of SChLAP1 and identifying structural elements that are critical to metastasis. Chemical probing experiments will be used to characterize the MALAT1 and SChLAP1 secondary and tertiary structural species while NMR will be used to further investigate MALAT1 triple helix dynamics. Preliminary MALAT1 results indicate that the triple helix is dynamic and can exist in multiple conformations while preliminary SChLAP1 data support the formation of independent folding domains that may be critical for the metastatic phenotype. This work will deepen our current understanding of MALAT1 triple helix dynamics in driving prostate cancer metastasis and provide the first biophysical and biochemical assessment of SChLAP1.

Poster 19

Examination of METTL16 RNA Targets and Cellular Location

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Methyltransferase-like 16 (METTL16, also METTL10D), identified only a few years ago, is responsible for depositing a methyl group on N^6 of adenosine (called m6A) in several different RNA targets. However, only a few of these targets have been intensely studied, including messenger RNA MAT2A, the long noncoding RNA MALAT1, and the snRNA U6. It has also been suggested that this methyltransferase is only found in the nucleus of the cell. Our goal was to verify these previous observations and discover additional RNA targets. Using both native and exogenously-introduced METTL16, we identified these and other RNA targets by immunoprecipitation. The results of these experiments differed, which led us to perform biochemical fractionation of the cells. It was revealed to us that METTL16 localized mostly to the cytoplasm, while only a small fraction was localized to the nucleus. RNA interference studies of METTL16 resulted in differential RNA expression of identified targets, suggesting this protein is involved in RNA regulation. More studies are needed to determine where the RNA targets are modified and the long-term effects of METTL16 knockdown on targets and general cell behavior. A quick method of m6A identification will expedite target identification and potentially confirm knockdown efficiency. Finally, introducing mutations into the METTL16 sequence will confirm if targets are methylated exclusively by this protein, and whether it can shuttle between the nucleus and the cytoplasm.

Poster 20

Mechanotransduction and alternative splicing regulation in skeletal muscle cells

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Skeletal muscle is a mechanosensitive tissue that exhibits high levels of developmental alternative splicing. RNA-binding proteins (RBPs) control splicing by binding to motifs within pre-mRNA. Numerous genes encoding for force transmitting membrane trafficking proteins are regulated by splicing in muscles, but the regulators are unknown. Defects in mechanical properties and splicing lead to devastating muscular dystrophies. Our goal is to understand the relationship between splicing regulation and mechanotransduction in muscles.

First, we found that during muscle cell differentiation splicing of numerous trafficking genes was controlled by two RBPs, polypyrimidine tract binding protein 1 (PTBP1) and quaking (QK). To determine how splicing regulation impacts mechanical properties of muscle cells, we depleted PTBP1 and QK and measured membrane stiffness using atomic force microscopy. Cells lacking PTBP1 and QK were stiffer than controls suggesting that transcriptional programs controlled by these RBPs affect cell stiffness. In the future, we plan to probe cell stiffening response using 3D force microscopy.

Second, we aim to identify mechanosensitive genes and alternative splicing networks. The Yes-associated protein (YAP) is a mechanosensitive, transcriptional activator that under stretching conditions translocates to the nucleus to activate downstream genes. We stretched undifferentiated and differentiated muscle cells using a Flexcell apparatus to mimic physiological stretching conditions. We observed that stretching resulted in expression changes of YAP-regulated genes. We plan to use RNA-seq to identify splicing and gene expression programs activated by mechanical forces in muscle cells. Ultimately, these studies will provide clues about molecular mechanisms of healthy muscle development and muscular disease onset.

Poster 21

Alternative splicing of the *Snap23, Tmed2,* and *Trak1* trafficking genes during striated muscle development

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Alternative splicing is a critical contributor of muscle development. Networks of alternative splicing transitions are coordinated in space and time by RNA-binding proteins (RBPs) to maintain muscle physiology. Further, dysregulation of splicing networks has repercussions associated with severe diseases. Genes encoding membrane trafficking proteins represent one network that exhibits developmental stage-specific splicing patterns. Surprisingly, few splice isoforms have been characterized and little is known about their functional roles. Here, we characterize three proteins involved in membrane trafficking that are alternatively spliced during striated muscle development: SNAP23, TMED2, and TRAK1. The synaptosome-associated protein 23 (SNAP23) mediates vesicle fusion with the plasma membrane during exocytosis. The transmembrane emp23 domain-containing protein 2 (TMED2) regulates vesicle budding during secretion. And the trafficking kinesin binding protein 1 (TRAK1) contributes to mitochondrial transport. Snap23 and Tmed2 pre-mRNAs undergo splicing of a single cassette exon, while Trak1 is subjected to alternative polyadenylation. At the mRNA and protein levels, there is a shift in isoform expression during muscle development: the Snap23 and Tmed2 cassette exons are increasingly retained, and the Trak1 proximal polyadenylation site is preferentially used. These splicing transitions are regulated by the RBPs

polypyrimidine tract binding protein 1 (PTBP1) and quaking (QKI), suggesting that these events comprise a functionally related splicing network. Further, depletion of Snap23 reveals its importance for muscle cell viability and differentiation, while Tmed2 depletion impacts only differentiation. Elucidating the mechanisms of regulation and the functional consequences of splicing networks will be fundamental to our understanding of muscle development and disease.

Poster 23

Investigating RNA structural motifs using small molecule-based pattern recognition

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The discovery that long non-protein coding (lnc) RNA have important regulatory roles has revolutionised molecular biology in recent years. As new functional and regulatory aspects of lncRNA are being investigated, these RNAs are also emerging as novel targets for disease diagnosis and therapies. However, a dearth of information for designing drugs targeting RNA is available.

The Hargrove lab has developed a pattern-based sensing technique (PRRSM) to determine and classify complex properties of small molecule-RNA recognition. PRRSM uses the somewhat promiscuous binding of aminoglycosides to RNA to sense the differential binding properties of RNA secondary structures (i.e., hairpin, stem, bulges, symmetric and asymmetric loops), which are measured through fluorescence variation of the label 5-benzofuranyl-uridine (BFU) that is placed at the most flexible position of the motifs. After performing principal component analysis (PCA), each secondary structure is correctly classified with 100% predictive power suggesting that underlying principles for the rational design of motif-selective RNA ligands are present.

Here, we use molecular dynamic simulations to identify the structural determinants at the basis of the RNA differentiation observed in PRRSM. Preliminary results delineate the size, shape and dynamics of each RNA motif and identify them as the major factors contributing to small molecule-based pattern recognition. This critical insight into discernible structural patterns of common RNA topologies will provide the basis for the rational design of selective RNA ligands to target therapeutically important lncRNA fragments. Ultimately, this knowledge will allow researchers to target full-length lncRNA with known implications in diseases such as cancer.

Poster 24

Using small-molecule antibiotics against a unique transcription regulating target in Gram-positive bacteria to fight antibiotic resistant biofilms

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Biofilm-associated Gram-positive bacteria cause infections in patients with implant devices and rapidly become resistant to antibiotics. Identifying effective methods to inhibit antibiotic resistance is needed. One promising approach is to target with small molecules the tRNA-dependent regulatory, T-box, system controlling aminoacyl-tRNA synthetase genes crucial for Gram-positive bacteria survival. The highly conserved specifier loop within the 5'UTR T-box element is found in many genes of the biofilm-producing pathogens, reducing the chances of bacteria mutating and becoming resistant to small-molecule antibiotics. A family of small molecules, PKZ18, inhibits growth of MRSA in biofilms. It was selected based on its ability to bind to the specifier loop in silico , potentially form favorable hydrogen bonds with the bases, and

destabilize the mRNA. UV-monitored thermal stability analysis experiments using chemically-synthesized wild type (WT) and mutant Stem 1 RNA constructs with and without the presence of PKZ18 revealed that PKZ18 decreases the stability of the mRNA when it binds to the WT Stem 1 region. PKZ18 did not bind to the mutant construct lacking the specifier loop, suggesting that the molecule specifically binds to the specifier loop region.
To confirm the exact region to which PKZ18 binds, we will synthesize multiple mutant versions of WT Stem 1, where different bases in the specifier loop have been deleted. Afterwards, we will use mass spectrometry to confirm the presence of PKZ18/RNA complexes and determine which nucleotides are essential for PKZ18 binding, based on whether or not the small molecule binds to the different mutants.

Poster 25

Investigating the Impact of Epitranscriptomic Modifications on RNA Dynamics

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Post-transcriptional modifications play essential roles in the biological functions of coding and non-coding RNAs. More than 100 chemically-modified RNA nucleosides have been identified to date that can impact RNA stability, translation efficiency, splicing, RNA-protein interactions, and folding. These modifications are highly abundant in rRNA, tRNA, snRNA, mRNA cap and more recently discovered in mRNA coding regions with the advancements in mapping techniques. Our work aims to determine whether epitranscriptomic modifications have the potential to trap transient short-lived and low-abundance RNA excited states (ESs) utilizing the transactivation response element (TAR) RNA from the human immune deficiency virus type 1 (HIV-1) as a model system. TAR exists in dynamic equilibrium with two excited states (ES1 and ES2) accompanied by reshuffling of base pairs in and around non-canonical motifs. NMR relaxation dispersion (RD) and chemical exchange saturation transfer (CEST) experiments show that 2'-*O*-methyl modifications in TAR increase the stability and lifetime of both excited states and thus have the potential to modulate RNA structural dynamics. Consequently, these modifications can expose or sequester key regulatory elements and therefore control the biological activity of structurally complex RNAs.

Poster 26

Differential Splice Form Stability of Trafficking Genes in Skeletal Muscle

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Alternative splicing of pre-mRNA occurs in approximately 90% of human genes and expands proteome diversity from a limited genome. RNA-binding proteins (RBPs) regulate this process to maintain physiology. Skeletal muscle has a high degree of alternative splicing, and genes encoding trafficking proteins are alternatively spliced during postnatal development.
C2C12 muscle myoblasts differentiate into myotubes (myogenesis) and reproduce developmental splicing transitions observed *in vivo*. Therefore, we used this model to identify factors that control splicing and determine how splice forms are regulated. We first observed that the RBPs MBLN1, MBLN2, PTBP1, and PTBP2 are downregulated during myogenesis while CELF2 is upregulated, suggesting that these RBPs might control alternative splicing during muscle cell differentiation. Second, splice forms from several trafficking genes exhibited differential stabilities. One example occurs in the clathrin-heavy chain (*Cltc*) gene, the main driver of clathrin-mediated endocytosis. Cltc exon 31 is skipped in myoblasts and is included during myogenesis. Interestingly, Cltc transcripts containing exon 31 are more stable than those lacking exon 31. Finally, we identified two microRNA (miRNA) binding sites, miR-7019-3p and miR-7658-3p, that span Cltc exons 30 and 32; only miR-7658-3p is expressed in C2C12 cells. MicroRNA binding sites may be a novel mechanism to explain differential splice form stability. Future studies will investigate if miRNAs directly regulate splice form stability. Together, our data suggests that RBPs contribute to alternative splicing during myogenesis. Furthermore, alternative exons may contribute to splice form stability, suggesting that splicing may impact protein isoform expression and function.

Poster 27

Dynein light chain dependent dimerization of Egalitarian is required for oocyte specification and mRNA localization.

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Egalitarian (Egl) is a RNA binding protein that is required for specification of oocyte fate in *Drosophila melanogaster*. Null alleles of Egl arrest oogenesis at very early stages. At later stages of egg development, Egl is required for the specific localization of numerous mRNAs within the oocyte. In addition to binding mRNA, Egl also directly interacts with Bicaudal-D (BicD) and Dynein light chain (Dlc). BicD is known to be an adaptor of the Dynein motor. Dlc is a core component of the Dynein motor but has also been shown to have functions that are independent of Dynein.
Similar to Egl, loss of Dlc and BicD also block oocyte specification. Thus, Egl, Dlc and BicD are thought to function together to specify oocyte fate and to mediate mRNA localization, likely by coupling RNA cargo to the Dynein motor. However, the specific role of BicD and Dlc in this pathway is not known. Using a mutational analysis, we demonstrate that Dlc is required for Egl dimerization. Dimerization of Egl is in turn required for efficient RNA binding activity. Once bound to cargo, Egl is able to interact with BicD and the Dynein motor. Thus, disrupting the Egl-Dlc interaction effectively uncouples cargo from Dynein, thereby blocking oocyte specification and disrupting mRNA localization.

Poster 28

Interplay between chromatin and myogenic alternative splicing

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Alternative splicing is a ubiquitous gene regulatory mechanism by which diverse transcripts are derived from a finite genome. During development, complex splicing transitions occur, leading to varied transcript profiles in fetal and adult tissues. Extensive splicing transitions take place during postnatal mouse heart and skeletal muscle development. Like splicing transitions, epigenetic patterns are cell-type specific and dynamic during organ maturation, presenting a convenient mechanism for splicing regulation. The interplay between chromatin and splicing has been described in neuronal, cancer and immune cell lines. However, the connection between chromatin and splicing in the context of muscle has not been investigated. These observations led us to hypothesize that histone modifications contribute to alternative splicing decisions in striated muscles.
First, we investigated this connection *in vitro*, by using the C2C12 cell line which reproduces splicing transitions observed in striated muscle. We observed that inhibition of histone deacetylation using trichostatin A promoted skipping of specific alternative exons. Second, we found a role for H3K36me3 in regulating the splicing of several genes. Third, bioinformatics analysis of our RNA-sequencing data in mouse heart development, and chromatin immunoprecipitation-sequencing (ChIP-seq) data (ENCODE), revealed altered histone modifications proximal to alternatively spliced exons. Lastly, we uncovered several chromatin modifiers that are alternatively spliced during C2C12 differentiation, as well as postnatal heart and skeletal muscle development.
Together, our data suggest that chromatin contributes to splicing regulation in striated muscle development. Moving forward, we will evaluate the direct connection between H3K36me3 and splicing, and the functional consequence of alternatively spliced chromatin modifiers.

Poster 29

Epigenetic regulation of transcription and pre-mRNA processing by histone PTMs

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Histone post-translational modifications (PTMs) modulate the organization of chromatin and are hypothesized to be carriers of epigenetic information. Until recently, it has been impossible to rigorously test this premise in multicellular eukaryotes, due to the repetitive nature of histone gene clusters. We have developed a BAC-based genetic platform in *Drosophila melanogaster* allowing direct interrogation of histone residue function. Here, we outline our studies of histone H3K36 methylation function (H3K36me) in maintaining transcriptome fidelity.

Yeast studies show that mutation of H3K36 or deletion of Set2, the H3K36 methyltransferase, causes genome-wide hyperacetylation of histone H4 and aberrant transcription initiation. The prevailing view is that H4 acetylation (H4Ac) aids nucleosome dissociation, allowing transcription initiation at cryptic sites in gene bodies. However, we found that mutation of the replication dependent H3 (H3.2) genes appears to uncouple these two phenotypes. That is, H3K36R (K36R) flies exhibit global hyper-H4ac but not cryptic

transcription in genes. Rather, transcription start site (TSS) profiling in K36R flies reveals cryptic initiation primarily in gene-poor regions. Studies of the mammalian Set2 ortholog, SETD2, also suggest a role for H3K36 trimethylation in regulation of splicing. However, our work mutating H3.2K36 demonstrates that this modification is neither a significant contributor to the regulation of alternative splice site choice, nor to canonical intron removal efficiency.

One explanation for differences between our results and studies of yeast H3K36 and Set2/SETD2 might be that trimethylation of histone variant, H3.3, which more closely resembles yeast H3, is the primary mediator of cryptic initiation and splicing phenotypes. Another is that Set2/SETD2 methylation of non-histone substrates may mediate these processes. Therefore we generated an H3.3BK36R mutant in a H3.3AΔ null background to probe these hypotheses. We expect comparison of H3.3BK36R, H3.3AΔ null double mutants with both Set2 mutants and H3.3BK36R, H3.3AΔ, H3.2K36R triple mutants to definitively parse these relationships.

Poster 30

Regulation of cell size by Rpl13a snoRNAs

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C/D box small nucleolar RNAs (snoRNA) are a multifunctional family of non-coding RNA that play a critical role in guiding 2'-O-methylation of ribosomal RNA (rRNA). In addition to their rRNA targets, current work from our laboratory suggests that snoRNAs of the Rpl13a locus (Rpl13a snoRNAs) direct methylation of distinct mRNA species, further regulating protein translation in vitro and in vivo. Interestingly, we discovered that germline knockout of four C/D box Rpl13a snoRNAs (U32a, U33, U34 and U35a) in adolescent mice produced developmentally smaller hearts. Similarly in H9C2 rat cardiomyoblasts, locked nucleic acid (LNA) antisense oligonucleotide knockdown of Rpl13a snoRNAs significantly reduced H9C2 cell size. Based on the findings, we hypothesized that Rpl13a snoRNAs may influence cardiac cell size through 2'-O-methylation. Interestingly, both mRNA and protein expression of a central regulator of cellular growth were significantly reduced in hearts from Rpl13a snoRNA knockout mice. Further, LNA antisense knockdown of Rpl13a snoRNAs in H9C2 cardiomyoblasts also significantly reduced mRNA expression of this particular regulator. Taken together, these results suggest that Rpl13a snoRNAs regulate cardiac cell size mediated through a central cell growth pathway.

Poster 31

Determining the importance of initiation and elongation on translation efficiency

Ivan Jimenez-Ruiz and Alain Laederach

The advent of next-generation sequencing has improved our ability to evaluate the presence and amount of ribosomal complexes that physically bound to RNA. However, establishing structure/function relationships in non-coding regions of human messenger RNAs (mRNA) is still a challenge. We used polysome profiling experiments on different cell types in order to understand how mRNAs control their translation. Specifically, human lymphoblast cells from the 1000 Genomes project and Human Embryonic Kidney (HEK293T) cells were processed through RNA-sequencing at multiple ribosomal fractions. Quantification results were obtained from the assembled transcripts produced by Sailfish as Transcripts per Million (TPMs) and used to optimize computational models based on distinct RNA features, including Kozak sequence strengths and Codon Adaptability Indexes. We show how these features are now beginning to reveal different classes of polysome-associated genes, based on their impact on translation

efficiency. These general models provide a draft workflow through which disease-related mutations in these tissues and their respective effects on the folding and translation of RNA are studied.

Poster 32

Xist Repeat A can disrupt multiple aspects of mRNA metabolism

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The long non-coding RNA *Xist* orchestrates X-chromosome inactivation (XCI), an epigenetic process that is essential during development and that provides an archetype for understanding interplay among various gene regulatory systems in health and disease. It is well-established that *Xist* silences gene expression by promoting the spread of heterochromatic marks along the X chromosome. However, these marks cannot spread over actively transcribed genes without the function of a critical domain of *Xist*, called Repeat A. What Repeat A does to initiate stable gene silencing remains an outstanding question central to understanding *Xist* and XCI. While Repeat A is essential for XCI, we unexpectedly found that, when expressed in a short standalone transcript, Repeat A is unable to stably silence transcription. Instead, we revealed that Repeat A can perturb multiple aspects of mRNA metabolism, including transcriptional elongation, splicing, and nuclear export. These disruptions to mRNA metabolism require the conserved, GC-rich repeat elements in Repeat A and structural elements immediately downstream of Repeat A, but they can occur in the absence of the key Repeat-A-interacting factor SPEN. Repeat A binds large amounts of SRSF1, a protein with well-characterized roles in promoting transcriptional elongation, splicing, and nuclear export of mRNAs. Our data lead us to hypothesize that Repeat A is an SRSF1 binding decoy that disrupts mRNA metabolism to initiate transcriptional silencing at the onset of XCI. Thus, SRSF1 sequestration by Repeat A may be the long-sought-after initial trigger that reduces transcriptional output to enable the spread of heterochromatin over active genes.

Poster 33

Nol9 is a Spatial Regulator for the Human ITS2 pre-rRNA Processing Complex

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The ribosome plays a universal role in translating the cellular proteome. Defects in the ribosome assembly factor Las1L are associated with congenital lethal motor neuron disease and X-linked intellectual disability disorders, yet its role in processing precursor ribosomal RNA (pre-rRNA) is largely unclear. The Las1L endoribonuclease associates with the Nol9 polynucleotide kinase to form the internal transcribed spacer 2 (ITS2) pre-rRNA endonuclease–kinase machinery. Together, Las1L–Nol9 catalyzes an essential tandem RNA cleavage and phosphorylation step to mark the ITS2 spacer for degradation by Xrn2 and the RNA exosome. While ITS2 processing is critical for the production of functional ribosomes, the regulation of the mammalian Las1L–Nol9 complex remains obscure. Here we characterize the human Las1L–Nol9 complex and identify critical molecular features that regulate its assembly and spatial organization. Yeast homologues of Las1L and Nol9 have been shown to form a hetero-tetramer that is required for pre-rRNA processing, but it has been unclear if this ordered assembly is conserved in humans. We establish that human Las1L and Nol9 form a higher-order complex and we further identify the regions responsible for orchestrating this intricate architecture. Structural analysis by high-resolution imaging defines the intricate spatial pattern of Las1L–Nol9 within the human nucleolar sub-structure linked with late pre-rRNA processing events. Furthermore, we uncover a Nol9-encoded nucleolar localization sequence that is

responsible for nucleolar transport of the assembled Las1L–Nol9 complex. Together, these data provide a mechanism for the assembly and nucleolar localization of the human ITS2 pre-rRNA endonuclease–kinase complex.

Poster 34

Visualizing a transient RNA protonation state that modulates oncogenic microRNA-21 maturation

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MicroRNAs are evolutionarily conserved small, non-coding RNAs, ~20-22 nucleotides in length, which serve as critical gene regulators in many aspects of biological processes. In the canonical biogenesis pathway, microRNAs are initially transcribed into long primary transcripts and cleaved by Drosha and Dicer to yield ~20 base-pair microRNA duplexes before being loaded into the functionally active mRNA-induced silencing complex. Despite their importance, most of these RNA elements are highly dynamic and have evaded high-resolution structural studies by conventional structural biology techniques. Here, we studied the structure and dynamics of microRNA-21, which also functions as an oncogene involved in tumorigenesis, progression, and metastasis. By applying low spinlock R1rho relaxation dispersion experiments, we uncovered a rare, protonationdependent conformational equilibrium encoded in the precursor element of microRNA-21, which involves a sparsely populated and transiently lived conformational state. By developing and applying nucleic-acid-optimized CEST NMR experiments, we were able to directly characterize this dynamic process. This dynamic equilibrium enables transient access to a rare, protonated conformational state, where long-range communication across the RNA reshuffles base pairing identities and stabilizes the structural region that is critical for biogenesis machinery recognition and activity. Via mutagenesis and microRNA processing assays, we further demonstrated that the structural properties of these distinct conformational states in microRNA-21 directly modulate the overall activity of the microRNA processing machinery. Together, these results indicate that microRNA themselves can encode a complex conformational landscape to direct functional outcomes, exemplifying a novel RNA-centric regulation of microRNA biogenesis.

Poster 35

RNA Structural Ensembles at the Exon-Intron Boundary *In Vivo*

Jayashree Kumar, Lela Lackey and Alain Laederach

The process of splice site selection by the spliceosome is dependent on the recognition of cis-sequence elements by trans-factors. These cis-elements include accessory sequences, like splicing enhancers or silencers, which are modulated by RNA structure around functional elements. RNA structures can promote or block trans-factor recognition and modify the effectiveness of cis-elements. The majority of disease-associated single nucleotide variants (SNVs) that occur close to splice junctions in intronic sequences cannot be explained by direct disruption of a cis-element. SNVs that change the structure of the RNA and impair splicing elements, but do not directly alter canonical splicing elements, are the focus of our study. Of 127,445 disease-associated SNVs within the Human Gene Mutation Database we predicted that 13.1% are predicted to disrupt splicing, and 1.6% (>2,000 SNVs) of those are likely to function by changing RNA secondary structure. One particularly salient example is in *MAPT*, a gene that codes for the Alzheimer's associated Tau protein. Alternatively spliced isoforms of *MAPT* separate into two groups based on the presence or absence of exon 10. These two groups are found in a 1:1 ratio in healthy brain tissue and the ratio is disrupted in the presence of intronic disease-associated SNVs. It is not well understood how RNA

structural ensembles dictate alternative splicing around the exon-intron junctions, and whether this mechanism is at play at the *MAPT* exon-intron 10 junction. We developed a computational model that combines the sequence of splicing regulatory *cis*-elements and their structural ensemble context. This model is derived from novel *in-vivo* chemical probing data collected from *MAPT* pre-cursor mRNA. We found that mutations have different effects on the structure and sequence context of splicing elements and this in turn affects the ratio of isoforms. Our model accurately captured the effects of all known disease-associated variants and also correctly predicted de novo effects of novel mutations in the intron. We found that the larger structural context around the *MAPT* exon-intron 10 junction plays a critical role in regulating its alternative splicing, suggesting a role for complex RNA structures in splicing regulation. Specifically, our use of element representations of structures sampled from the Boltzmann suboptimal ensemble using *in vivo* structure probing reveals the complex interplay of structure and cis-elements in controlling splicing.

Poster 36

Profiling the Epitranscriptome in Glioblastoma

Jennifer H. Simpson, Daniel Todd, Jian Teng, Bakhos A. Tannous, and Norman H.L. Chiu

RNA modifications are decorations on the chemical structure of the four canonical RNA bases, sugar, or even the phosphate group that makes up the monomeric units of RNA known as a nucleotide. Over 170 of these distinct modifications have been discovered to date. These evolutionarily controlled modifications require a significant energy cost to maintain cellular functions and overall health, suggesting their importance. Conversely, disruptions or alterations in modifications that lead to gene under-or- over expression occurs in disease states, such as found in the N6-methyladenosine (m6A) mRNA modification that is implicated in the self-renewal and tumorigenesis of glioblastoma (GBM). The arduousness in RNA modification research has been limited methodologies for detecting, quantifying and identifying specific RNA molecules that carry RNA modifications.
In this analytical study, ultra performance liquid chromatography in tandem with high resolution mass spectrometry (UPLC-HRMS) was employed for the profiling of the epitranscriptome in glioblastoma cell lines U87 and LNZ308, as well as patient derived primary cells MGG6, MGG8 and PN157. UPLC-HRMS was carried out in a Water's Acquity LC system equipped with a polar end-capped reverse phase Acquity HSS T3 column and a Thermo Fisher Scientific Q Exactive Plus. Positive identification of 40 modified RNS were confirmed by MS/MS fragmentation. Biological and technical replicates were used to account for biological variability. This novel characterization identified a comparable profile of modified RNA between the cell lines used in the study in both total RNA and small RNAs from 18 – 200 NTs in length.

Poster 37

Dysregulated ESRP2-mediated RNA Splicing Accumulates Immature Hepatocytes in Severe Alcoholic Hepatitis

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Severe alcoholic hepatitis (SAH) is a deadly liver disease without an effective medical therapy. Although SAH mortality is known to correlate with hepatic accumulation of immature liver cells, why this occurs, and how it causes death is unclear. We hypothesized that mechanisms controlling when fetal programs switch on and off are dysregulated in SAH, resulting in accumulation of fetal-like cells that are functionally immature. Here, we focused on epithelial splicing regulatory protein-2 (ESRP2), an RNA splicing factor that maintains the non-proliferative, mature phenotype of adult hepatocytes. Alternative RNA splicing of ESRP2-target mRNAs was analyzed in liver explants of 10 patients with SAH and in livers of alcoholic liver disease (ALD) mouse models. Rare hepatocytes retained ESRP2; fetal splice variants accumulated at the expense of mature splice variants; massive adult-to-fetal reprogramming was evident in human SAH. In mouse ALD models, levels of fetal splice variants correlated with severity of inflammation and liver damage. The inflammatory cytokines released by excessive alcohol ingestion suppressed ESRP2, increased fetal variants, induced stem/progenitor markers and enhanced hepatocyte growth in cultured hepatocytes. Ammonia detoxification was impaired in these fetal-like hepatocytes: they expressed lower levels of ammonia-detoxifying enzymes and released more ammonia but less urea into culture medium than more mature hepatocytes. In conclusion, sustained loss of ESRP2 by inflammatory cytokines permits re-emergence of a fetal RNA splicing program enabling surviving hepatocytes to shed adult hepatocyte functions and become more regenerative but threatens overall survival by populating the liver with functionally-immature hepatocytes.

Poster 38

A functional role for the cancer disparities-linked genes, CRYbB2 and its pseudogene, CRYbB2P1, in the promotion of breast cancer

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In the U.S., the breast cancer (BC) mortality rate is 41% higher for African American (AA) than White American (WA) women. While numerous gene expression studies have classified biological features that vary by race and may contribute to survival, few studies have experimentally tested these associations. CRYβB2 expression draws interest because of its association with decreased survival and AA ethnicity in multiple cancers. However, several reports indicate overexpression of the CRYβB2 pseudogene, CRYβB2P1, and not CRYβB2, is linked with race and poor outcome. It remains unclear whether either or both genes are linked to BC outcomes. We investigated CRYβB2 and CRYβB2P1 expression in human BCs and BC cell models, with the goal of elucidating mechanistic contribution of each gene to racial disparities. Methods: Custom scripts for CRYβB2 or CRYβB2P1were generated and used to identify reads that uniquely aligned to either gene. Gene expression according to race and tumor subtype were assessed using TCGA BC RNA-sequencing alignment samples (n=1,221), and BC models were engineered to have each gene overexpressed or knocked-out and evaluated by in vitro/biochemical and in vivo assays to

identify functions. Results demonstrate CRYβB2P1 is expressed at higher levels in breast tumors compared to CRYβB2, but only CRYβB2P1 is significantly increased in AA relative to WA tumors. We show that independent of CRYβB2, CRYβB2P1 enhances tumorigenesis in vivo. Results also reveal CRYβB2P1 may function as a ncRNA to regulate CRYβB2. Our data underscore both CRYβB2 and CRYβB2P1 promote tumor growth, but their mechanisms for tumor promotion are likely distinct.

Poster 39

Jon Kastan, Duke University

Eukaryotic protein synthesis control at multiple levels allows for dynamic, selective responses to diverse conditions, but spatial organization of translation initiation machinery as a regulatory principle has remained largely unexplored. Here we report on a role of Constitutive Repressor of eIF2α Phosphorylation (CReP) in translation of the endoplasmic reticulum (ER)-resident chaperone Binding immunoglobulin Protein (BiP), cell-cycle regulator p27, and poliovirus, at the ER. Functional, proximity-dependent labeling, and cell fractionation studies revealed that CReP, through binding eIF2α, anchors translation initiation machinery at the ER and enables local protein synthesis in this compartment. This ER site was protected from the suppression of cytoplasmic protein synthesis by acute stress responses, e.g. phosphorylation of eIF2α(S51) or mTOR blockade. We propose that partitioning of translation initiation machinery at the ER enables cells to maintain active translation during stress conditions associated with global protein synthesis suppression.

Poster 40

Drugging the Transcriptome: Transcriptome-Wide Capture and Amplification of RNA-Ligand Binding Sites

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Currently, only 0.05% of the human genome has been drugged with fewer than 700 gene products targeted. RNAs can fold back on themselves to form complex structures that can contain pockets or clefts with sufficient structural sophistication to allow specific and high-affinity binding by small molecules. Targeting mRNAs could modulate protein gene products by upregulating or downregulating translation efficiency or by altering mRNA abundance or stability. Previous studies have revealed that diverse motifs within a transcriptome can be highly structured and may represent an untapped opportunity for discovering therapeutically useful small molecule binding sites. Therefore, a robust method for discovering ligands and mapping RNA-ligand binding sites across the transcriptome would have high value, both in developing novel tool compounds and in identifying RNA motifs that might be targeted therapeutically. Our goal is to develop methodology for transcriptome-wide capture and amplification of RNA-ligand binding sites and discovery of novel function-modulating small molecules that bind RNA. This strategy will employ a photo-reactive ligand carrying an alkyne that can be crosslinked to an RNA binding site. Subsequent probe enrichment, amplification and next-generation sequencing will be used to reveal RNA-ligand binding sites. Once fully implemented, we hope this technology will enable rapid identification of therapeutically relevant RNA motifs with high-information-content structures able to bind novel small molecule ligands.

Poster 41

Cotranscriptional RNA strand invasion mediates ligand sensing in the *E. coli thiB* thiamine pyrophosphate riboswitch

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RNA folds immediately as it is transcribed by RNA polymerase, thereby creating RNA structures faster than new nucleotides being added to an emerging RNA chain. Consequently, cotranscriptionally folded RNA structures can differ greatly from equilibrium folded structures. Riboswitches are structured RNA elements that respond to small molecules, ions, and other metabolites to regulate transcription, translation, splicing, and mRNA degradation. Riboswitches take advantage of cotranscriptional folding to regulate gene expression by altering the folding pathway depending on the presence or absence of a ligand. The thiamine pyrophosphate (TPP) riboswitch, a highly conserved riboswitch family found in all kingdoms of life, has been shown to regulate translation via changes in cotranscriptional RNA structure. In this work, we have investigated the hypothesis that the *Escherichia coli thiB* TPP riboswitch functions through an RNA strand invasion mechanism by which TPP binding blocks a key rearrangement event that allows 'sequestering stem' to form around the ribosome binding site, preventing translation. To examine this hypothesis, we have used cotranscriptional SHAPE-seq, a technique which combines RNA chemical probing, precise polymerase arrest and high-throughput sequencing, to study intermediate structures in the folding pathway of the *E. coli thiB* TPP riboswitch. A series of deletion mutants created to favor or disfavor an intermediate structure predicted to facilitate this mechanism demonstrated efficient strand invasion to be essential to *thiB* riboswitch function. This work along with recent work on the ZTP riboswitch and the signal recognition particle (SRP) RNA have demonstrated the prevalence of strand invasion in RNA cotranscriptional folding pathways.

Poster 42

H/ACA snoRNAs are key determinants of cell identity and stem cell homeostasis

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Pseudouridine regulates RNA stability and function. H/ACA small nucleolar RNAs (snoRNAs) guide pseudouridylation within a small nucleolar ribonucleoprotein complex (snoRNP). We seek to reveal critical links between H/ACA snoRNA expression, H/ACA snoRNP function, and differentiation. Quantification of H/ACA snoRNAs revealed that H/ACA snoRNA expression is cell-type dependent and regulated during differentiation. Strikingly, we found that regulation of specific snoRNAs largely overlapped in three different cellular models of differentiation: differentiation of mouse embryonic stem cells (mESCs) by retinoic acid treatment, differentiation of mESCs to cardiomyocytes, and myoblast differentiation into myotubes. To assess the role of the H/ACA snoRNP in differentiation, we depleted Dkc1, the catalytic subunit, or Nop10, which anchors the snoRNA in the complex. Knockdown of either protein disrupted mESC homeostasis and promoted differentiation, but the effect was more severe upon depletion of Dkc1. All H/ACA snoRNAs were sensitive to Dkc1 knockdown. In contrast, only six snoRNAs were sensitive to Nop10 depletion, five of which were also downregulated upon differentiation. In myoblasts, Nop10 knockdown promoted differentiation into myotubes, and the changes in snoRNA expression upon Nop10 knockdown parallel those observed during myogenesis. Northern blot analysis revealed changes in pre-rRNA processing upon mESC differentiation. We are investigating whether regulation of specific H/ACA snoRNAs leads to differences in pseudouridylation of target sites in the rRNA and alterations in ribosomal function. Together, these data suggest that H/ACA snoRNAs and the H/ACA snoRNP are important determinants of cell identity, potentially through regulatory pseudouridylation of ribosomal RNA and other, as yet unidentified target RNAs.

Poster 43

Regulation of HIV-1 gene expression by post-transcriptional acetylation of cytidine in viral RNA transcripts

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As obligate parasites, viruses need to navigate a variety of cellular regulatory systems while infecting and replicating in the host cell. Post-transcriptional modifications have recently emerged as an important layer of regulation of viral RNA function. For example, our lab and others have shown that the RNA modification N^6-methyladenosine (m^6A) can enhance the replication of multiple viruses in *cis*, including Human Immunodeficiency virus 1 (HIV-1), Influenza A virus, SV40 and Kaposi's sarcoma-associated herpesvirus (KSHV). Recent reports have revealed the presence of another RNA modification, N^4-acetylcytidine (ac4C) on cellular mRNAs and have shown that ac4C can enhance mRNA stability and translation. Here, we demonstrate that ac4C is present at multiple sites on HIV-1 mRNAs and on the viral genomic RNA. Through ac4C RNA immunoprecipitation (RNA-IP) and RNA-seq, we found ac4C on HIV-1 mRNAs as well as the virion genomic RNA, with ac4C sites in the coding regions of the *pol, env, nef* genes, and the trans-activation response (TAR) hairpin. Phenotypically, we observe that increasing the expression level of the ac4C acetyltransferase NAT10 leads to an increase in viral replication that is dependent on the RNA binding and enzymatic domains of NAT10. Moreover, both CRISPR-depletion of NAT10 (ΔNAT10) and treatment with the small molecule NAT10 inhibitor Remodelin, diminishes HIV-1 replication in T cells. Lastly, silent mutations introduced to prevent ac4C deposition on the viral genome indeed diminished HIV-1 replication. Our data suggest that HIV-1 has evolved to incorporate ac4C in essential viral gene coding regions and regulatory RNA structures, and that NAT10-dependent ac4C addition enhances HIV-1 replication. We will present the latest developments from this project addressing how ac4C regulates HIV-1 infection, providing us with new potential points of intervention.

Poster 44

A mechanistic study of the *pbuE* adenine riboswitch from the perspective of cotranscriptional chemical probing

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Riboswitches are non-coding RNAs that create unique RNA-based regulatory systems found in all domains of life and especially prevalent in bacteria. Riboswitch sequences regulate genes in response to specific environmental factors such as small molecule toxins, chemical alarmones, and metabolic cofactors. To regulate these genes, riboswitches respond to ligand binding by folding into structures that control transcription, translation, or RNA cleavage. Among the 40 diverse classes of known riboswitches, a subset regulates transcription by making their regulatory decisions during the fast timescales of transcription, requiring structures to fold cotranscriptionally. While we have a mature understanding of the chemical basis of RNA-ligand interactions and the structural characteristics of riboswitch aptamers, we lack a general understanding of the step-by-step structural transitions that occur during cotranscriptional folding that enable rapid genetic decisions. Here, I use the *Bacillus subtilis pbuE* adenine riboswitch as a model system to examine how cotranscriptional folding events and intermediate structures lead to functional gene expression outcomes. To understand this process, I optimize and apply cotranscriptional SHAPE-Seq, a technique that combines chemical probing and next generation sequencing to uncover the ligand-dependent folding pathway of the *pbuE* adenine riboswitch at a single nucleotide resolution. We specifically examine the structural order of events for aptamer folding, terminator nucleation and strand invasion by the

expression platform. We aim to use these results, in combination with recent studies on the ZTP and fluoride riboswitches, to further develop hypotheses for more generalized principles of the role of cotranscriptional structures in riboswitch gene regulation.

References

1. Mandal, M., Boese, B., Barrick, J. E., Winkler, W. C., & Breaker, R. R. (2003). Riboswitches control fundamental biochemical pathways in Bacillus subtilis and other bacteria. *Cell*, *113*(5), 577–586.
2. Batey, R. T. (2012). Structure and mechanism of purine-binding riboswitches. *Quarterly Reviews of Biophysics*, *45*(3), 345–381.
3. Frieda, K. L., & Block, S. M. (2012). Direct observation of cotranscriptional folding in an adenine riboswitch. *Science*, *338*(6105), 397–400.
4. Watters, K. E., Strobel, E. J., Yu, A. M., Lis, J. T., & Lucks, J. B. (2016). Cotranscriptional folding of a riboswitch at nucleotide resolution. *Nature Structural & Molecular Biology*, 1–10.
5. Strobel, E. J., Cheng, L., Berman, K. E., Carlson, P. D., & Lucks, J. B. (n.d.). (2019). A mechanism for ligand gated strand displacement in ZTP riboswitch transcription regulation. *BioRxiv*.

Poster 45

The role of RNA exosome in pluripotency

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The role of different RNA binding proteins in cell fate transitions is not clear. Here we study the role of the RNA degradation component, EXOSC2 in pluripotency. We have previously shown that EXOSC2 is targeted and is upregulated by the pluripotency factor ZSCAN10 during reprogramming. We have also shown that one of the functions of exosome in ESC cells is to regulate the redox levels by regulating the levels of GPX2 mRNA, an enzyme involved in glutathione metabolism. In this study, we downregulate EXOSC2 in embryonic stem cells, and we unravel a connection between EXOSC2 regulation and cell plasticity. Our data show that downregulation of EXOSC2 in ESC blocks differentiation without affecting the self-renewal capacity of mouse ESC cells. Our data also reveal novel targets of cytoplasmic RNA exosome function. The importance of characterizing the function of RNA exosome in pluripotency is critical for basic stem biology, regenerative medicine, and cancer.

Poster 46

The role of an Intellectual Disability gene, DDX3X, in regulating translation required for neural progenitor fate decisions during brain development

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Mutations in the RNA helicase *DDX3X* are associated with a wide range of developmental deficits and brain malformations and account for 1-3% of Intellectual disability (ID) cases in females. We recently found that DDX3X is required for cortical development in mice and that loss of *Ddx3x* impairs neural progenitor proliferation and their propensity to form neurons. **However, the distinct requirements for DDX3X in neural progenitors and neurons have not been characterized.** Using a conditional *Ddx3x* (cKO) mouse model, we are disentangling the distinct consequences of *Ddx3x* depletion in neural progenitors and neurons during cortical development. Our preliminary data indicates *Ddx3x* depletion from neural progenitors and their neuronal progeny leads to more progenitors in the ventricular zone and less neurons in the cortical plate. Loss of *Ddx3x* in neural progenitors also causes microcephaly. Because DDX3X is an RNA helicase with known roles in translation regulation, **we hypothesize that DDX3X controls neural progenitor fate decisions through regulation of translation.** We are currently employing ribosome footprinting to identify DDX3X translational targets relevant for progenitor proliferation in *Ddx3x* cKO mice. These studies will enhance our understanding of RNA regulation required for normal brain development and the mechanism by which aberrations in translation can lead to ID.

Poster 47

Conserved RNA genome structural elements in multiple dengue virus serotypes regulate infection

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RNA viruses encode the information required to usurp cellular metabolism and gene regulation and to enable their own replication in two ways: in the linear sequence of their RNA genomes and in complex higher-order RNA structures. Yet, the full extent to which viral RNA genomes contain true higher-order structural elements whose functions are critical to viral processes and are conserved across multiple viruses remains poorly understood. In this study, we use SHAPE chemical probing and single molecule correlated chemical probing (RING-MaP) to define and identify structural elements conserved across the RNA genomes of the four major dengue virus (DENV) serotypes. Conserved structural elements are found across dengue genomes, both in 5' and 3' untranslated regions and in the coding regions for viral structural and non-structural proteins. Many of these conserved structural elements contribute to viral fitness, and their disruption impairs DENV replication and infectivity. DENV is a serious mosquito-borne pathogen that threatens more than one-third of the world's population, and the future of a current vaccine is in question. Functional RNA structural elements discovered in this study to be conserved across DENV serotypes may be exploited in the development of critically needed and broadly effective anti-DENV therapeutic strategies and tetravalent vaccines based on attenuated viruses.

Poster 48

m^{6}A Controls mRNA Localization in Neurons

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RNA modifications have emerged as a pervasive feature of cellular mRNAs broadly impacting gene expression. For example, the most abundant mRNA modification, N^6-methyladenosine (m^{6}A), can control mRNA processing, turnover and translation through binding of various “reader” proteins. In the nervous system, loss of m^{6}A results in severe neurodevelopmental and neurocognitive abnormalities. Specifically, the m^{6}A methyltransferase *Mettl3*, demethylase *Fto* and reader *Ythdf1* are implicated in the control of learning, memory formation and response to drugs of abuse. Mechanistically, m^{6}A-containing mRNAs are found in dendrites of hippocampal neurons, contribute to synapse formation, and are required for the long-term potentiation (LTP) that underlay synaptic plasticity. Furthermore, the levels of m^{6}A in the neuronal transcriptome are controlled dynamically in space and time following fear conditioning, suggesting that rapid changes in m^{6}A may contribute directly to memory formation. Yet, it remains unclear how m^{6}A contributes to these processes in neurons. mRNA transport to synapses and their local translation following activity contributes to synaptic plasticity. We hypothesize that m^{6}A modification of mRNAs directly contributes to their localization to synapses and local translation in response to synaptic plasticity. To investigate the contribution of m^{6}A to mRNA localization, we are using a combination of global transcriptome analysis, reporter assays, tethering and single molecule imaging in dissociated primary hippocampal cultures. Our work will help better define how m^{6}A controls mRNA homeostasis at synapses, contributes to synaptic plasticity and brain function at the molecular level.

Poster 49

The Mechanism of N6-Methyladenosine Deposition on Hepatitis C Viral RNA

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The positive sense ssRNA virus hepatitis C (HCV) uses a variety of RNA regulatory mechanisms to carefully coordinate its lifecycle. Recently we have shown that the RNA base modification N6-Methyladenosine is present on the viral genome and that disruption of m^{6}A sites within the viral RNA as well as perturbation of the host m^{6}A machinery impacts the production of infectious viral particles. In contrast to the canonical models of how m^{6}A methylation occurs on host mRNAs, within the nucleus and co-transcriptionally during association with Pol II, HCV has a totally cytoplasmic lifecycle and transcription of its RNA is mediated by the viral polymerase NS5B. We hypothesized that viral proteins might be interacting with components of the methyltransferase complex to localize it to HCV RNA. We first perturbed components of the methyltransferase complex such as the depositing enzymes METTL3+14 and the coordinating protein WTAP and discovered that depletion of both results in a similarly increased viral titer. We then used a chimeric WTAP construct that is fused with a promiscuous biotin ligase to test interaction of WTAP and a set of HCV proteins. Curiously we found that there is an interaction between the HCV E1 protein and WTAP. HCV E1 is a structural protein important for binding and entry of virions and is not thought of as a traditional member of the HCV RNA replication complex. These preliminary findings suggest that HCV E1 might be involved in a viral mechanism that relocalizes the methyltransferase complex to facilitate HCV m^{6}A modification.

Poster 50

Matthew Tegowski and Kate D. Meyer

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N^6-methyladenosine (m^6A) is the most common post-transcriptional modification in mammalian mRNAs. Its deposition has been shown to regulate many steps of mRNA biogenesis and function, and it has important effects on both development and disease. Although discovered in the 1970s, little was known about the effects of m^6A on mRNAs due a lack of effective tools to detect and study it. Recently, new methods that can be combined with next generation sequencing to detect m^6A have led to foundational discoveries of the regulation, distribution, and function of m^6A. However, most m^6A-detection methods depend on the use of antibodies to enrich m^6A-containing RNAs, and this requires large quantities of RNA. To avoid these issues, we developed deamination adjacent to RNA modification targets (DART-seq), which is an antibody-independent method of detecting m^6A sites transcriptome-wide. Importantly, DART-seq requires the use of little input RNA, which makes it possible to detect global m^6A distribution in new sample types, including cellular compartments and potentially even single cells. This highly flexible technique allows for the investigation of fundamental questions of m^6A regulation, as well as its effects on the biology of diverse cell types.

Poster 51

Single-molecule investigations of oxidative modifications in RNA

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Epigenetic modifications to RNA are varied and plentiful and thought to be able to fine-tune expression through controlling translation rates.[1] In addition, the solvent exposure of RNA makes it especially vulnerable to oxidative modifications. Studying RNA modifications has proven challenging because of the low copy number of RNA in cells, the low prevalence of these modifications, and randomness in modification location. Nanopore technology has recently been used to sequence individual molecules of DNA and RNA. Specifically, the latch zone of alpha hemolysin can be used to monitor individual molecules and hold them stationary while measurements are made.[2] This technique is particularly advantageous for something like the study RNA modifications especially if said modifications are at specific sites such as those in tRNA. The anticodon loop of tRNA would be especially prone to oxidative modifications because it is requisitely solvent-exposed. Additionally, tRNA exerts its function in the cytosol where oxidants are more likely to be found than in the nucleus. Research to date in this area has been made using mass spectrometry on digested RNAs however this prevents sequence information from being retrieved.[3] The purpose of this study is to develop mutants of alpha hemolysin with altered dimensions to investigate the presence and prevalence of RNA modifications, especially oxidative modifications, by holding DNA/RNA hybrids stationary in the nanopore and to examine one individual base pair on a single duplex.

This work was supported by a grant from the NIH (R01 GM093099).

Poster 52

RNA-Ligand Binding Signatures: A tool to probe the structural basis of selective RNA-ligand binding and predict hits from RNA-ligand docking

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Noncoding RNAs (ncRNAs) are a growing class of biomolecules that are increasingly being revealed as important drug targets. However, there is a lack of information about what structural features govern affinity and selectivity in RNA-ligand binding. We hypothesized that identifying patterns in hydrogen bonding interactions would reveal key differences between RNA-ligand complexes that exhibit varying affinities and selectivities. We built a tool that identifies all hydrogen bonds in a complex and determines the number of interactions for each RNA atom type, which creates a Binding Signature. It then determines similarity between multiple complexes based on these signatures using both principle components analysis and hierarchical clustering. We applied this tool to a subset of RNA-ligand complexes from the Protein Data Bank (PDB), which had two groups: complexes with known low, and high selectivity binding. Interestingly, the clustering algorithm found these two classes to be different based on their hydrogen bonding patterns, without prior knowledge of the selectivity data. The low selectivity complexes were marked by hydrogen bonding to the backbone and major groove-accessible atoms, which high selectivity complex were enriched in bonds to Watson-Crick base pairing atoms. We then asked if we could use this tool to analyze computationally docked RNA-ligand complexes and identify differences between compounds that had high scores with validated in vitro activity, and those with high scores but no in vitro activity. We did find differences between Binding Signatures for these groups, suggesting this could be used as a method to increase enrichment from RNA-Ligand virtual screens.

Poster 53

Development of a nanomolar-affinity RNA-targeting ligand by fragment-based ligand discovery

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The transcriptome represents an attractive but underutilized set of targets for the development of small-molecule ligands. Small-molecule ligands targeted to messenger RNAs and to non-coding RNAs have the potential to modulate cell state and disease. We have created a technology that leverages fragment-based screening strategies and SHAPE-MaP RNA structure probing to discover small-molecule fragments that bind an RNA structure of interest. We identified fragments and cooperatively binding fragment pairs that bind to the TPP riboswitch with millimolar to micromolar affinities. We then used structure-activity-relationship information to efficiently design a linked fragment ligand that binds to the TPP riboswitch with high nanomolar affinity. Principles from this work are readily applicable to other RNA structures of interest, leveraging cooperativity and multisite binding to develop high-quality ligands for diverse RNA targets.

Poster 54

Post-transcriptional Regulation of Antiviral Gene Expression

Michael J. McFadden, Alexa B.R. McIntyre, Nandan S. Gokhale, Christopher E. Mason, Stacy M. Horner

Type I interferon (IFN) drives the induction of hundreds of IFN-stimulated genes (ISGs). The expression of these genes must be carefully regulated to allow for efficient production of antiviral effectors and controlled shut-off of inflammatory factors. The RNA base modification N6-methyladenosine (m^6A) has emerged as an important regulator of mRNA expression. We hypothesized that m^6A could regulate the expression of ISGs in response to viral infection. To investigate the role of m^6A in the IFN response, we perturbed the cellular enzymes responsible for its deposition, METTL3 and METTL14. Depletion of METTL3/14 followed by IFN-β treatment led to decreased protein abundance of a subset of ISGs through decreased translation, as determined by immunoblotting and polysome profiling. Conversely, overexpression of METTL3/14 led to increased protein production of the same subset of ISGs. To determine whether METTL3/14 regulates these ISGs through m^6A, we used meRIP-seq and found that certain ISGs are m^6A-modified during the IFN response, including the ISGs that were regulated by METTL3/14. For example, IFITM1 was found to contain m^6A within its 3'UTR and is regulated by METTL3/14, while PKR did not contain m^6A and was not regulated by METTL3/14. Additionally, the m^6A reader protein YTHDF1 was found to promote IFITM1 expression in an m^6A-dependent fashion. Finally, METTL3/14 depletion led to increased infection by vesicular stomatitis virus, specifically after IFN pre-treatment, suggesting METTL3/14 promotes the antiviral IFN response. Overall, our findings suggest that m^6A is an important node for antiviral gene production and the establishment of an antiviral cellular state.

Poster 55

Role of the Stem-Loop Binding Protein (SLBP) in histone pre-mRNA processing and mRNA localization in *Drosophila melanogaster*

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Histone mRNAs are the only eukaryotic mRNAs that are not polyadenylated. They end instead in a conserved 3' stem-loop. The 3' stem-loop participates in cell cycle regulation of histone mRNAs, with both processing and stability of the mRNA, being tightly regulated. The stem-loop binding protein (SLBP) is a key factor in this process. SLBP binds the stem loop and is required to properly process histone mRNAs and stays bound while the mature mRNA is translocated to the cytoplasm where it is essential for translation. To study the biological function of dSLBP, I created a null mutant SLBP in Drosophila melanogaster with Fly-CRISPRCas9. Genetic analyses show that the null dSLBP dies at first instar larvae. Western Blot analysis demonstrates that at 16-20hrs of development the embryo runs out of maternal SLBP; resulting in misprocessed histone mRNA. Within the same CRISPR experiment, I also obtained a 30nt deletion. This 10aa deletion is viable over a deficiency of dSLBP but female sterile. Only a small amount of histone mRNA and protein is loaded into the egg. Oogenesis proceeds normally until stage 10B, when the maternal histone mRNA is synthesized. At this stage there are low SLBP levels in wild type ovaries, and immunofluorescence data shows that the mutant SLBP is mainly localized in the cytoplasm at this stage, and histone mRNA is not produced. The 10aa deletion may remove a nuclear import signal from SLBP, resulting in failure to produce mature histone mRNA.

Poster 56

The Effects of M6A RNA Modification on Breast Cancer Progression and EMT

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Metastatic breast cancer is the number two killer cancer in women in the United States. The 5-year survival rate drops drastically as the cancer progresses and late diagnosis require drastic and less efficient treatments. Despite these facts, little is known on the regulation that causes this metastatic cancer to be come to become so aggressive as well as invasive. In recent years, it has become clear that posttranscriptional regulation plays a key role in the aberrant gene expression underlying malignancy and metastasis. The mRNA posttranscriptional modification N6-methyladenosine (m6A) is involved in many post-transcriptional regulation processes including mRNA stability and translational efficiency. It has been reported to be involved in many different cancer types, including breast cancer, as well as Epithelial to Mesenchymal Transition (EMT). Currently there is data to suggest that m6A is both a tumor suppressor and facilitator for progression and migration in several cancer types. By using the MCF10 breast cancer model with progression in mind, we have begun to sort through these discrepancies within the field by using Crispr-Cas9 to knock out Mettl3 in three cell lines, MCF10A, MCF10AT1 and MCF10CA1H. Phenotypic studies suggest that decreased m6A increases proliferation and migration in the MCF10A cells while decreases proliferation and migration in the more aggressive MCF10Ca1h cells. With these studies, we can begin to understand how changes in m6A lead to phenotypic changes in cancer cells and potentially manipulate this mRNA modification as a novel breast cancer treatment.

Poster 57

Maximizing quantitative structural information from high-throughput RNA structure probing

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RNAs enact numerous cellular functions through the formation of intricately folded structures. High-throughput RNA structure probing experiments couple chemical probing of RNA structure with high-throughput sequencing. These experiments can be used to determine signatures of biologically relevant structures in order to construct models of functional RNA folds. While this experimental approach has so far yielded useful data, several major limitations have precluded our ability to obtain precise and quantitative RNA structural information. These limitations include a lack of standards for experimental and data processing steps that result in inconsistent generation and interpretation of the primary chemical probing 'reactivity' data that is collected from these experiments. This in turn has prevented rigorous comparison of experimental results within and between laboratories.

Here, we have designed and begun to characterize a standard benchmark panel of RNAs of known structure that can be used as experimental calibration standards to allow comparison of reactivities within and between experiments. By implementing calibration standards, measurements of reactivity in RNA structure probing experiments will become more quantitative, allowing the maximal amount of structural information to be extracted from these experiments. Our preliminary studies have shown the value of standards to correct for experimental variation, and we have additionally used these standard RNAs to compare and evaluate current experimental methods of high-throughput RNA structure probing. These RNAs serve as a strategy to develop and validate an accurate and quantitative definition of chemical probe reactivity that is directly linked to RNA structure.

Poster 58

Active Site Coordination Within a Multienzyme pre-rRNA Processing Complex

Monica C. Pillon, Allen L. Hsu, Juno M. Krahn, Jason G. Williams, Kevin H. Goslen, Mack Sobhany, Mario J. Borgnia, and Robin E. Stanley

Ribosome assembly is a complex process reliant on the coordination of trans-acting enzymes to produce functional ribosomal subunits and secure the translational capacity of the cell. Las1 is a recently discovered endoribonuclease that assembles into a multienzyme complex with the Grc3 polynucleotide kinase to orchestrate the targeted removal of a transcribed spacer (ITS2) from precursor ribosomal RNA (pre-rRNA). The essential Las1 endoribonuclease cleaves the ITS2 spacer at a defined site to initiate pre-rRNA processing. The Grc3 polynucleotide kinase subsequently phosphorylates the resulting 5'-hydroxyl RNA to signal for 5'- and 3'-exoribonucleases to degrade the ITS2. Disruption of mammalian Las1-Grc3 has been linked to congenital lethal motor neuron disease and X-linked intellectual disability disorders, thus highlighting its importance in human health; yet, its mechanism of action remains unclear. Here we report that the Las1 endoribonuclease assembles into a higher-order tetrameric complex with its binding partner the Grc3 polynucleotide kinase, which is essential for the activation of its nuclease and kinase functions. To understand how Las1-Grc3 achieves its strict nuclease specificity and coordinates its dual enzymes, we determined a series of high-resolution Las1-Grc3 structures in multiple conformational states. Structural characterization of Las1-Grc3 reveals its molecular architecture harboring a composite nuclease active site flanked by two discrete RNA kinase sites. Coupled with functional studies, we identify molecular features crucial for RNA specificity and two molecular switches that coordinate nuclease and kinase function. Together, our structures and corresponding functional studies establish how Las1-Grc3 couples its enzymatic functions to drive ribosome assembly.

Activities: I am a postdoctoral trainee at the National Institute of Environmental Health Sciences. My work aims to understand the regulation and activation of essential eukaryotic enzymes involved in processing precursor ribosomal RNA. This conference provides a unique opportunity to meet and network with influential scientists in my field of nucleic acid enzymes. I also aim to display my work at this conference by submitting an abstract for a poster presentation, as well as, request the opportunity to give an oral presentation during the Gordon Research Seminar.

Poster 59

The RNA binding protein Rbfox2 is necessary for cardiovascular development

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Loss of function mutations in alternative splicing regulator RBFOX2 are strongly associated with congenital heart defects in human genetics studies. However, the role of RBFOX2 in cardiovascular development remains unknown. Here, we show that conditional ablation of *Rbfox2* in mouse cardiac progenitor cells causes embryonic lethality and an arrest in cardiovascular development. *Rbfox2* mutants displayed

avascular yolk sac and defects in cardiac chamber and outflow tract formation, associated with cell proliferation and endocardial mesenchymal transition defects. RNA-seq analysis identified aberrant splicing of genes involved in cell proliferation, adhesion and migration in Rbfox2 mutant embryos. Switching the splicing pattern of only three Rbfox2 targets to mimic splicing patterns of Rbfox2 mutant embryos, impaired cell cycle progression. Furthermore, Rbfox2 depletion in endothelial cells altered focal adhesions, negatively impacting cell adhesion but promoting faster migration. Dissecting the mechanism revealed that Rbfox2 controls endothelial cell migration through Hippo signaling. Modulating YAP, the downstream effector of Hippo signaling, rescued Rbfox2-induced fast migration phenotype. Consistent with the involvement of Hippo pathway, transcriptome analysis uncovered dysregulation of downstream Hippo transcriptional networks in *Rbfox2* mutant embryos. In sum, our results uncovered the alternative splicing regulator Rbfox2 as a novel upstream modulator of Hippo signaling essential for early cardiovascular development.

Poster 61

Recruitment of PRC2 by repressive long non-coding RNAs and RNA-binding proteins

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Non-coding RNAs are emerging as important regulators of biological processes, including gene expression. *Xist* is the classical long non-coding RNA (lncRNA) that recruits the Polycomb Repressive Complexes 1 (PRC1) and 2 (PRC2) to the X chromosome for gene silencing during X chromosome inactivation. While it is well understood that *Xist* is required for PRC recruitment, the mechanism of this recruitment is still unknown, and some discrepancies of its function have yet to be resolved. For example, PRC2 binding to RNA inhibits its catalytic activity, and its interaction between RNA and nucleosomes is antagonistic. Therefore, we sought to identify additional factors that may bridge PRC2 and lncRNAs that allow PRC2 to maintain its catalytic activity while being recruited by RNA. We have identified the RNA-binding protein, SAFB1, as being important for the recruitment of PRC2 to the X chromosome. Loss of SAFB1 abolishes PRC2 association with *Xist,* as well as other non-coding RNAs that recruit PRC2 for gene silencing. Additionally, the epigenetic histone mark left by PRC2, H3K27me3, is lost on the inactive X chromosome in cells lacking SAFB1. These findings improve our mechanistic understanding of *Xist* function in X chromosome inactivation and infer additional insight on how similar non-coding RNAs are able to control chromatin modifying enzymes for genome regulation.

Poster 62

m^6A regulation in response to cocaine administration

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Adenosine methylation (m^6A) is the most abundant mRNA modification, playing a key role in gene expression regulation by recruiting additional factors for mRNA stability, localization, and translation. Emerging evidence indicates that m^6A modifications contribute to changes in synaptic activity upon exposure to drugs of abuse by altering dopaminergic neuron signaling. I hypothesize that m^6A regulates local gene expression at the synapse by recruiting novel recognition proteins in response to drugs of abuse, specifically cocaine. To examine changes to synaptic gene expression, RNA sequencing alongside m^6A

sequencing will be performed in striatal synaptoneurosome (SN) preparations and striatal homogenates before and after cocaine administration. This strategy will reveal whether synaptic changes in the striatal methylome are induced by exposure to drugs of abuse. To identify novel proteins recognizing synaptic m^6A, I will utilize the iBioID system which will allow for specific biotinylation of transient m^6A reader proteins. I will then subject SN lysates to pulldown and mass-spectrometry to identify novel interactors. This will provide the first identification of m^6A readers specifically at the synapse in addition to uncovering drug-induced m^6A reader dynamics. Collectively, these studies hope to reveal novel mechanisms of drug-induced gene expression control mediated by m^6A and further our understanding of synaptic plasticity during addiction.

Poster 63

Creation of novel synthetic circRNAs with optimized protein expression in cultured cells and animals

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Circular RNAs (circRNAs) are a novel class of RNAs which are prevalent in multicellular organisms. Many circRNAs are generated by the backsplicing of exons in pre-mRNAs. Endogenously circularized exons often contain inverted repeats (such as *Alu* elements) in the flanking introns, which promote backsplicing. However, there is tissue-specific and temporal regulation of circRNA expression, indicating the presence of additional regulatory mechanisms. Using three model intron pairs (HIPK3, Laccase2, and ZKSCAN1) and split GFP exons, we determined requirements for spacing of intronic repeats relative to the splice junctions. These studies resulted in a synthetic double deletion construct based on the HIPK3 intron pair which increased circRNA expression 5-fold in cultured cells. We tested this double deletion using our AAV-delivered circRNA expression system in mice and showed increased circRNA expression in many tissues. We also tested the ability of various viral IRES elements to drive translation of the GFP exon. The Poliovirus IRES was more efficient in the cells and tissues tested than the EMCV or KSHV IRES. This was verified by polyribosome analysis of circRNA on polyribosome gradients. Analysis by Northern blotting revealed that there were multiple additional circular and linear species expressed (both larger and smaller than the major circular RNA) that varied with the IRES used. We increased the exon size by incorporating a larger ORF into the circRNA. This construct produced equivalent amounts of circRNA, while expressing both proteins. We introduced the double deletion into the larger exon construct, and saw the same stimulation of expression.

Poster 64

exRNA Atlas analysis reveals distinct extracellular RNA cargo types and their carriers present across human biofluids

Roger P. Alexander, Pacific Northwest Research Institute

To develop a map of cell-cell communication mediated by extracellular RNA (exRNA), the NIH Extracellular RNA Communication Consortium created the exRNA Atlas resource (https://exrna-atlas.org). The Atlas version 4P1 hosts 5,309 exRNA-seq and exRNA qPCR profiles from 19 studies, as well as a suite of analysis and visualization tools. To analyze variation between profiles, we applied computational deconvolution. The analysis led to a model with six exRNA cargo types (CT1, CT2, CT3A, CT3B, CT3C,

CT4), each detectable in multiple biofluids (serum, plasma, CSF, saliva, urine). Five of the cargo types associate with known vesicular and non-vesicular (lipoprotein and ribonucleoprotein) exRNA carriers. To validate utility of this model, we re-analyze an exercise response study by deconvolution to identify physiologically relevant response pathways that were not detected previously. To enable wide application of this model, as part of the exRNA Atlas resource, we provide tools for deconvolution and analysis of user-provided case-control studies.

Poster 65

Tumor Angiogenesis and Metastasis Regulation Through microRNA-200b, Quaking, and Cyclin D1

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Angiogenesis is critical to cancer development and metastasis. However, anti-angiogenic agents have only had modest therapeutic success, partly due to an incomplete understanding of endothelial cell (EC) biology. Noncoding RNAs have been demonstrated to regulate many different hallmarks of cancer. We previously reported that the microRNA (miR)-200 family inhibits metastasis through regulation of tumor angiogenesis, but the underlying molecular mechanisms are poorly characterized. Here, an integrated analysis of miR-200b:mRNA relationships in Cancer Genome Atlas datasets and in ECs transfected with miR-200b mimic identified the conserved RNA binding protein Quaking (QKI) as a lead miR-200b endothelial target with previously unappreciated roles in the tumor microenvironment. We confirmed QKI is a target of miR-200b by qPCR, western blot and luciferase assay and found QKI knockdown mimics miR-200b overexpression in inhibiting EC proliferation and sprouting. QKI was also upregulated in tumor vs. normal endothelium in an orthotopic lung cancer model. In addition, both cancer cell and endothelial QKI expression in patient samples significantly corresponded with angiogenic indices and poor survival. To characterize QKI's mechanism of endothelial regulation, we performed RNA-Seq following QKI knockdown in ECs and observed a downregulation in many cell cycle-related genes. Cyclin D1 (CCND1) was among the most potently decreased. QKI supported angiogenesis by stabilizing CCND1 mRNA to promote G1/S cell cycle transition and proliferation. *In vivo,* both nanoparticle-mediated RNA interference of endothelial QKI expression and palbociclib blockade of CCND1 function inhibited metastasis in concert with significant effects on tumor vasculature. Altogether, this work demonstrates the clinical relevance and therapeutic potential of a novel, actionable miR/RBP axis in tumor angiogenesis and metastasis.

Poster 66

Transcriptional activation of HIV by structure-switching the 7SK RNA

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Department of Chemistry[1], Department of Medicine[2] and UNC HIV Cure Center[3], University of North Carolina

Transcription in eukaryotic cells is highly regulated, controlled in part by the positive transcription elongation factor complex, P-TEFb. The non-coding 7SK RNA binds P-TEFb and selectively sequesters the complex, preventing transcriptional activation. The cycle of binding and release of P-TEFb is important for both transcriptional activation in general and, because Tat protein-dependent transcription from the HIV-1 promoter is uniquely sensitive to P-TEFb, disruption of 7SK-dependent P-TEFb sequestration represents a plausible strategy for HIV latency reversal. Using single molecule in-cell chemical probing, we have identified a conformational switch in the structure of the 7SK RNA that modulates P-TEFb release and consequent transcriptional activation. Structural characterization of the 7SK conformations enabled intelligent design of antisense oligonucleotides (ASOs) that promote formation of either of the two 7SK RNA conformations. In a human cell-based assay that measures gene expression from the HIV long terminal repeat (LTR) promoter, we show that structure-switching 7SK-targeting ASOs can enhance Tat-dependent reporter expression. Induced structure-switching of the 7SK non-coding RNA enables transcriptional reprograming in cells and represents a potential strategy to reverse latency and contribute to a functional cure for HIV.

Poster 67

Examination of RNA-Privileged Small Molecule Chemical Space and Elucidation of Important Recognition Properties

Wicks SL, Morgan BS, Hargrove AE.

The development of RNA-targeted chemical probes has been hindered due in part to a lack of guiding principles for achieving specific RNA:small molecule interactions. To elucidate these guiding principles, our laboratory has analyzed molecular descriptors of bioactive RNA-targeted ligands and identified trends that support the existence of RNA-privileged chemical space. Consequently, this work aims to validate the existence and examine the boundaries of RNA-privileged chemical space through rapid evaluation of small molecules against RNA targets and analysis of molecular properties that lead to specific recognition. Towards these goals, we have rationally designed an RNA-focused small molecule library that encompasses putative RNA-privileged chemical space and validated a fluorescent indicator displacement assay as a reliable high-throughput screening tool to identify ligand interactions with four RNA targets. Current work is focused on screening the library and employing computational methods to analyze leads as well as distinguishing molecular properties that are important for specific RNA binding. In parallel, we are evaluating specific RNA-binding of a general, drug-like small molecule library that has been extensively screened against protein targets. Binding profiles of both libraries will be compared to identify recognition properties of specific RNA-binding small molecules which in turn will serve as input to refine algorithms and apply machine-based learning for the prediction of RNA-targeting ligands. Altogether, this work will help to define the RNA-binding preferences of thousands of ligands, identify new RNA-targeting scaffolds and leads for therapeutically-relevant RNAs, and further elucidate the molecular properties that contribute to specific RNA recognition.

Poster 68

Codon usage bias in a complex multicellular organism: one size does not fit all

Scott Allen, Mike Rogers, Erez Cohen, Jessica Sawyer, Ivan Jimenez Ruiz, Alain Laederach, Chris Counter, Don Fox

For years it was believed that mutations changing one codon to another synonymous codon were “silent” in terms of biological impact. We have since come to appreciate that not all synonymous codons are created equal. Within an organism’s transcriptome, some synonymous codons occur more frequently than others – a phenomenon termed codon usage bias. A prevailing view in the field is that RNAs enriched in rare occurring synonymous codons are less stable and poorly translated compared to their more common counterparts. Most studies supporting this model have been performed in single-celled systems, however, leaving it unclear how codon usage bias impacts distinct tissue biology in complex multicellular organisms. Our lab developed a fluorescent-reporter-based system to identify tissue-level differences in codon usage bias in *Drosophila* and found that the testes and brain are resistant to the impacts of rare codon usage. We next used bioinformatic approaches to examine endogenous genes in these codon bias-resistant tissues. We find that genes highly expressed and unique to the testis in both *Drosophila* and humans are enriched for rare codons compared to other tissues, suggesting that resistance to codon usage bias has biological significance to the male germline and is a conserved feature across species.

Poster 69

m^{6}A readers in the nervous system

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N^6-methyladenosine (m^{6}A) is an abundant RNA modification found in thousands of cellular RNAs, and it controls the fate of these RNAs at every step of their life cycle. m^{6}A acts primarily through m^{6}A reader proteins, which recognize modified RNAs and elicit their reader-specific functions. We have identified a nuclear m^{6}A reader expressed primarily in the nervous system, where m^{6}A is most abundant among mammalian tissues. Our transcriptome-wide studies have revealed that this reader binds m^{6}A sites largely in the introns of pre-mRNAs and in non-coding RNAs, with evidence that it influences pre-mRNA splicing and the stability of non-coding RNAs. It is also highly expressed during neurodevelopment and our preliminary efforts indicate that it may regulate cellular proliferation. Prior studies have also demonstrated the importance of m^{6}A in the developing brain. However, we still lack a clear mechanistic understanding of how m^{6}A regulates neurodevelopmental proliferation and differentiation. Thus, further study of this m^{6}A reader will help us better understand how m^{6}A functions to regulate pre-mRNA splicing and non-coding RNA stability and may also provide mechanistic insight into how RNA modifications regulate the processes and pathways vital to the developing brain.

Poster 70

Structural RNA components of ZFP36L2-*Lhr* mRNA interaction

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We are interested in mRNA regulation mediated by the adenine uridine rich element binding proteins, ARE-BPs. When one of the TTP-family members, ZFP36L2 (or L2), is expressed at low levels in a mouse model, we observed complete female infertility accompanied by decreased ovulation rate. The role of L2 in ovulation is mediated by its effect on at least one transcript, luteinizing hormone receptor (*Lhr*) mRNA. Using different biological assays we had confirmed the modulatory effect of L2 on *Lhr* mRNA at the post-transcriptional level. *Lhr* mRNA contains three potential ARE sites, however L2-binding is specific to a single ARE located in the 3'-UTR, which we term the 'functional ARE'. Other TTP family members do not bind to the *Lhr* mRNA, suggesting binding specificity of L2 to this particular transcript. We found that RNA structure mediates a significant component of the specificity in this interaction. Our structural data using *in vitro* SHAPE-MaP support a model in which maximum binding occurs only in a specific structural context, where the functional ARE is presented in a loop. Also, the proximal stem in this loop seems to be a key modulator of the binding affinity to L2. To explore this RNA-protein interaction *in vivo* we used multiple chemical probing methods in cells. Our preliminary results suggest that L2 is bound uniquely to one ARE of *Lhr* mRNA *in vivo*. Understanding of L2-*Lhr* mRNA interactions may shed light into novel RNA therapeutics and thus a provocative new way to modulate ovulation independent of hormone therapy.
Key words: RNA structure, ARE-BP, infertility

Poster 71

Role of 5-InsP$_7$ in mRNA decay and P-Body formation: The regulation of migratory and inflammatory genes

Soumyadip Sahu and Stephen B. Shears

Homeostasis mechanisms against metabolic stress in eukaryotic cells often stabilize mRNAs by reversibly forming non-membranous, cytoplasmic processing bodies (PBs) to store the mRNAs. We state a novel observation that mRNA stability and PB formation are coordinated by a small-molecule, the "inositol pyrophosphate", IP$_7$. This work follows prior identification of 5'-mRNA decay by NUDT3. This enzyme hydrolyzes the triphosphate between 7-methylguanosine cap and the 5'-mRNA, in a transcript subset: ITGB6, Fibronectin, LCN2, S100A8 expressing inflammatory and migratory proteins. Decapping *in vitro* was inhibited by IP$_7$. We elevated IP$_7$ levels in intact HCT116 and HEK293 cells, through CRISPR knockout of PPIP5Ks which metabolize IP$_7$. qPCR analysis revealed inhibition of NUDT3-mediated decapping in *PPIP5K*$^{-/-}$ cells; target mRNAs decay slower after ActD addition vs WT cells. Pharmacological (TNP) and genetic silencing (using RNAi) of IP$_7$ synthesis, reverted transcript levels WT levels, validating that the inhibition of NUDT3 decapping is IP$_7$-mediated. Despite higher target mRNA levels in the *PPIP5K*$^{-/-}$ cells, levels of the corresponding proteins were like WT cells. These data indicate translational repression in *PPIP5K*$^{-/-}$ cells, raising a potential connection of decapping inhibition with PB assembly. We immunostained a PB marker, DCP1a. Total PBs/cell is 2.4-fold higher in *PPIP5K*$^{-/-}$ vs WT cells. We incubated WT HCT116 cells with liposomes containing exogenous IP$_7$; this novel method for intracellular delivery of IP$_7$ elevated

PBs/cell 3.4-fold compared to uptake of empty liposomes. Supervision of mRNA stability and PB assembly by a small-molecule signal is a new paradigm for control over gene expression.

Poster 72

Elucidating the Role of H3K36me3 in post-transcriptional mRNA processing

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In metazoans, histone posttranslational modifications (PTMs) play a crucial part in the regulation of gene expression by modifying the accessibility of chromatin. Studying how these epigenetic modifications control different aspects of gene expression is essential to understand organismal development, physiology and disease progression. The tri-methylation of Histone 3 on Lysine 36 (H3K36me3) is implicated in the co-transcriptional regulation of transcription and pre-mRNA processing. Previous work on H3K36me is primarily based on studies that involve mutations in various effector proteins (e.g. K36 writers and readers). Using a BAC-based histone replacement system in *Drosophila melanogaster*, we mutated all of the endogenous H3.2K36 into non-modifiable H3K36R, enabling direct interrogation of the functions of H3K36me3. RNA-Seq analysis of H3K36R flies revealed global dysregulation of transcription. Moreover, some of the highly expressed transcripts in nuclear RNA fraction showed reduced expression in polyA RNA fraction, indicating that H3K36me3 is potentially regulating polyA tail length and mRNA stability. To unravel the role of H3K36me3 in post-transcriptional regulation, we aim to determine if and where the regulators of pre mRNA processing enrich genome-wide in H3K36R animals versus wildtype, using either ChIP-Seq or CUT&RUN methods. We hypothesize that positive regulators will be recruited and negative regulators will be excluded from regions that are normally enriched in H3K36me3.

Poster 73

SHAPE-JuMP: Detecting RNA-RNA Interactions via Covalent Linkage and Reverse Transcription

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For many RNAs, the higher-order structure of the molecule is critical to its function. However, discerning this structure, especially for large molecules, is a challenging and unresolved problem. We have developed SHAPE-JuMP (selective 2´-hydroxyl acylation analyzed by primer extension and juxtaposed merged pairs) to probe RNA tertiary structure at large scales. A bi-functional small molecule is used to covalently link two neighboring nucleotides that are close in three-dimensional space. The site of this linkage is then reported via an engineered reverse transcription enzyme that “jumps” across the crosslinked nucleotides, creating a cDNA with a deletion that identifies the site of the crosslinked nucleotides. Massively parallel sequencing and alignment analysis are then used to report tertiary contacts all across RNA molecules under a diverse array of biologically informative conditions. These tertiary contacts will improve our understanding of RNA

structure and have been used to improve three-dimensional modeling of RNA structures and characterize long range interactions, such as UTR circularization in the Dengue virus genome.

Poster 74

Levels of the RNA Binding Protein DND1 Identifies Subpopulations of Murine Male Germ Cells During Fetal Development

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The importance of RNA-binding proteins (RBP) in the adult testis for male germ cell (MGC) development is well established. More recently, mounting evidence indicates that RBPs are also critical for MGC development during embryonically. Dead End 1 (DND1), first expressed in the mouse at embryonic day (E) 7.5, is a bi-modal (transcript protection or destruction) germ cell specific RBP. Studies on the *Ter* mutation in *Dnd1* have demonstrated that this RBP is involved with MGC survival: specifically in relation to balancing germ cell pluripotency with differentiation; and regulation of the cell cycle and chromatin. We recently used CRISPR knock-in to produce a transgenic mouse with a DND1-GFP fusion protein. Characterization studies with these mice showed that DND1-GFP expression in germ cells is negatively correlated with their entry into meiosis. RNA immunoprecipitation (RIP) studies against the GFP tag in arresting MGCs from the fetal testis, identified DND1 transcript targets involved with pluripotency, male fate specification, cell cycle and chromatin modification. During confocal imaging and FAC sorting work we observed a heterogenous population of germ cells expressing lower or higher levels of DND1-GFP, DND1-GFP(+)lo and DND1-GFP(+)hi respectively. Quantification and RT-qPCR analysis of these two populations showed that DND1-GFP(+)hi MGCs are in the minority but appear represent more robust germ cells. Preliminary expression work did not positively characterize DND1-GFP(+)lo MGCs. Further studies with DND1-GFP(+)lo MGCs are focused on accessing expression levels of genes associated with apoptosis and somatic populations. Work with DND1-GFP(+)hi MGCs is concentrated on determining their level of stemness.

Poster 75

Mapping of RNA Nm sites and interrogation of functional relevance using high-throughput methods

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Box C/D snoRNAs are conventionally known to guide RNA ribose 2'-*O*-methylation on rRNAs and snRNAs and contribute to structural rigidity. However, increasing evidence of snoRNAs' miRNA-like functions, regulation of mRNA splicing, and guidance of Nm modifications on mRNA suggests expanded roles. As high-throughput methods for mapping known and novel Nm sites such as Ribometh-seq, RibOxi-seq and Nm-seq had just become available in recent years, our lab is interested in utilizing these tools to mechanistically study snoRNA guided Nm modifications in general, and at the same time with a focus under the context of cardiovascular biology. We have established mice and tissue culture genetic snoRNA KO models to investigate loss of novel Nms (such as previously unmapped mRNA sites) associated with the snoRNA KOs. We successfully used both RibOxi-seq/Nm-seq and Ribometh-seq to verify loss of known Nm modification on rRNAs corresponding to snoRNA guides that were KO; we have subsequently investigated differential methylation in U6 snRNA, which was shown to be involved in congenital heart diseases (CHD). We have also undertaken extensive methodology and data analysis pipeline optimizations for RibOxi-seq aiming to achieve reliable mapping of mRNA Nm modifications. Additionally, continued

analysis of data generated in my previous lab with *Trypanosoma brucei* indicated differential methylation status between life stages, which can provide insight to future studies.

Poster 76

Exploiting codon usage identifies RpS21 as an *in vivo* amplitude-specific RAS/MAPK regulator

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The RAS small GTPases, composed of HRAS, NRAS, and KRAS, is mutated in one third of all human cancers. KRAS is the most mutated isoform, suggesting some features of this gene render it more likely to initiate tumorigenesis. Our group linked the high mutational frequency of KRAS to its rare codons enrichment and poor translation. This suggests the novel concept that low, rather than high RAS signaling induces tumor initiation. Consequently, proteins that modify the level of oncogenic RAS signaling should dictate whether an oncogenic mutation becomes tumorigenic or not. These modifiers may offer therapeutic targets in clinic.

To find modifiers of Ras signaling intensity *in vivo*, *Drosophila* whole genome haploinsufficiency screen was performed by altering codon usage as a novel platform to control signaling output. Fifteen deficiencies were identified as new intensity-specific modifiers of Ras^{V12} signaling. One deficiency was mapped to a single gene Rps21, small ribosomal subunit protein 21, a known tumor suppressor in flies. RpS21 preferentially influences low Ras signaling outputs, and negatively regulates Ras/MAPK signaling in multiple cell/tissue and signaling settings. Interestingly, RpS21 haploinsufficiency or RNAi knockdown increases the level of Ras^{V12} expression suggesting it acts as tumor suppresser through inhibition of Ras^{V12} expression.

Taken together, exploiting codon usage successfully identified RpS21 as an *in vivo* signal strength-dependent Ras/MAPK regulator. Moreover, the role of RpS21 in regulating oncogenic Ras expression suggests a new mechanism by which ribosomal proteins may act as tumor suppressors.

Poster 77

Driving Factors in Amiloride Recognition of HIV RNA Targets

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Noncoding RNAs are increasingly promising drug targets yet ligand design is hindered by a paucity of methods that reveal driving factors in selective small molecule:RNA interactions, particularly given the difficulties of high-resolution structural characterization. HIV RNAs are excellent model systems for method development given their targeting history, known structure-function relationships, and the unmet need for more effective treatments. Herein we report a strategy combining synthetic diversification, profiling against multiple RNA targets, and predictive cheminformatic analysis to identify driving factors for selectivity and affinity of small molecules for distinct HIV RNA targets. Using this strategy, we discovered high affinity and selective ligands for multiple targets and the first ligand for ESSV, an exonic splicing silencer critical to replication. Computational analysis revealed guiding principles for future designs and one of the first predictive models of small molecule:RNA binding. These straightforward methods are expected to facilitate progress toward selective targeting of disease-causing RNAs.
