Welcome

to the

3rd Annual

Rocky Mountain RNA Symposium

Thursday, April 25
2024

CU Anschutz
Aurora, CO

hosted by

Colorado RNA Club

in partnership with

RNA Bioscience Initiative

Special Thanks

Sponsors:

RNA Bioscience Initiative
SCHOOL OF MEDICINE
UNIVERSITY OF COLORADO **ANSCHUTZ MEDICAL CAMPUS**

RNA Society

RNA Club

LEXOGEN
The RNA Experts

Organizing Committee
(Alphabetical Order by Last Name)

Mary Baitinger	Events Coordinator	Univ of Colorado – Anschutz
Matthew Taliaferro, PhD	Assistant Professor	Univ of Colorado – Anschutz
Kathryn Walters	Doctoral Student	Univ of Colorado – Anschutz
Ning Zhao, PhD	Assistant Professor	Univ of Colorado – Anschutz

RNA Club Organizing Committee

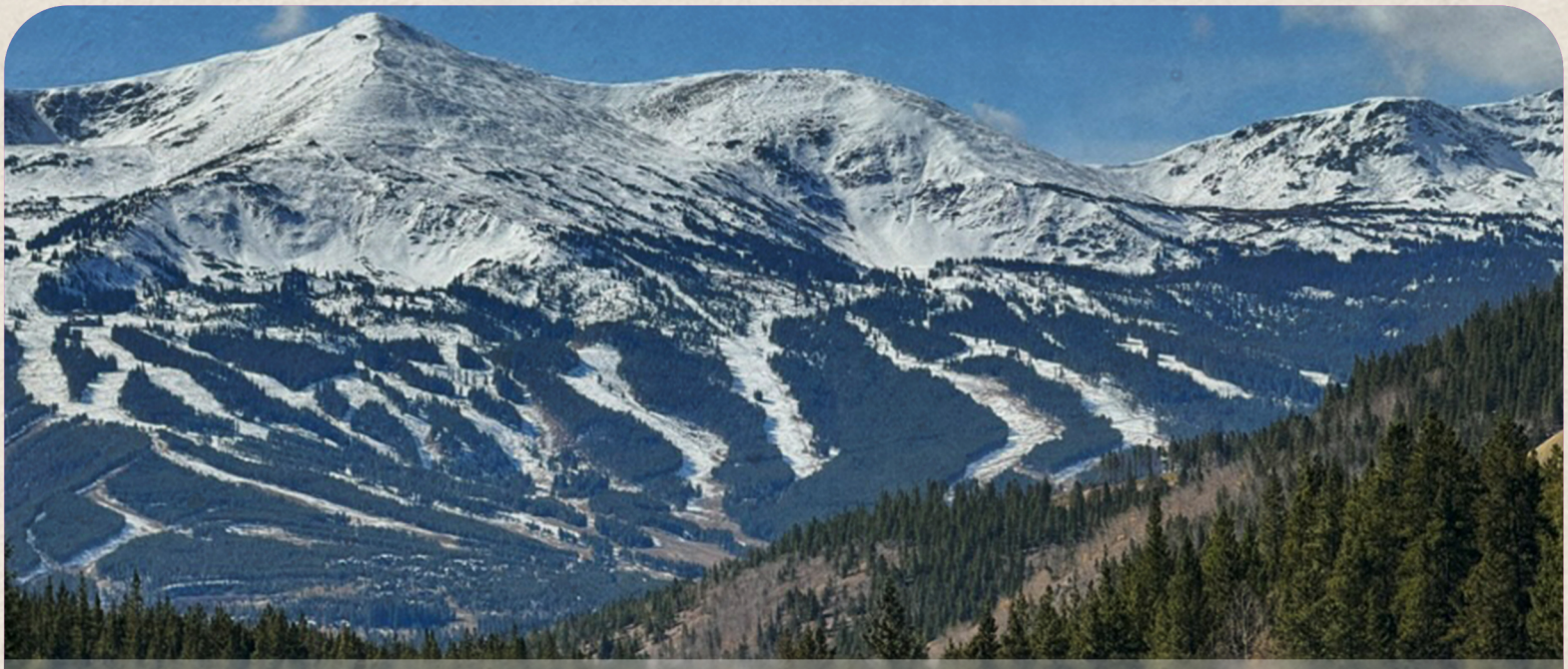
Matthew Taliaferro, PhD	Faculty Lead	Univ of Colorado – Anschutz
Kathryn Walters	President	Univ of Colorado – Anschutz
Eliza Lee, PhD	Vice President	Univ of Colorado – Boulder
Gaia Bublitz	Event Coordinator	Univ of Colorado – Boulder
Logan McCoy	Event Coordinator	Univ of Colorado – Boulder
Kate Matlin	Social Media Chair	Univ of Colorado – Anschutz

Rocky Mountain RNA Symposium Hosts
(Alphabetical Order by Last Name)

Amber Baldwin	Mukherjee Lab	Univ of Colorado – Anschutz
Chloe Barrington-Ham, Phd	Rissland Lab	Univ of Colorado – Anschutz
Joelle Lo	Taliaferro Lab	Univ of Colorado – Anschutz
Oscar Munoz	Jaganathan Lab	Univ of Colorado – Anschutz
Katie Vaeth	Taliaferro Lab	Univ of Colorado – Anschutz

THIRD ANNUAL

ROCKY MOUNTAIN RNA SYMPOSIUM



Join us for a fantastic day of science and networking!

Activities include:
An evening social, poster prizes, and much more!

April 25, 2024

Featuring talks from:

Homa Ghalei, PhD
Emory University

Myriam Gorospe, PhD
Laboratory of Genetics and Genomics - NIA

Heather Hundley, PhD
Indiana University

Kristin Patrick, PhD
Texas A&M University

Brought to you by:

RNA CLUB

RNA SOCIETY

LEXOGEN
The RNA Experts

RNA Bioscience Initiative
SCHOOL OF MEDICINE
UNIVERSITY OF COLORADO **ANSCHUTZ MEDICAL CAMPUS**

PROGRAM

2024 ROCKY MOUNTAIN RNA SYMPOSIUM

Thursday, April 25, 2024

Elliman Conference Center, AHSB, CU Anschutz

8:00 am	Check-In/Poster Setup/Breakfast
8:45 am	Introductory Remarks
9:00 am	***Homa Ghalei, Phd***; Emory University
9:45 am	Conner Purdy; CU Anschutz
10:05 am	Morning Poster Session
11:15 am	***Kristin Patrick, PhD***; Texas A&M University
12:00 pm	Lunch
1:00 pm	Kristin Fluke, PhD; Colorado State University
1:20 pm	***Heather Hundley, PhD***; Indiana University
2:05 pm	Laura White, PhD; CU Anschutz
2:30 pm	Afternoon Poster Session
3:35 pm	Eliza Lee, PhD; CU Boulder
3:55 pm	***Myriam Gorospe, PhD***; NIH
4:40 pm	Poster Awards and Closing Remarks
5:00 pm	Networking Social, TStreet

COLORADO RNA CLUB

LEXOGEN
The RNA Experts

RNA SOCIETY

RNA Bioscience Initiative
SCHOOL OF MEDICINE
UNIVERSITY OF COLORADO **ANSCHUTZ MEDICAL CAMPUS**

Speaker Abstracts
(In Order of Presentation)

Homa Ghalei, PhD – 9:00am
Associate Professor, Emory University School of Medicine

Molecular Consequences of Deregulated rRNA Processing and Modification in Biology and Disease

Ribosome biogenesis is a complex and highly regulated process involving the action of over 200 assembly factors bringing together a total of 79 proteins and 4 ribosomal RNAs in yeast. The maturation of rRNAs from precursor transcripts is a critical aspect of ribosome biogenesis involving a series of orchestrated processing steps by nucleases and over a hundred RNA chemical modifications. Despite tight regulation and quality controls, rRNA processing and chemical modifications go awry in several human diseases. Missense mutations in genes encoding structural subunits of the RNA exosome, the ribonuclease complex required for rRNA processing, cause a growing family of diseases with diverse pathologies, collectively termed RNA exosomopathies. Similarly, pathogenic mutations that impact rRNA modifications are associated with neurodevelopmental disorders and implicated in the onset and progression of cancer. I will present data on recent findings made by my research team and our collaborators that shed light on the molecular consequences of dysregulated rRNA processing and modifications. These molecular defects have a profound impact on both the quantity and quality of ribosomes within the translating pool, thereby disrupting the delicate balance of cellular protein homeostasis. Our findings underscore the intricate interplay between ribosome biogenesis, RNA processing, modifications, and disease pathogenesis, providing crucial insights and a deeper understanding of these complex cellular processes.

Conner Purdy – 9:45am
Doctoral Student, Ford Lab, University of Colorado-Anschutz

Determining the Role of eIF3d/e-Mediated Stress-Induced Translation in Breast Cancer Metastasis

Nearly all breast cancer-related deaths are caused by metastatic disease instead of the primary tumor. Unfortunately, there are currently no effective targeted therapies for metastasis. It has been shown that genomic alterations are not the main drivers of metastasis, and that instead, tumor cell plasticity, due to changes in gene expression in response to external cues, plays a critical role. While much research has focused on transcriptional/epigenetic means to induce tumor cell plasticity and metastasis, the role of protein translation in metastasis has been underappreciated. Importantly, recent studies have highlighted drastic differences in translational demand between tumor cells at the primary versus metastatic sites. The goal of this project is to understand the role of stress-induced translation in breast cancer metastasis, with a focus on eIF3e. eIF3e is one of 13 subunits of the eIF3 complex (eIF3a-m) involved in translation in mammalian cells. eIF3e, and its binding partner eIF3d, are critical for the translation of mRNAs that regulate key processes such as response to stress, epithelial to mesenchymal transition (EMT), proliferation and survival. These processes also play key roles in tumorigenesis and metastasis; and it is known that eIF3e is dysregulated in numerous cancers, including breast cancer. Excitingly, we recently showed that a novel compound, NCGC00378430 (abbreviated 8430), inhibits breast cancer-associated EMT and metastasis in vivo, and discovered that it binds to eIF3e. We show that 8430 does not alter global translation but can inhibit non-canonical translation of mRNAs such as c-Jun and HIF1α during nutrient deprivation or hypoxia, respectively, similar to eIF3e, or its binding partner, eIF3d. We show that 8430 binds to eIF3e and decreases the amount of eIF3d within the eIF3 complex, without altering the composition of other subunits. Because 8430 targets eIF3e and inhibits EMT and metastasis, these data implicate eIF3d/e as important contributors to metastasis, and potential therapeutic targets. We have also developed closely related analogs of 8430 such as “209” which displays similar phenotypes in vitro and has better solubility and potency than 8430. To further understand the role of eIF3d/e in hypoxia and metastasis, we have recently performed ribosome sequencing (RiboSeq) experiments +/- hypoxia, +/- 209, and +/- eIF3e and

eIF3d knockdown. This work implicates eIF3d/e in the regulation of specialized translation in response to stressful and changing microenvironments, such as hypoxia, during the metastatic cascade. Furthermore, our novel compounds, 8430 or 209 (and future analogs), may provide a starting point for determining whether inhibition of eIF3d/e will decrease tumor cell plasticity and metastasis, while conferring limited side effects.

Kristin Patrick, PhD – 11:15am
Assistant Professor, Texas A&M College of Medicine

Nuclear RNA binding proteins: Concertmasters of the macrophage innate immune response

When innate immune cells like macrophages encounter a pathogen, they massively upregulate hundreds of inflammatory and antimicrobial genes in a matter of minutes. This response is executed by specialized transcription factors (e.g. NFkB, IRF3, AP-1) that are activated by pathogen sensing signaling cascades. Under this current "transcription-focused" paradigm, the potential contribution of post-transcriptional control mechanisms to tuning inflammatory gene expression has been largely neglected. My lab has implicated several RNA binding proteins (RBPs) in the SR and hnRNP families of splicing factors in controlling the kinetics and amplitude of inflammatory gene expression. Collectively, our work highlights RNA processing as a key regulatory node in shaping the macrophage response to pathogens.

Recently, we have started investigating ways that macrophages could quickly remodel their nuclei to redistribute RBPs and prioritize processing of inflammatory genes. There is a growing appreciation for stress-responsive membraneless organelles (MLOs) regulating various steps of eukaryotic gene expression in response to extrinsic cues. We found that the nuclear paraspeckle, a highly ordered biomolecular condensate that nucleates on the Neat1 lncRNA, is a critical component of the macrophage antimicrobial response. Specifically, we report that lipopolysaccharide (LPS) treatment triggers dynamic remodeling of macrophage paraspeckles and that loss of paraspeckles, via Neat1 KO, results in damped pro-inflammatory responses and a failure to control replication of both bacterial and viral pathogens. We are currently working to understand how paraspeckles are remodeled in response to additional pathogen-associated molecular patterns and how other nuclear MLOs respond to pathogen-mediated cues. Collectively, our work supports a model whereby dynamic assembly and disassembly of MLOs reorganize the nuclear landscape to enable macrophage responses to different pathogens.

Kristin Fluke, PhD – 1:00pm
Postdoctoral Researcher, Santangelo Lab, Colorado State University

Unique and extensive epitranscriptomic profiles in heat-loving Archaea enhance thermophily

The extraordinary quantity of known RNA modifications and their ubiquity in all life strongly suggests the epitranscriptome provides tangible benefits to cellular fitness. Ribosomal RNA is among the most heavily modified RNAs in a cell; modifications to rRNA are known to have profound impacts on ribosome function, and in turn, proteostasis. Recent investigations into archaeal epitranscriptomes have demonstrated that ribosomes from Thermococcus kodakarensis, a heat-loving archaeon, are densely modified with 4-acetylcytidine (ac4C) and 5-methylcytidine (m5C), and that the epitranscriptome supports hyperthermophilic growth. Using LC-MS/MS, bisulfite-sequencing, and high-resolution cryo-EM structures of the archaeal ribosome, we identified a unique epitranscriptomic mark in the T. kodakarensis 16S rRNA that includes a new RNA modification, m4,2C. We characterized and structurally resolved a novel class of RNA methyltransferase that generates m4,2C whose function is critical for hyperthermophilic growth. The phylogenetic distribution of the newly identified m4,2C synthase family implies m4,2C is biologically relevant in each Domain. Resistance of m4,2C to bisulfite-driven deamination suggests that efforts to capture m5C profiles via bisulfite sequencing are also capturing m4,2C.

Heather Hundley, PhD – 1:20pm
Associate Professor, Indiana University

Mechanisms of in vivo Target Recognition by the ADAR family of RNA Modification Enzymes

The ADAR family of RNA binding proteins binds double-stranded RNA (dsRNA) and catalyzes the deamination of adenosine (A) to inosine (I). As the A-to-I conversion changes hydrogen bonding specificity of the base, most enzymes and cellular factors recognize inosine as guanosine. Hence, A-to-I RNA editing can alter the coding potential, splicing, and small RNA-mediated silencing of mRNA. The ability of ADARs to change the genome-encoded information present in RNA provides an important means to diversify the transcripts expressed in an organism's tissues over time and is being harnessed for personalized medicine approaches to correct mutations at the RNA level and improve human health. However, details of how ADARs bind specific transcripts as well as how certain adenosines are selected within the bound transcript for editing remain largely unknown, especially at a molecular level in vivo. Using the C. elegans model system, we have been able to demonstrate that the A-to-I editing machinery involves RNA binding specificity dictated by one protein, while the enzymatic activity is contributed by a second protein. Our insights gained from recent enhanced crosslinking and immunoprecipitation coupled to high-throughput sequencing specific experiments will be presented. In addition, recent work on how editing and RNA binding of transcripts in specific tissues is influenced by the environment and development will be discussed.

Laura White, PhD – 2:05pm
Research Associate, Hesselberth Lab, University of Colorado-Anschutz

So many mods in so little time: >45 RNA modifications profiled by direct RNA-seq

Epitranscriptomic marks on nucleic acids produce disruptions in ion flow when they are fed through biological nanopores. In principle, this effect enables the identification of any modification that generates a differentiable signal distortion; however, distinguishing the signals produced by the >170 distinct chemical modifications present on RNA molecules is a non-trivial technical challenge. We leveraged the diverse chemical repertoire of tRNAs, the most abundantly modified class of RNA, to evaluate the signals produced at known modification sites across a broad range of viral, prokaryotic, and eukaryotic species. We evaluated signals from more than 45 distinct RNA modifications using both first and second generation Oxford Nanopore direct RNA sequencing chemistry, and further report a proof of concept approach for detecting low abundance mitochondrial and viral tRNA reads using the higher library throughputs enabled by the new RNA004 chemistry. This work provides a roadmap to guide future efforts towards de novo detection of RNA modifications across the tree of life using nanopore sequencing.

Eliza Lee, PhD – 3:35pm
Postdoctoral Associate, Cech Lab, University of Colorado Boulder

N-6-methyladenosine (m6A) Promotes the Nuclear Retention of mRNAs with Intact 5' Splice Site Motifs

In eukaryotes, quality control of mRNA represents an important regulatory mechanism for gene expression. Misprocessed mRNAs that contain an intact 5' Splice Site (5'SS) motif are retained in the nucleus and targeted for decay. Previously, we showed that the nuclear retention of these transcripts requires ZFC3H1, a component of the Poly(A) Exosome Targeting (PAXT) complex, and U1-70K, a component of the U1 snRNP. In S. pombe, the ZFC3H1 homolog, Red1, binds to the YTH-domain containing protein Mmi1 to target certain RNA transcripts for nuclear retention and decay. Here we show that ZFC3H1 and U1-70K interact with YTHDC1 and YTHDC2, two YTH domain-containing proteins that bind to N-6-methyladenosine (m6A) modified RNAs. We then show that YTHDC1 and YTHDC2 are required for the nuclear retention of mRNAs with intact 5'SS motifs. Furthermore, disruption of m6A methyltransferase activity inhibits the nuclear retention of these transcripts. Using m6A-miCLIP analysis, we map m6A methylation marks to intronic polyadenylated (IPA)

transcripts, which contain intact 5'SS motifs and are nuclear retained and degraded in a ZFC3H1-dependent manner. We find that m6A is enriched near intact 5'SS motifs and the poly(A)-tail. Overall, this work suggests that the m6A modification acts as part of an evolutionarily conserved quality control mechanism that targets misprocessed mRNAs for nuclear retention and decay.

Myriam Gorospe, PhD – 3:55pm
Chief, Laboratory of Genetics and Genomics, National Institute on Aging Intramural Research Program, NIH

Long noncoding RNA LANCL1-AS1 in aging muscle regeneration

With advancing age, the skeletal muscle experiences a gradual loss of function resulting from declining number, quality, and size of muscle fibers. In turn, the loss of muscle is associated with frailty, fractures, and increased risk of metabolic diseases. Muscle mass and function are maintained through myogenesis, a process whereby muscle stem cells regenerate muscle fibers in the adult. As skeletal muscle ages, declining function of muscle stem cells (satellite cells) to form myofibers contributes to the loss of muscle mass (sarcopenia). Given that muscle function and regeneration are increasingly compromised with age, we have a long-standing interest in understanding myogenesis.

Among the many regulators of myogenesis, our lab has focused on RNA-binding proteins (RBPs) and noncoding (nc)RNAs for many years. In this presentation, I discuss some of the long noncoding RNAs [1] implicated in controlling myogenesis that we have studied over the past 5 years. Human myogenesis was found to be fostered by lncRNA OIP5-AS1, abundant in the cytoplasm of myoblasts, which scaffolds HuR and MEF2C mRNA, thereby enhancing the production of myogenic transcription factor MEF2C [2]. The same lncRNA, OIP-AS1, was further discovered to lower miR-7 levels by target-directed microRNA degradation (TDMD); the loss of miR-7 derepressed production of the fusogenic protein MYMX, which is crucial for myoblast fusion and myotube generation [3]. During human myogenesis, a linear lncRNA residing in the nucleus, lncFAM, was found to accumulate and promote differentiation by recruiting HNRNPL to the MYBPC2 promoter, in turn rising MYBPC2 levels [4]. Interestingly, we also found a circular lncRNA (circSamd4) that promoted myogenesis by increasing during myogenesis, as circSamd4 inactivated the transcriptional repressors PURA and PURB and enabled transcription of the Myh gene [5].

In an ongoing study, we have focused on lncRNAs directly implicated in skeletal muscle aging. We examined the transcriptomes of skeletal muscle biopsies from a cross-sectional study of healthy individuals 22 to 83 years old to identify lncRNAs changing in expression levels. After contrasting them with the transcriptomes changing during human myogenesis in culture, a top candidate emerged, LANCL1-AS1, which increased with myogenesis and declined with muscle aging. Interestingly, silencing LANCL1-AS1 attenuated myogenesis and globally reduced the levels of mRNAs transcribed from the mitochondrial genome (mt-mRNAs). Molecular characterization revealed that LANCL1-AS1 associated with the mitochondrial RBP LRPPRC, which, together with SLIRP, regulates mt-mRNA levels by preserving the length of their poly(A) tails and prolonging their half-lives. Accordingly, overexpressing LANCL1-AS1, but not a mutant LANCL1-AS1 unable to interact with LRPPRC, restored myogenic capacity to primary myoblasts from muscle of old monkeys, where LANCL1-AS1 is conserved. In light of the finding that mt-mRNAs in old human skeletal muscle had shorter poly(A) tails than those in young muscle, we propose that LANCL1-AS1 helps to preserve myogenesis across the life span by improving mitochondrial function.

[1] Herman et al., Mol Cell (2022). https://pubmed.ncbi.nlm.nih.gov/35714586/
[2] Yang et al., Nucleic Acids Res (2020). https://pubmed.ncbi.nlm.nih.gov/33270893/
[3] Yang et al., Nucleic Acids Res (2022). https://pubmed.ncbi.nlm.nih.gov/35736212/
[4] Chang et al., Nucleic Acids Res (2023). https://pubmed.ncbi.nlm.nih.gov/36533518/
[5] Pandey et al., Nucleic Acids Res (2020). https://pubmed.ncbi.nlm.nih.gov/31980816/

Morning Poster Session

01	Predicting where Transcription Factors are functioning via eRNAs	**Mary Allen**
02	A Prophage Terminase With Trnase Activity Sensitizes ... Stress	**Siva R Uppalapati**
03	The role of chromatin state in intron retention: a case study in ...models	**Asa Ben-Hur**
04	Short Linear Motifs within the ZαZβ unit modulate domain ... specificity	**Jeffrey Krall**
05	Integrating Single-Cell Experiments and Stochastic... Dynamics	**Eric Ron**
06	The Unkempt RNA binding protein reveals a local ... overduplication	**Abraham Martinez**
07	Investigating estrogen receptor alpha's exchange of nucleic acids	**Halley Steiner**
08	Diffusion of messenger RNA in the cytoplasm of HeLa cells	**A. Pacheco-Pozo**
09	The West Nile virus infectious unit contains multiple virus particles	**Marina Fujii**
10	Regulation of transposition by the essential ribosome ... Bcd1	**Brandon Wehmiller**
11	Unveiling tRNA-like mimicry utilized in MHV68 ... infection	**Elizabeth Spear**
12	Stuck in the middle with U: RNA localization to the midbody	**Katie Vaeth**
13	Identification of an essential gene for Borrelia burgdorferi that ... activity	**Taylor Van Gundy**
14	Age-associated decline in LANCL1-AS1 impairs mitochondrial.... muscle	**Dimitrios Tsitsipais**
15	Understanding the mechanism of eIF3d-mediated selective ... hypoxia	**Kate Matlin**
16	Role of Active Site Adjacent Positively Charged Residues in ... Activity	**Ryan Coops**
17	Development of the Pin-point™ platform: a versatile base ... generation	**Shannon Hinsdale**
18	Expanding the druggable genome: targeting RNA with small molecules	**Lukasz Olenginski**
19	Transcriptional bursting underlies RNA repeat foci formation	**JP Ouyang**
20	Alternative Splicing Alters the Female and Male Germ ... Development	**Victor Ruthig**
21	TDP43 prevents RNA accumulation in neurites	**Charlie Moffatt**
22	TBD	**Gabriel Galindo**
23	Determining the elusive mechanism of gene regulation by Musashi-2	**Kathryn Walters**
24	Characterizing the Nucleic Acid Binding Properties of ... N-terminus	**Lily Beck**
25	Feedback from mRNA decay to the transcriptional machinery	**Michael Cortázar**
26	Ribosome profiling reveals translational reprogramming and ... myeloma	**Neel Mukherjee**
27	hnRNPA2/B1 regulates motor protein RNA localization and ... neurites	**Joelle Lo**

Afternoon Poster Session

28	A RelBbu-dependent small antisense RNA regulates ... operon	**Marisa Foster**
29	Myelodysplatic Syndrome-Associated Splicesome ... Response	**Johnathon Schafer**
30	How Do Right-Handed Helices Become Left-Handed and ... ADAR1	**Parker Nichols**
31	Unveiling the bat signal: How flavivirus sfRNAs affect bat ... responses	**Lauren Malsick**
32	Exploring Transcription Dynamics through Spatial Stochastic Modeling	**Luis Aguilera**
33	Identification of potential riboswitch elements in H. Sapians ... learning	**W. Scott Raymond**
34	DNA methyltransferase 1 (DNMT1) and its possible regulation by RNA	**Ella Tommer**
35	Hemotopoietic Transcription Factor GATA1 is a ... Protein	**Daniella Ugay**
36	PDGFRalpha signaling regulates Srsf3 transcript binding ... trafficking	**Thomas Forman**
37	Dissecting molecular mechanisms of dsRNA recognition and ... vivo	**Boyoon Yang**
38	Leaky scanning as a developmental buffering mechanism	**Grace Gustafson**
39	Stress- responsive membraneless organelles ... macrophages	**Kaitlyn Armijo**
40	RNA Polymerase III transcription in cranial neural crest cell development	**Kristin Watt**
41	Proteogenomic discovery of novel and regulatory ORFs in... beta-cells	**Neel Mukherjee**
42	Reactivity of PNPase towards oxidized RNA ... 7,8-dihydro-8oxoguanine	**Brody Reynolds**
43	TRUB1-mediated RNA modification networks regulate ... production	**Nadine Koertel**
44	Humanizing the zinc-finger domain in U1C protein of the ... changes	**S. Chalivendra**
45	Insights into "scaffolded" selection revealed from the crystal ... aptamer	**Shea Siwik**
46	Steroid hormone disruption of RNA polymerase III at tRNA ... cancer	**J. Finlay-Schultz**
47	Evolutionary dynamics of polyadenylation signals and their ... protists	**Marcin Sajek**
48	The SRSF1 and HNRNPU RNA Binding proteins regulate ... Splicing	**Frank Lee**
49	Quantification of subcellular RNA localization through direct ... oxidation	**Hei-Yong Lo**
50	Quantifying translational effects of SARS-CoV-2 UTRs with ... cells	**Adam Koch**
51	HIV Under the Lens: Real-time Fluorescent Imaging of ... Dynamics	**Sam Klink**
52	Role of mTOR in RP mRNA Localization	**Megan Pockalny**

01 - Mary Allen

Predicting where Transcription Factors are functioning via eRNAs

Taylor Jones1,2, Rutendo F. Sigauke1, Lynn Sanford1, Dylan J. Taatjes2, Mary A. Allen1*, Robin D. Dowell1,3,4*

1*BioFrontiers Institute, University of Colorado Boulder 2 Biochemistry, University of Colorado Boulder 3 Computer Science, University of Colorado Boulder. 4 Molecular, Cellular and Developmental Biology, University of Colorado Boulder

*Corresponding author(s). E-mail(s): mary.a.allen@colorado.edu; robin.dowell@colorado.edu; dylan.taatjes@colorado.edu

Transcription factors (TFs) orchestrate transcription and play a critical role in cellular maintenance, identity, and response to external stimuli. TFs generally comprise two domains: a DNA binding domain and an activation/repression domain. While ChIP assays have measured DNA binding, they do not identify when and where TFs are actively regulating transcription. We designed TF profiler to infer transcription factor regulatory activity, i.e., when a TF is present and actively regulating transcription. TF profiler uses RNA polymerase activity (GRO-seq or PRO-seq) to infer TF activity, which was applied across hundreds of data sets and TFs. From this application we identified three distinct classes of TFs: Ubiquitous factors are expressed and active across all cell types, play important roles in maintaining cellular homeostasis, and bind primarily at promoters.Tissue-specific factors are regulated at transcription and act almost exclusively at enhancers. Stimulus-responsive TFs are regulated post-transcriptionally but act predominantly at enhancers.These three TF classes lead us to hypothesize that while gene transcription combines the effects of both enhancers and promoters, tissue-specific genes are more influenced by enhancers than other genes.

02 - Siva R Uppalapati

A Prophage Terminase With Trnase Activity Sensitizes Salmonella To Oxidative Stress

S. Uppalapati, K. Sashi , L. Liu , J. Kim , D. Orlicky , M. McClelland , A. Vazquez-Torres

Phage virulence factors define the virulence properties of their bacterial hosts. In the present study, we have found that the Gifsy-1 prophage ameliorates Salmonella enterica virulence in mice and macrophages by sensitizing its bacterial host to oxidative stress rendered from innate immune responses. Surprisingly, ROS induces a novel moonlighting function of anticodon tRNase to a canonical DNase, terminase encoded on Gifsy-1 prophage. The ensuing tRNA fragmentation compromises bacterial translation, intracellular survival and recovery from oxidative stress. To counter the adverse effects of Gifsy-1 terminase on tRNA fragmentation Salmonella adapts by transcribing the RNA repair Rtc system. The ‘antivirulence’ prophage may persist in Salmonella because it provides a selective advantage to its host by stalling the bacterial translation while repressing viral maturation, providing opportunities for DNA and RNA repair as a way of maintaining genome integrity and ultimately bacterial host survival.

03 - Asa Ben-Hur

The role of chromatin state in intron retention: a case study in leveraging large scale deep learning models.

Ahmed Daoud and Asa Ben-Hur

Complex deep learning models trained on very large datasets have become key enabling tools for current research in natural language processing and computer vision. By providing pre-trained models that can be fine-tuned for specific applications, they enable the creation of accurate models with minimal effort and computational resources. Large scale genomics deep learning models come in two flavors: the first are large language models of DNA sequences trained in a self-supervised fashion, similar to the corresponding natural language models; the second are supervised learning models that leverage large scale genomics datasets from ENCODE and other sources. We argue that the latter models are the equivalent of foundation models in natural language processing in their utility, as they encode within them chromatin state in its different aspects, providing useful representations that allow quick deployment of accurate models of gene regulation. We demonstrate this premise by leveraging the recently created Sei model to develop simple, interpretable models

of intron retention and demonstrate their advantage over models based on the DNA langauage model DNABERT-2.

On the biological side, our work demonstrates the impact of chromatin state on the regulation of intron retention: our model is able to discover the involvement of numerous transcription factors and chromatin marks in regulating intron retention, providing better accuracy than a recently published custom model developed for this purpose.

Availability: The source code for this work is available at https://github.com/Addaoud/IntronRetention

Preprint: https://www.biorxiv.org/content/10.1101/2024.01.26.577402v1.abstract

04 - Jeffrey Krall

Short Linear Motifs within the ZαZβ unit modulate domain interactions and substrate specificity

Jeffrey B. Krall, Parker J. Nichols, Morkos A. Henen, Quentin Vicens, and Beat Vögeli

All isoforms of Adenosine Deaminase Acting on RNA 1 (ADAR1) contain a Zβ domain, while the interferon inducible ADAR1p150 isoform contains an additional N-terminal Zα domain connected to Zβ via a ~100 residue intrinsically disordered region (IDR). Both domains have been characterized through biophysical and structural techniques individually, however, few studies have investigated the domains in the context of a larger protein construct. Schwartz et. al (1998) proposed that ZαZβ could act as a single bipartite domain, however, further characterization of the two domains in tandem have not been undertaken. Here, we use a variety of biophysical experiments to investigate the structure, dynamics, and potential roles of the Z-domains as it is found within full length ADAR1. Our results reveal two previously unreported Short Linear Motifs (SLiMs) of ~14 residues in the IDR, which exhibit higher-than-average compactness. Both SLiMs bind to the Z-RNA binding interface on Zα and the structurally homologous region on Zβ, leading to a compact conformation in solution. The SLiMs compete with Z-RNA for binding to the Zα domain, presenting a potential role for the autoregulation of Zα by increasing RNA-binding specificity.

05 - Eric Ron

Integrating Single-Cell Experiments and Stochastic Models to Understand and Predict Glucocorticoid Receptor Transport and DUSP1 mRNA Expression Dynamics

Eric M. Ron1,3, Luis U. Aguilera2,3, Linda S. Forero Quintero, Joshua Cook1,3, Brian Munsky1,3

1Biomedical Engineering, 2Biochemistry & Molecular Biology, 3Chemical & Biological Engineering Colorado State University, Fort Collins, CO, USA.

Overactivation of mitogen-activated protein kinase (MAPK) signaling pathways is key to multiple inflammatory responses, and synthetic glucocorticoids, such as Dexamethasone (Dex), have long been used to treat inflammatory pathologies. Upon cell entry, Dex binds to the glucocorticoid receptor (GR) and initiates GR translocation to the nucleus. Nuclear GR interacts with Glucocorticoid Regulatory Elements (GRE's) to promote transcription of anti-inflammatory genes, including dual-specificity phosphatase 1 (DUSP1). In turn, DUSP1 encodes for MAPK phosphatase 1 (MKP-1), which down-regulates the cell's inflammatory response through dephosphorylation of the P38 and JNK MAPKs. Predictive understanding of these Dex-GR-DUSP1 dynamics could yield new insight into steroidal treatments for inflammation.

We build a predictive and mechanistic spatiotemporal understanding for Dex modulation of GR localization and DUSP1 transcription. Combining Immunocytochemistry (ICC) and single-molecule fluorescent in situ hybridization (smFISH), we quantify the sub-cellular temporal dynamics of GR localization and DUSP1 transcription for hundreds of individual HeLa cells at 12 different times following continuous Dex stimulation. We observe that GR localization and DUSP1 expression depend on the concentration and time of Dex stimulation. Moreover, although GR translocation dynamics is similar among neighboring cells, the downstream activity of DUSP1 is highly heterogeneous. Using a chemical master equation framework, we infer a discrete stochastic model that simultaneously and quantitatively reproduces the time-varying probability distributions of the GR and DUSP1 spatiotemporal dynamics – at all times and for multiple Dex concentrations.

Finally, we validate that our parameterized model accurately predicts GR and DUSP1 responses in several, previously unmeasured stimulus conditions.

06 - Abraham Martinez

The Unkempt RNA binding protein reveals a local translation program during centriole overduplication

Abraham Martinez, Alexander J. Stemm-Wolf, Ryan M. Sheridan, Matthew J. Taliaferro, Chad G. Pearson

Cell division depends on the two centrosomes that nucleate and organize the bipolar mitotic spindle. During S-phase, the Polo-like kinase, Plk4, promotes centriole duplication for centrosome assembly. Promiscuous elevations of Plk4 promote centrosome amplification, a founding event of tumorigenesis. Here, we investigated the transcriptional consequences of elevated Plk4, and found Unkempt (Unk), an RNA binding protein, to be one of only two mRNAs that were upregulated by more than 1.5-fold. Unk localizes to centrioles and centrosomes, in addition to membrane-less cytoplasmic transport structures called centriolar satellites. RNA binding activity of Unk is required for Plk4-induced centriole overduplication and the localization of centriole assembly proteins and centriolar satellites to the centrosome. The major centriolar satellite protein, Cep131, also promotes centriole overduplication. We discovered centrosomes and centriolar satellites to be sites of enriched protein translation. Further, Cep131 promotes centrosome localized translation, while Unk suppresses it. The translation suppressor complex CCR4-NOT, and PABPC4 localize to the centrosome in an Unk-dependent manner. Plk4-induced centrosome amplification also requires translation suppression. We propose that centriolar satellites and Unk promote and repress local translation, respectively, creating a translational program to ensure centriole duplication.

07 - Halley Steiner

Investigating estrogen receptor alpha's exchange of nucleic acids

Halley Steiner, Nickolaus Lammer, Wayne Hemphill, Robert Batey, Deborah Wuttke

Estrogen receptor alpha (ERα) is a ligand-responsive transcription factor critical for sex determination and development. Recent reports challenge the canonical view of ERα function by suggesting an activity beyond binding dsDNA at estrogen-responsive promotor elements: association with RNAs in vivo. Whether these interactions are direct or indirect remains unknown, which limits the ability to understand the extent, specificity, and biological role of ERα-RNA binding. Here we demonstrate that an extended DNA-binding domain of ERα directly binds a wide range of RNAs in vitro with structural specificity. ERα binds RNAs that adopt a range of hairpin-derived structures independent of sequence, while interacting poorly with single- and double-stranded RNA. RNA affinities are only four-fold weaker than consensus dsDNA and significantly tighter than nonconsensus dsDNA sequences. Moreover, RNA binding is competitive with DNA binding. Together, these data show that ERα utilizes an extended DNA-binding domain to achieve a high-affinity/low-specificity mode for interacting with RNA.

08 - Adrian Pacheco-Pozo

Diffusion of messenger RNA in the cytoplasm of HeLa cells

Adrian Pacheco-Pozo, Ryan Roessler, Arturo Matamoros Volante, O'neil Wiggan, Tim Stasevich, Diego Krapf

Messenger RNA (mRNA), once out of the cell nucleus, must diffuse through the cell cytoplasm until reaching the various places where protein synthesis takes place. Given the complexity and heterogeneity of the cytoplasm, the interaction of the mRNA with its environment plays a role in its diffusion, thus resulting in different modes of motion. In this work, we study the statistical properties of mRNA trajectories diffusing in the cytoplasm of HeLa cells. These trajectories were obtained using high-resolution single-particle tracking experiments which were analyzed using statistical technics to then compare the results with theoretical models. We have found that mRNA, while diffusing through the cytoplasm, exhibits heterogenous anomalous diffusion. The study of single trajectories reveals that the mRNA randomly switches between two states. Each state is characterized by a distribution of sojourn times: one is an exponential distribution while the other is heavy tailed.

09 - Marina Fujii

The West Nile virus infectious unit contains multiple virus particles

Marina Fujii, Emily Fitzmeyer, Bradly Burke, Emily Gallichotte, Marcela Henao-Tamayo, Brian Geiss, Greg Ebel

An “infectious unit” is a set of virus particles that starts infection in a cell. The infectious unit is often represented as a plaque-forming unit in virology and is widely considered a single virus particle. However, a growing body of research indicates presence of multiple virus particles in an infectious unit. We previously determined that a mean of 10 Zika virus particles are associated with one plaque-forming unit, demonstrating polyinfection in flavivirus.

To expand our understanding of what an infectious unit in flaviviruses is, we examined the number of genomes associated with West Nile virus (WNV) using a genetically barcoded WNV containing 11 degenerate codons in the NS4b gene. Using the barcode WNV library, we infected cells at low multiplicity of infection to generate well-isolated plaques, extracted RNA, and analyzed barcode diversity with next-generation sequencing. Our results suggested that, on average, 5 to 7 WNV virus particles/genomes comprise a WNV plaque-forming unit. Some plaque-forming units, however, were highly diverse, containing more than 20 discrete viral sequences. Since multiple virus particles are unlikely to enter the same cell with low multiplicity of infection, we hypothesize that WNV aggregates are responsible for poly-infection. To visualize WNV virus aggregation, we are currently developing a “flow virometry” -assay that will allow us to quantify the number of virus particles present in virus aggregates. Using flow virometry, we plan to correlate physical information about viral particle number in aggregates with our plaque assay data to better define the nature of the WNV infectious unit.

10 - Brandon Wehmiller

Regulation of transposition by the essential ribosome biogenesis factor, Bcd1.

Wehmiller, Brandon; Ghalei, Homa

Dysregulation of transposable element (TE) genome integration, known as transposition, accelerates aging and contributes to the development of neurodegenerative diseases. Class I TEs, or retrotransposons, are capable of “copy-and-paste” insertions into the cellular genome that alter gene expression, leading to potential genome instability. Regulation of TE genome integration is therefore critical to combat the development of NDs. Saccharomyces cerevisiae serves as an ideal system to study retrotransposition because its genome contains self-encoded TEs known as Ty elements. Among these, Ty1 is the most active and best studied, providing a robust model for studying the mechanisms that control its insertion into the genome. Although studies have elucidated several steps needed for Ty1 integration, the roles of many host factors during transposition remain unclear. A key gap in knowledge remains in understanding the mechanisms by which host factors influence transposition pathways. Using immunoprecipitation coupled with mass spectrometry, our lab has identified a novel interaction network between the conserved ribosome biogenesis factor Bcd1, Ty1-encoded proteins and RNA Pol III subunits. Based on these data, I hypothesize that Bcd1 limits TE genome integration through interactions with Ty1 Gag-Pol polyproteins and PolIII-transcribed RNAs. My data, based on in vivo transposition assays, show that Bcd1 plays a prominent role in regulating transposition. Future studies will address the role of Bcd1 within the retrotransposition pathway through in vitro binding assays, co-localization studies, retromobility assays, and unbiased sequencing approaches. Completion of this study will provide a foundation for designing therapeutics to restrict undesired genome integrations in NDs.

11 - Elizabeth Spear

Unveiling tRNA-like mimicry utilized in MHV68 gammaherpesvirus infection

Elizabeth Spear, Brittany Gomez, Rachael Kostelecky, Kyra Noell, Gabrielle Vragel, Ashley Tseng, Eric Clambey, Linda van Dyk, Madeline Sherlock, Jeffrey Kieft

Certain DNA viruses are known to cause oncogenesis and pose a risk to human health. Among these cancerous DNA viruses are the gammaherpesvirus subfamily of herpesviruses such as Epstein-Barr virus (EBV) and Kaposi’s sarcoma-associated herpesvirus (KSHV). Both establish lifelong infection in their host and cycle between two activity phases, productive lytic replication, and quiescence through viral gene regulation via a

latent state. The murine gammaherpesvirus MHV68 serves as a small animal model for gammaherpesvirus infection and pathogenesis in the can Dyk lab. Interestingly, gammaherpesviruses generate non-coding RNAs during infection that are important for viral pathogenesis, for example the EBERs (Epstein-Barr Encoded RNAs), and the TMERs (MHV68 tRNA-miRNA Encoded RNAs). Within the MHV68 TMER, there is a tRNA like structure that is processed as a canonical tRNA through RNase Z cleavage and CCA processing, but its ability to be recognized and processed by tRNA machinery appears to halt at this point, and these structures do not go on to be aminoacylated. By using biochemistry and structural biology, this new project will investigate the role of this tRNA-like mimicry in MHV68 infection.

12 - Katie Vaeth

Stuck in the middle with U: RNA localization to the midbody

The midbody (MB) is a microtubule-rich organelle that forms between two dividing cells and is a critical mediator of the conclusion of mitosis, abscission. Sequential recruitment of specific abscission regulating proteins to the MB is required for abscission, yet it is unknown how abscission regulators are localized to and activated at the MB. We have shown that MBs contain a specific subset of mRNAs and MB localized mRNAs can be locally translated. Local translation results in the accumulation of abscission-regulating proteins at the site of abscission. To understand how RNAs are localized to the MB, we began by validating the necessity and sufficiency of previously identified cis-elements within the 3'UTRs of RNAs to drive localization to the MB. Thus, these cis-elements are conserved across cell type and species for plus-end directed mRNA localization. Using a massively parallel reporter assay, we will identify new cis-elements driving RNA localization to the MB by tiling across the 3'UTRs of MB localized RNAs in 260 nucleotide windows. For each new element identified, we will then assess the necessity and sufficiency of the cis-element to localize RNAs to the MB. We have identified a unique subset of RNAs present at the MB, suggesting a potential means of abscission regulation via mRNA localization to the MB and local translation of specific RNAs at the MB.

13 - Taylor Van Gundy

Identification of an essential gene for Borrelia burgdorferi that has RNA chaperone activity

Taylor Van Gundy1, Kevin Brandt1, Dhara Patel2, Gavin Chambers2, Bradley Guilliams3, Richard Marconi2 and Meghan C. Lybecker1

1Bacterial Diseases Branch, Division of Vector Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado, 2Virgina Commonwealth University, Department of Microbiology and Immunology, School of Medicine, Richmond, Virgina,3Colorado State University, ARC Core facility, Fort Collins, Colorado

Borrelia (Borreliella) burgdorferi, the causative agent of Lyme disease, transverses two vastly different environments between Ixodes ticks and vertebrates. The spirochete regulates gene regulation as it cycles between its tick vector and vertebrate hosts. Many mechanisms of post-transcriptional gene regulation rely on RNA chaperones, a group of RNA binding proteins that modulate RNA structure. We recently identified proteins with in vitro RNA chaperone activity, including, PlzA, BosR, CheY2 and several uncharacterized proteins including BB0749 in Borrelia burgdorferi. BB0749 accelerates RNA annealing and strand displacement activity in vitro but did not unwind RNA in vivo in a heterologous system. We demonstrate that BB0749 is an essential gene for Borrelia burgdorferi using a conditional knockdown mutant strain. Our data demonstrate that growth rate and cell morphology are impaired in the mutant strain. We are currently identifying the RNA targetome and regulon of BB0749.

14 - Dimitrios Tsitsipais

Age-associated decline in LANCL1-AS1 impairs mitochondrial activity by poly(A) tail shortening of mt-mRNAs in human skeletal muscle

Dimitrios Tsitsipatis1, Jen-Hao Yang1,2*, Elizabeth K. Izydore1, Krystyna Mazan-Mamczarz1, Luigi Ferrucci1, and Myriam Gorospe1

Myogenic regeneration of skeletal muscle preserves physiologic muscle mass and function. Here, we sought to identify long noncoding (lnc)RNAs involved in skeletal muscle regeneration. Cross-sectional analysis of skeletal muscle transcriptomes from healthy, 22- through 83-year-old individuals revealed lncRNA LANCL1-

AS1 among the top declining transcripts. Conversely, LANCL1-AS1 increased robustly during skeletal myogenesis, a cell culture model of muscle regeneration, and promoted myogenesis. Comprehensive identification of proteins by mass spectrometry (ChIPR-MS) revealed an interaction of LANCL1-AS1 with the mitochondrial protein leucine-rich PPR motif-containing protein (LRPPRC), enhancing the formation of chaperone complex LRPPRC-SLIRP, which maintains long poly(A) tails in mRNAs transcribed in mitochondria (mt-mRNAs). Accordingly, old skeletal muscle mt-mRNAs exhibited short poly(A) tails and lower mitochondrial activity, while increasing LANCL1-AS1 levels in primary myoblasts from old non-human primates restored mitochondrial activity and myogenesis. We propose that the age-associated reduction in LANCL1-AS1 contributes to the decline in mitochondrial function in old human skeletal muscle.

15 - Kate Matlin

Understanding the mechanism of eIF3d-mediated selective translation in hypoxia

Kate Matlin, Connor Purdy, Amber Baldwin, Chris Alderman, Rui Zhao, Heide Ford, Neelanjan Mukherjee

Throughout development, cells frequently encounter environmental stressors, such as hypoxia. Cells respond to hypoxia by modulating gene expression, and they can respond quickly to acute stressors by controlling mRNA translation. Cancer cells are particularly well equipped to respond to stress, which can enable them to survive, gain an invasive phenotype, and metastasize more efficiently. We are interested in understanding the mechanism by which cancer cells regulate translation in response to hypoxia so that we can devise means to inhibit it to treat cancer metastasis. Under hypoxia, canonical translation is largely inhibited. Yet, some mRNA translation can still occur through noncanonical pathways, which is critical for cell survival and plasticity but not well understood. Recently, an alternative mRNA cap-binding protein, eIF3d, was found to initiate translation when mTOR was inhibited in stress conditions. eIF3d is a member of a 13-subunit protein complex, eIF3, which plays an essential scaffolding role in translation initiation. We sought to determine the role of eIF3d in our model of acute hypoxic stress. We subjected breast cancer cells to hypoxia and used ribosome profiling in parallel with RNA sequencing to detect changes in translational efficiency. We observed hundreds of changes at the translational level, and few at the transcriptional level, indicating the acute hypoxic response occurs primarily at the translational level. Then, through knockdown of eIF3d, we ablated the translational response, indicating eIF3d is required for the acute translational response. Our current work aims to determine why certain transcripts are selected for eIF3d-mediated translation over others. To do so, we will first determine direct mRNA binders of eIF3d and compare these transcripts to mRNAs translationally regulated by eIF3d. After defining regulated and nonregulated transcripts, we will identify features in the 5' UTR of transcripts in each category, which include secondary structure, upstream open reading frames, alternative start codons, and motifs for RNA binding proteins. Our goal is to develop a predictive model to determine whether a given mRNA transcript will be regulated by eIF3d and later adopt this model to stress conditions to determine which features will make transcripts regulated by eIF3d in stress. Further, we aim to elucidate the mechanism of eIF3d-mediated translation by interrogating the role of functional domains of eIF3d, including its cap-binding domain, and potential other required elements for eIF3d-mediated translation. Ultimately, this research will provide mechanistic insight into the noncanonical translational mechanisms that promote cancer metastasis and offer new avenues for therapeutic intervention.

16 - Ryan Coops

Role of Active Site Adjacent Positively Charged Residues in Maintaining Poliovirus 3C Protease Activity

Ryan Coops, Olve Peersen

Dept. of Biochemistry and Molecular Biology, Colorado State University

Most positive-strand RNA viruses encode their genome in a single open reading frame, translated as a polyprotein. This polyprotein is cleaved into smaller proteins by viral proteases, which allow for the creation of smaller polyprotein intermediates. These intermediates can provide different functionality than terminal proteins that allow for a highly compact genome. In picornaviruses, this process is carried out by the 3C protease, which has a canonical catalytic triad of cysteine, histidine, and an acidic amino acid. In addition to this conserved active site, many 3C proteases include a basic residue placing a positive charge proximal to the active site, though this residue exists in many locations within the protein. We have examined the importance of this residue via mutagenesis studies of Arg 130 in poliovirus 3C protease. Our data suggests that the presence of a positive charge is necessary to maintain efficient cleavage of polyprotein peptide junctions in vitro, and that

arginine is the preferred residue for this activity at most cleavage sites. Similarly, we present data investigating the role of viral RNAs in stimulating the 3C protease, including junction specific activation arising from interactions with the 5' UTR cloverleaf and the internal cis-replication element.

17 - Shannon Hinsdale

Development of the Pin-point™ platform: a versatile base editing technology with broad Cas enzyme and deaminase compatibility ideal for optimized CAR-T generation

Shannon Hinsdale, Robert Blassberg, Paul Russell, Bronwyn Joubert, Olga Mielczarek, Natalia Gurule, Branden Smeester, Ryan Prestil, Anastasia Lomova Kaufman, Jesse Stombaugh, Žaklina Strezoska, Anja van Brabant Smith, Pablo Perez-Duran, Immacolata Porreca, Kevin Hemphill

Revvity, Lafayette, CO

The rise of next generation precision genome engineering tools, including base editing, have led to groundbreaking developments in immunotherapy and chimeric antigen receptor (CAR)-T cell engineering. Revvity's modular Pin-point base editing platform employs delivery of a nickase or deactivated Cas enzyme, a deaminase fused to an aptamer binding protein, and an aptameric guide RNA (gRNA) that act in conjunction to facilitate highly efficient and precise nucleotide conversion. We have developed mRNAs encoding the Cas and deaminase components while synthesizing chemically modified aptamer containing gRNAs capable of inducing highly efficient multiplex knockout of therapeutically relevant loci including: B2M, CD52, TRAC and PDCD1 in primary T and iPS cells. The modular nature of this system enables simultaneous site-specific knock-in of a CAR using aptamer-less gRNA, ideal for a single-step knock-in and knock-out approach without sequential delivery, gRNA crosstalk, or the need of orthogonal Cas enzymes. This approach significantly minimizes the level of indels and translocations found in cells generated from CRISPR-Cas9 mediated knockout, of particular advantage to iPSCs which are known for their high sensitivity to gene editing.

Because most pathogenic SNVs remain unreachable with published base editing systems, we then looked to further expand the reach of target nucleotide conversion by optimizing different components of the system for further target accessibility. We demonstrate that through use of additional deaminases, including the mutant Anolis APOBEC, and additional Cas enzymes, including the engineered deactivated Cas12f (dCasMINI), we can now target sites previously unattainable through nCas9 mediated base editing. Additional optimization of aptameric placement at different positions within the gRNA can further tailor the systems editing window, reducing the occurrence of bystander edits where desired. Using an arrayed screening platform and functional reporter assay developed for high-throughput study of gRNA function and design, we have started to further characterize these novel Pin-point configurations to streamline additional therapeutic pipeline development.

18 - Lukasz Olenginski

Expanding the druggable genome: targeting RNA with small molecules

Lukasz T. Olenginski and Robert T. Batey

The vast majority of clinically approved small molecule drugs target proteins. However, only a small percentage of proteins (~3.5%) have been successfully drugged, highlighting the need for new therapeutic directions. One popular alternative is to target RNA with small molecules, which offers the opportunity to modulate disease-related processes with the potential to vastly increase the druggable genome. However, success in the development of RNA-targeting small molecule therapeutics has been limited, in part because many of the basic principles governing RNA-ligand interactions are poorly understood. Fortunately, Nature has provided a robust system to study the details of RNA-small molecule recognition: the riboswitch. These bacterial mRNA elements bind a diverse assortment of small molecule metabolites and are therefore ideal models to explore the druggability of RNA. For example, recent work from the Batey group has identified a set of modified cobalamins (Cbls) that productively bind a methylCbl-selective riboswitch and regulate in-cell function. These Cbl derivatives host chemically distinct groups at their β-axial position that confer different RNA binding affinities. Interestingly, chemical probing data suggest that the recognition of these ligands likely involves the displacement of a nucleotide from the RNA core, a binding mechanism common to other medically relevant RNA-targeting lead compounds. To explore the chemical features that promote specific and high affinity displacement RNA binding modes, I synthesized an expanded library of novel Cbls with systematically varied β-axial moieties and quantified their binding to a representative methylCbl-selective riboswitch. These

data were then modeled to build a predictive and quantitative structure-activity relationship to reveal which chemoinformatic descriptors correlate with high affinity RNA binding. In addition, I solved the crystal structure of multiple RNA-small molecule complexes that further inform the design of future ligands. Together, the results from these studies will provide quantitative metrics for predicting the effect of different chemical modifications on displacement RNA binding interactions and high-resolution structures to visualize these principles in atomic detail. As such, this work can enable researchers to rapidly curate chemical space in lead compound identification and optimization.

19 - JP Ouyang

Transcriptional bursting underlies RNA repeat foci formation

John Paul Tsu Ouyang, Roy Parker

Repeat expansions of short nucleotide sequences underlie over 40 neuromuscular diseases. Common to many of these diseases is the accumulation of the transcribed repeat RNA into microscopically discernible foci. Previous analysis of the dynamics of these foci have suggested that they form through coalescence of individual RNAs into larger assemblies. Using quantitative single molecule RNA analysis in a patient-derived myotube model for the Myotonic dystrophy type 1 (DM1) CUG repeat expansion, we determined the number of RNAs within DM1 nuclear foci. We find that the majority of these foci are actually comprised of single RNAs. We find that rare foci can contain upwards of 25 distinct RNA species. However, we provide evidence that foci containing multiple RNAs are formed from transcriptional bursting and dissociate with time. Our data argue against a coalescence model of repeat foci formation and show that transcriptional bursting underlies RNA repeat foci formation.

20 - Victor Ruthig

Alternative Splicing Alters the Female and Male Germ Cell Splice-ome at Transition Points in Germ Cell Development

Andrew Yarilin1,2 and Victor A. Ruthig1

1Developmental Biology Section, Pediatrics Department, University of Colorado Anschutz Medical Campus, Aurora, CO, 2Brooklyn Technical High School, Brooklyn, NY, USA

During development alternative splicing, via isoform switching, influences differentiation through splice-ome shifts. In the reproductive tract the splice-ome is dynamic during adult male meiosis, spermatogenesis. However, less is known about the splice-ome of fetal germ cells and female meiosis, oogenesis. Using RNA immunoprecipitation sequencing (RIP-seq), we recently reported the germ cell specific RNA binding protein DND1 as a regulator of splicing genes, including Sf3b2. DND1 level in male germ cells also correlates with the level of the splicing regulator Rbfox2. We analyzed differentially spliced genes (DSGs) in our male germ cell transcriptome RNA-seq datasets using IsoformSwitchAnalyzeR. DSG analysis found drastic splice-ome changes (1,121 genes) as male germ cells enter cell cycle arrest. During arrest the splice-ome seems static (18 genes). We previously established female germ cell and male germ cell DND1 expression is negatively correlated with meiotic entry. We then used IsoformSwitchAnalyzeR to re-analyze published RNA-seq datasets on female and male meiosis. We identified switches in the female germ cell splice-ome at sex determination (121 genes) and a larger switch at meiotic entry (403 genes). The largest female germ cell shift in splicing occurred late in meiosis I (1,737 genes) with a sudden drop in splice-ome changes as female germ cells pause in meiotic diplotene (0 genes). In female and male meiosis, pathways related to differentiation, epigenetic regulation, cell cycle, and metabolism were affected specifically by splice-ome changes. Functionally many isoform switches in both sexes altered transcript coding potential and receptiveness to non-sense mediated decay. Collectively, our data indicate female and male germ cells successfully differentiate during development partly through switches in alternative splicing.

Study supported by the Lalor Foundation fellowship, NIH NIGMS F32-GM129956, Duke Trent Memorial Fund, and Theresa Dow Wallace Fund of the New York Community Trust to VAR; and Brooklyn Technical High School Weston Scholarship to AY.

21 - Charlie Moffatt

TDP43 prevents RNA accumulation in neurites

Charlie Moffatt, Ankita Arora, Bryan Guzman, Roberto Castro Gutierrez, Holger Russ, Daniel Dominguez, Matthew Taliaferro

Localization of RNA molecules to subcellular locations enables spatially restricted processes. While hundreds of RNA transcripts are trafficked to neuronal projections, the mechanisms underlying their localization are largely unknown. Mutations in TDP-43, an RNA-binding protein known to regulate RNA metabolism, are linked to amyotrophic lateral sclerosis (ALS). TDP-43 is implicated in the control of neuronal RNA trafficking. However, transcripts dependent on TDP-43 for transport are largely unknown and how TDP-43 recognizes those RNAs remains unclear. Using inducible TDP-43 knockout neuronal cells, subcellular fractionation, and high-throughput sequencing, we interrogated soma and neurite RNA populations in the presence and absence of TDP-43. In contrast to previous reports, we found that TDP-43 inhibits RNA accumulation in neurites. Many RNAs were significantly more abundant in neurites in knockout cells compared to wildtype, while their soma abundance remained unchanged. These mislocalized RNAs were significantly enriched for TDP-43 binding motifs and CLIP-defined binding sites in their 3' UTRs. Using a massively parallel reporter assay, we identified discrete elements within these 3' UTRs sufficient for TDP-43-dependent RNA mislocalization. These elements frequently overlapped established TDP-43 binding sites. Using a high-throughput RBP/RNA interaction assay, we found that TDP-43 strongly bound these RNA elements in vitro. Mutating TDP-43 sequence motifs in these elements abolished both in vitro binding and TDP-43-dependent RNA localization, supporting the model that loss of TDP-43 binding causes accumulation of its target RNAs in neurites. These results suggest a role for TDP-43 in the misregulation of axonal RNA metabolism often seen in ALS models.

22 - Gabriel Galindo

Not submitted

23 - Kathryn Walters

Determining the elusive mechanism of gene regulation by Musashi-2

Kathryn Walters1, Amber Baldwin1, and Neelanjan Mukherjee1

1RNA Bioscience Initiative, Department of Biochemistry and Molecular Genetics, Molecular Biology Program, University of Colorado School of Medicine, Aurora, Colorado.

RNA-binding proteins (RBPs) are proteins that are involved in post-transcriptional regulation of gene expression and important cellular processes such as cell differentiation and metabolism. RBPs exert post-transcriptional control by interacting with specific elements within target mRNAs and often recruit proteins/complexes that help to regulate the fate of the target RNA. Here we focus on the RBP, Musashi-2 (MSI2), which binds to the UAG sequences in the 3'UTR of its target transcripts. MSI2 plays an important role in stem cell identity, cell fate, and cell cycle regulation as well as a role promoting a variety of cancer types such as myeloid leukemia and breast cancer. The molecular mechanism by which MSI2 regulates target RNA translation and/or decay is unknown. Moreover, whether MSI2 acts as a repressor or activator appears to be context dependent. Here we find that MSI2 acts as a translational activator. We also identify a region in MSI2 which is essential for this function as a translational activator. Finally, we explore these findings in multiple cell lines and find little support for context dependent regulation. Instead, we find that MSI has isoform specific transcript regulation. This work will impact our understanding of fundamental principles of RBP-mediated regulation, as well as novel mechanisms underlying MSI2 regulatory function.

24 - Lily Beck

Characterizing the Nucleic Acid Binding Properties of ZBP1's N-terminus

Lily Beck, Jeff Krall, Parker Nichols, Quentin Vicens, Morkos Henen, Beat Vögeli

Detection of nucleic acids that are present in atypical conformations or subcellular locations is an important function of the cell as it can trigger the innate immune response. Human Z-DNA binding protein 1 (ZBP1)

specifically recognizes the alternative Z-form of nucleic acids as a sign of infection, and in turn it activates interferon (IFN) regulatory factors and downstream pathways that induce necroptosis of infected cells and trigger inflammation. At the start of this project, the ability of ZBP1 to bind and flip Z-DNA was well characterized, whereas there was limited knowledge about what it could do with RNA. In our experimental contexts, we were able to observe that ZBP1 can flip B-DNA, but not A-RNA, to the Z-conformation.

25 - Michael Cortázar

Feedback from mRNA decay to the transcriptional machinery

Michael A. Cortázar1 and Sujatha Jagannathan1, 2

1Department of Biochemistry and Molecular Genetics, University of Colorado Anschutz Medical Campus, Aurora, Colorado.
2RNA Bioscience Initiative, University of Colorado Anschutz Medical Campus, Aurora, Colorado.

Premature termination codons (PTCs) generally induce the degradation of mRNAs via the quality control mechanism nonsense-mediated mRNA decay (NMD), which prevents the production of potentially toxic truncated proteins. Intriguingly, it has also recently been proposed that activation of NMD can induce transcriptional upregulation of genes related in sequence to the PTC-containing genes to achieve a functional transcriptional compensation. This phenomenon has been termed Nonsense-Induced Transcriptional Compensation (NITC).

Whether mRNA decay triggers a global and/or a gene-specific transcriptional adaptation response remains unknown. To answer this question, I incorporate PTCs at the clinically relevant LMNA gene (encoding the Lamin A/C proteins) using CRISPR and directly measure transcriptional activity genome-wide by metabolic labeling of nascent transcripts via Bru-Seq. Strikingly, I found that LMNA is transcriptionally upregulated in response to the integration of PTCs in both mammalian HEK293 and myoblast MB135 cells consistent with NITC. Furthermore, PTCs in the LMNA gene resulted in transcriptional upregulation of the sequence-related LMNB2 gene, not simply explained by a genome-wide increase in transcriptional activity, suggesting a gene-specific response. Given that the PTC position along the mRNA is known to influence NMD activity, I investigated whether the position of the PTC along the LMNA gene can also influence the degree of this transcriptional upregulation. To this end, I used CRISPR to incorporate a pool of PTCs at different exons of the LMNA gene via non-homologous end-joining DNA repair. Surprisingly, PTCs in exons located towards the 3' end of the mRNA strongly induce transcriptional upregulation. To study the effect of position and identity of PTCs (UAA, UAG, and UGA stop codons) more specifically, I use Saturation Genome Editing (SGE) at specific exons of the LMNA gene. This approach involves homology-directed repair to generate a library of specific mRNA variants from chromatin in a population of cells. I validated SGE for the study of chromatin-derived PTCs by generating all possible LMNA mRNA variants with single PTCs between codons 626 and 656 (n=93 variants), a region of the LMNA gene that includes the site for deposition of the NMD-relevant last exon junction complex (EJC). SGE successfully captured the expected drop in NMD activity at the last EJC, and a more nuanced variability in NMD activity across NMD-sensitive variants. I am currently measuring variability in transcriptional upregulation across these variants and the role of the EJC in transcriptional upregulation. Finally, I am investigating the potential of NITC to induce transcriptional upregulation of multiple genes using measurements of transcriptional activity (Bru-Seq) and a splice-switching drug, risdiplam, that incorporates PTCs at a subset of risdiplam-sensitive genes. Importantly, confirmation and understanding of this mechanism might open novel avenues for future RNA therapies aiming to enhance gene expression of specific genes to suppress disease phenotypes.

26 - Neel Mukherjee

Ribosome Profiling Reveals Translational Reprogramming and mTOR Activation in Omacetaxine Resistant Multiple Myeloma

Zachary J. Walker1, Katherine Vaeth2,3, Amber Baldwin2,3, Denis J. Ohlstrom4, Lauren T. Reiman1, Beau M. Idler5, Brett M. Stevens1, Daniel W. Sherbenou*1,6, Neelanjan Mukherjee*2,3,6

Affiliations: 1Division of Hematology, Department of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA. 2Department of Biochemistry and Molecular Genetics, University of Colorado Anschutz Medical Campus, Aurora, CO, USA. 2RNA Bioscience Initiative, University of Colorado Anschutz Medical Campus, Aurora, CO, USA. 3Department of Medicine, Division of Medical Oncology, University of Colorado Anschutz Medical Campus, Aurora, CO. 4School of Medicine, Emory University, Atlanta, GA. 5Mayo Clinic Alix School of Medicine, Scottsdale, AZ. 6University of Colorado Cancer Center, University of Colorado Anschutz Medical Campus, Aurora, CO, USA.

Protein homeostasis is critical to the survival of multiple myeloma (MM) cells. While this is targeted with proteasome inhibitors, mRNA translation inhibition has not entered trials. Recent work illustrates broad sensitivity MM cells to translation inhibitor omacetaxine. We hypothesized that understanding how MM cells become omacetaxine resistant will lead to the development of drug combinations to prevent or delay relapse. We generated omacetaxine resistance in H929 and MM1S MM cell lines and compared them to their parental lines. Resistant lines displayed decreased sensitivity to omacetaxine, with EC50 > 100 nM, compared to parental line sensitivity of 24-54 nM. To adapt to omacetaxine, H929 and MM1S exhibited an increased percentage of multi-nucleated polyaneuploid cells that led to distinct molecular mechanisms of resistance Interestingly, both resistant lines showed a defect in oncologic potential via extended survival in a MM xenograft model. Since omacetaxine inhibits protein synthesis, we performed both RNA-sequencing and ribosome profiling (Ribo-seq) to identify shared and unique regulatory strategies of resistance. Transcripts encoding translation factors and containing TOP motifs in their 5' UTR were translationally upregulated in both resistant cell lines. The mTOR pathway promotes the translation of TOP motif containing mRNAs. Indeed, mTOR inhibition restored partial sensitivity to omacetaxine in both MM1S and H929 cells. Primary MM cells from patient samples were sensitive to combinations of omacetaxine and mTOR inhibitors rapamycin and Torin 1. These results provide a rational approach for omacetaxine-based combination in patients with multiple myeloma, which have historically shown better responses to multi-agent regimens

27 - Joelle Lo

Hnrnpa2/b1 regulates motor protein RNA localization and activity in neurites

Joelle Lo1, Lisa Wood2, Jeffrey K Moore2, J. Matthew Taliaferro1,3

1 Department of Biochemistry and Molecular Genetics, University of Colorado Anschutz Medical Campus, 2 Department of Cell and Developmental Biology, University of Colorado Anschutz Medical Campus, 3 RNA Bioscience Initiative, University of Colorado Anschutz Medical Campus

RNA localization is a post transcriptional regulatory process that enables the spatial and temporal control of gene expression. Although thousands of RNAs are known to be asymmetrically localized, the mechanisms underlying their transport remain unknown.

hnRNPA2B1 is an RNA binding protein involved in a variety of post transcriptional processes including RNA localization. However, it is not known what RNAs hnRNPA2B1 targets, how those targets are recognized and the contribution of proper localization of these RNAs to cell function. To study this, we created CAD mouse neuronal cell lines that had hnRNPA2B1 knocked out and a rescuing transgene that can be inducibly expressed

with doxycycline. Next, we sought to identify the RNA targets of hnRNPA2B1 by comparing subcellular RNAs in the soma and neurite fractions of these cells. We found that hnRNPA2B1 loss resulted in a large neurite specific increase in RNA levels for 301 genes. These RNAs were also significantly enriched for CLiP peaks and known 6mer sequences bound by hnRNPA2/B1. Among this group were the RNAs that encode for cytoskeletal transport proteins including kinesins and dyneins. To further understand how hnRNPA2B1 regulates these RNAs, we rescued knockout cells with a mutant hnRNPA2B1 that contained three R to A point mutants in the RGG domain, which resulted in the protein adopting a more cytoplasmic distribution. Interestingly, the RNAs previously identified as more neurite enriched in knockout cells became less neurite enriched in the RGG mutant rescue compared to the wildtype rescue, likely due to increased cytoplasmic hNRNPA2/B1 abundance. Next, we wanted to explore how mislocalization of motor protein encoding RNAs in knockout cells impact their function using live cell imaging to monitor lysosome transport. We found that both the speed and distance traveled by lysosomes was significantly impaired in KO cells compared to wildtype rescue cells. These results establish Hnrnpa2b1 as a negative regulator of neurite RNA accumulation, indicate the role of the RGG domain in controlling this effect, and demonstrate that RNA mislocalization can lead to defects in microtubule-based motor protein activity.

AFTERNOON POSTER SESSION

28 - Marisa Foster

A RelBbu-dependent small antisense RNA regulates expression of the glp operon

Marisa Foster, Laura Hall, Dan Drecktrah, Taylor Van Gundy, Kevin Brandt, Julia Hilliard, D. Scott Samuels, and Meghan C. Lybecker

Bacterial Diseases Branch, Division of Vector Borne Diseases, US Centers for Disease Control and Prevention, Fort Collins, Colorado, University of Montana, Missoula, Montana, Colorado State University, Fort Collins, Colorado

Borrelia (Borreliella) burgdorferi, the etiological agent of Lyme disease, is maintained in its natural vertebrate reservoir, usually small mammals, using Ixodes ticks as a vector. The spirochete utilizes different carbohydrates as carbon and energy sources as it transits this enzootic cycle between its vertebrate host and tick vector. Glycerol, a sugar alcohol, is important for B. burgdorferi survival in the tick midgut. B. burgdorferi shuttles glycerol into glycolysis using glycerol uptake facilitator (GlpF) to transport glycerol into the cell, glycerol kinase (GlpK) to phosphorylate it, and glycerol-3-phosphate dehydrogenase (GlpD) to oxidize it to dihydroxyacetone phosphate. The genes encoding these proteins constitute the glp operon. Both RelBbu, the RelA/SpoT homolog that controls the stringent response and is required for survival in the tick, and PlzA, the only known c-di-GMP binding protein in B. burgdorferi, regulate expression of the glp operon. We identified a RelBbu -dependent small RNA encoded opposite to the glpF open reading frame, termed antisense glpF (as-glp). The glpF mRNA and asRNA are reciprocally regulated by RelBbu, suggesting a potential mechanism of regulation via the asRNA. To determine the role of as-glp in regulation of the glp operon, we generated an as-glp mutant strain and assayed glp operon expression. Our data suggest that the structure and regulation of this operon is considerably more complex than previously recognized.

29 - Johnathon Schafer

Myelodysplatic Syndrome-Associated Splicesome Mutations' Impact on the Host Defense Response

Myelodysplastic Syndrome (MDS) is a hematopoietic stem cell disorder that has the potential for progression to Acute Myeloid Leukemia (AML). MDS is characterized by somatic mutations in hematopoietic stem cells that disrupt the generation and function of mature myeloid cells. There are several risks associated with MDS including increased susceptibility to infection. Conserved somatically acquired mutations in components of the pre-mRNA splicing machinery are commonly found in MDS patients. In our lab, we have utilized mouse models expressing MDS-associated spliceosome mutations and found that the mutation U2AF1-S34F induces defective neutrophil migration to the peritoneum during infection, resulting in lack of control of infection. I am investigating both a second mouse model that expresses another RNA splicing mutation, SF3B1-K700E, and looking at genotyped human PBMCs from MDS patients to determine if this mechanism or other mechanisms of immunodeficiency are present across other MDS genotypes.

30 - Parker Nichols

How Do Right-Handed Helices Become Left-Handed and What is Their Role In A-to-I Editing by ADAR1

Parker J. Nichols, Jeffrey B. Krall, Morkos A. Henen, Robb Welty, Quentin Vicens, and Beat Vögeli

The left-handed Z-conformation of nucleic acids can be adopted by both DNA and RNA when bound and stabilized by Zα domains found within a variety of innate immune response proteins, including Adenosine Deaminase Acting on RNA (ADAR1). The Zα domain of ADAR1 is important for upregulating A-to-I editing during the interferon response. How the Zα domain promotes increasing editing is poorly understood. We made mutations to the Zα domain of ADAR1 which prevents its ability to stabilize Z-conformations and measured A-to-I editing through RNA-seq. We see little impact to sparse editing events, however, hyper-editing is significantly decreased. Through a combination of Nuclear Magnetic Resonance and biophysical measurements, we develop a model by which right-handed helices must first "melt" before transiently sampling the left-handed Z-conformation, which is then recognized and stabilized by Zα domains. Our results yield some insight into what contexts Z-conformations would be expected to be adopted in the cell, and what their function may be in the innate immune response.

31 - Lauren Malsick

Unveiling the bat signal: How flavivirus sfRNAs affect bat cell innate immune responses

Lauren Malsick, Amanda Bartels, and Brian Geiss

Microbiology, Immunology, and Pathology Department at Colorado State University

Flaviviruses cause significant morbidity and mortality across the globe and include mosquito-borne pathogens such as dengue (DENV), West Nile virus (WNV) and Zika (ZIKV). Many species of bats can be infected with flaviviruses as determined by serological, molecular, or virus isolation evidence, but bats typically do not develop severe disease due to infection. Why bats are infectable by these important human pathogens but do not get disease is unclear. Bats are vectors for a number of human viral pathogens, and definition of how bats interact with and control the viruses they encounter in nature will help clarify their role in viral transmission cycles between different species and their potential as reservoirs for future spillover events.

Subgenomic flaviviral RNAs (sfRNAs) have been recently detected in wild bats captured in Uganda. Flaviviral sfRNAs are produced through exoribonuclease 1 (XRN1) stalling on the highly structured 3' untranslated region (UTR) of the flavivirus genome. Detection of sfRNAs in wild bats indicates that flavivirus genomes interact with the bat RNA decay pathway as they do in humans. Flavivirus sfRNAs produced in human and mouse infection are associated with suppression of host immune responses and increased host pathogenesis, but because bats do not show disease we hypothesize that flavivirus sfRNAs may interact with bat cells differently than human cells. The role of the RNA decay pathway in controlling viral infection in bat cells and impact of flavivirus sfRNAs in bat infection has not been investigated and represents an interesting new area of inquiry.

We have determined that infection of primary Artibeus jamaicensis bat cells (AJ6) with West Nile virus induced strong innate immune responses, suggesting similar responses to infection with human cells. We will discuss our ongoing work examining the effect of sfRNAs on West Nile virus infection and how sfRNAs affect innate immune responses in bat cells, including characterization of sfRNA species in bat cells, how disruption of sfRNA structures alters viral replication, and how sfRNA disruption affects host gene innate immune response gene expression. The results presented represent the first exploration of the role of bat RNA decay pathway and its interaction with viral genomes during viral infections.

32 - Luis Aguilera

Exploring Transcription Dynamics through Spatial Stochastic Modeling

Alison Shad, Jack Forman, Eric Ron, Linda Forero-Quintero, Luis Aguilera, and Brian Munsky.

In the mammalian nucleus, RNA synthesis, diffusion, and degradation are governed by complex stochastic interactions. Traditional modeling efforts have focused on transcriptional kinetics, neglecting RNA temporal and spatial variation. Here, we aim to study the spatial dynamics of RNA near transcription sites (TS) alongside

the mechanisms governing its diffusion and degradation. We hypothesize that RNA clusters more densely near TS, forming brighter spots observable via fluorescent probes, which diminish in intensity with increasing distance from the TS due to RNA degradation. By integrating single-cell experimental data and spatial stochastic models, we aim to generate a more complete model of gene expression. Our preliminary results show that the RNA abundance and brightness are increased around the TS, suggesting a clustering effect.

33 - William Scott Raymond

Identification of potential riboswitch elements in Homo Sapiens mRNA 5'UTR sequences using Positive-Unlabeled machine learning

Riboswitches are a class of noncoding RNA secondary structures that interact with a target ligand to induce a conformational change. These changes are regularly used for regulatory purposes within a cell's transcriptome and translatome. Riboswitches are ubiquitous and well characterized in bacteria and prokaryotes, with some examples also being described in certain eukaryotes: fungi, plants, and yeast. To date, no solely RNA-small molecule riboswitch has been discovered in Homo Sapiens. Several analogous riboswitch-like mechanisms have been described within the H. Sapiens translatome as well as other regulatory RNA mechanisms with greater and less complexity prompting an open question: Is there a H. Sapiens riboswitch dependent on only small molecule ligands? In this work, we set out to train Positive-Unlabeled (PU) machine learning classifiers on known riboswitch sequences. We then applied an ensemble classifier to H. Sapiens mRNA 5'UTR sequences taken from the 5'UTR database, UTRdb, in the hope of identifying a set of mRNAs to investigate for riboswitch functionality. 67,683 riboswitch sequences were obtained from RNAcentral and sorted for ligand type and used as positive examples and 48,031 5'UTR sequences were used as unlabeled, unknown examples. 20 positive-unlabeled classifiers were trained on sequence and secondary structure features while withholding one or two ligand classes from the positive examples. Cross validation was then performed on the withheld ligand sets to obtain a validation accuracy range of 75\%-99\% across the 20 classifiers. The joint sets of 5'UTRs identified as potential riboswitches by all 20 classifiers were then stored and analyzed. 15333 sequences were identified as a riboswitch by one or more classifier(s) and 436 of the H. Sapiens 5'UTRs were labeled as harboring potential riboswitch elements by all 20 classifiers. These 436 sequences were mapped back to the most similar riboswitches within the positive data and examined. An online database of identified and ranked 5'UTRs, their features, and their most similar matches to known riboswitches, are provided to guide future experimental endeavors to identify H. Sapiens riboswitches.

34 - Ella Tommer

DNA methyltransferase 1 (DNMT1) and its possible regulation by RNA

Ella Tommer, Linnea Jansson-Fritzberg, Jessica Song, Camila Sousa, Eliza Lee, Vignesh Kasinath, Tom Cech

DNMT1 is the main mammalian maintenance methyltransferase, tasked with maintaining DNA methylation patterns throughout somatic cell division. Methylation fidelity is crucial for cellular health and identity. Thus, this work aims to elucidate how DNMT1's activity is regulated in cells. Many previous studies done on DNMT1 have used truncations lacking parts of the N-terminal domains (amino acids 1-621) for ease of expression and purification. We find that N-terminal truncations greatly reduce the affinity of DNMT1 for hemi-methylated DNA (Figure 1A) and ablate its ability to methylate hemi-methylated DNA (Figure 2). This could indicate the N-terminal domains are playing a mechanistic role involving product release, anchoring, and/or scanning for substrates. Given that previous literature has described DNMT1 as a processive enzyme, we compared the activity of the full-length enzyme versus the truncations on a hemi-methylated DNA substrate with varied number of hemi-methylated CpG sites (Figure 3, 4). While the 351 truncation's methylation activity plateaus at a lower level than full-length, its overall pattern of activity mimics the full-length enzyme's (the greatest activity was in the reaction with the 3 CpG substrate, second greatest in the reaction with the 7 CpG substrate etc., Figure 4A-C). This may indicate that the RFTS domain (amino acids 351-597) is involved in whatever process governs DNMT1's substrate preference, while the low-complexity domain (amino acids 1-351) could be involved in substrate binding and/or product release.

Many reports have described DNMT1 binding to RNA. However, a systematic test of RNA sequence and structure motifs found DNMT1 binds most tightly to a non-canonical G-quadruplex forming transcript made of poly-(UG) repeats, called a pUG fold. Previously published work in the lab established that pUG fold binding is competitive for hemi-methylated DNA binding (Figure 5C), despite binding data with truncated enzymes indicating that DNA is primarily bound to residues in the N-terminal while pUG fold RNA is bound to residues

in the C-terminal (Figure 1). Additionally, pUG fold RNA binding inhibits the methyltransferase activity of DNMT1 (Figure 5A-B). Given that the KDapp for pUG fold RNA is ~10-fold lower than the KDapp for hemi-methylated DNA, this interaction seems probable in cells. Taken together, our preliminary data indicate DNMT1 may be regulated by pUG fold RNA in vivo. Indeed, it was recently established that pUG fold transcripts play a signaling role in the C. elegans RNAi pathway.

35 - Daniella Ugay

Hemotopoietic Transcription Factor GATA1 is a Non-Canonical RNA-Binding Protein

Daniella A. Ugay, Robert T. Batey, Deborah S. Wuttke

A growing number of global unbiased RNA-binding protein discovery studies and systematic surveys suggest that many diverse families of transcription factors (TFs) are capable of binding RNA in cells. By mining publicly available databases, we have found GATA-family TFs as a potential target of direct RNA binding. Recently, GATA1 and GATA2 TFs have been shown to UV-crosslink with RNA in cells. While these observations reveal a potential RNA binding activity of GATA-family TFs, the scope and functional relevance of these RNA interactions have yet to be determined. Motivated by these observations, we have set out to investigate RNA binding of GATA1 TF, which is the founding member of the family and mater regular of hematopoiesis. We have found that GATA1 is capable of binding RNA in vitro through its DNA-binding domain. Unlike sequence-specific DNA binding, GATA1 is selective toward structural RNA elements. Additionally, DNA and RNA binding is mutually exclusive, suggesting the existence of overlapping binding surfaces for DNA and RNA. Collectively, these data strongly support the in vivo crosslinking experiments showing that GATA1 binds RNA directly and competitively with DNA, thus challenging the previously proposed model for the simultaneous binding of DNA and RNA to a TF.

36 - Thomas Forman

PDGFRalpha signaling regulates Srsf3 transcript binding to affect PI3K signaling and endosomal trafficking

Thomas E. Forman, Marcin Sajek, Eric D. Larson, Neelanjan Mukherjee, and Katherine A. Fantauzzo

Signaling through the platelet-derived growth factor receptor alpha (PDGFRalpha) plays a critical role in craniofacial development, as mutations in PDGFRA are associated with cleft lip/palate in humans and Pdgfra mutant mouse models display varying degrees of facial clefting. Phosphatidylinositol 3-kinase (PI3K)/Akt is the primary effector of PDGFRalpha signaling during skeletal development in the mouse. We previously demonstrated that Akt phosphorylates the RNA-binding protein serine/arginine-rich splicing factor 3 (Srsf3) downstream of PI3K-mediated PDGFRalpha signaling in mouse embryonic palatal mesenchyme (MEPM) cells, leading to its nuclear translocation. We further showed that ablation of Srsf3 in the murine neural crest lineage results in severe midline facial clefting, due to defects in proliferation and survival of cranial neural crest cells, and widespread alternative RNA splicing (AS) changes. Here, we sought to determine the molecular mechanisms by which Srsf3 activity is regulated downstream of PDGFRalpha signaling to control AS of transcripts necessary for craniofacial development. We demonstrated via enhanced crosslinking and immunoprecipitation (eCLIP)-seq of MEPM cells that PDGF-AA stimulation leads to preferential binding of Srsf3 to exons and loss of binding to canonical Srsf3 CA-rich motifs. Through the analysis of complementary RNA-seq data, we showed that Srsf3 activity results in the preferential inclusion of exons with increased GC content and shorter intron to exon length ratio. Moreover, we found that the subset of transcripts that are bound by Srsf3 and undergo AS upon PDGFRalpha signaling commonly encode regulators of PI3K signaling and early endosomal trafficking. Functional validation studies further confirmed that Srsf3 activity downstream of PDGFRalpha signaling leads to retention of the receptor in early endosomes and increases in downstream PI3K-mediated Akt signaling. Finally, we generated an Srsf3 phosphomutant knock-in allele (Srsf3A7) through mutagenesis of the seven Akt consensus motifs in Srsf3 and demonstrated that trans-heterozygous Srsf3A7/fl;Wnt1-Cre+/Tg embryos develop severe midline facial clefting. Taken together, our findings reveal that growth factor-mediated phosphorylation of an RNA-binding protein underlies gene expression regulation necessary for mammalian craniofacial development.

37 - Boyoon Yang

Dissecting molecular mechanisms of dsRNA recognition and A-to-I editing of RNA editing enzymes in vivo

Boyoon Yang, Heather Hundley

All animals generate transcriptomic diversity by modifying genetic information through RNA editing. A-to-I RNA editing is mediated by Adenosine deaminases that act on RNA (ADARs). ADARs convert specific adenosines into inosines in double-stranded RNA (dsRNA). ADARs have a high potential as therapeutic means to correct specific G-to-A mutations at the RNA level without modifying the genome. Therefore, it is crucial to understand how ADARs bind specific transcripts and select an individual adenosine for editing in vivo.

In Caenorhabditis elegans, ADR-2 is the sole editing enzyme with weak dsRNA binding affinity, whereas ADR-1 is an editing-deficient cofactor which provides dsRNA binding affinity via the dsRNA binding domain (dsRBD) and recruits ADR-2 to most ADR-2 targets. While in vitro studies have shown that ADARs bind dsRNA in a structure-dependent manner, whether a given dsRBD recognizes specific sequences or structures in vivo is poorly understood. To identify binding sites of ADARs, we performed enhanced crosslinking immunoprecipitation (eCLIP) coupled to high-throughput sequencing. Herein, we identified in vivo binding sites of the first dsRBD of ADR-1, most of which occurs in 3' UTRs. Surprisingly, the vast majority of ADR-2 binding event occur within intronic regions, suggesting that ADR-1 and ADR-2 may exhibit different substrate selectivity. Elucidating the mechanism of how ADR-1 and ADR-2 co-occupy target mRNAs will provide new insights into how A-to-I editing is mediated in vivo.

38 - Grace Gustafson

Leaky scanning as a developmental buffering mechanism.

Grace Gustafson, Raisa Bailon-Zambrano, Abi Mumme-Monheit, Juliana Sucharov, and James T. Nichols

Deleterious mutations can display a range of severity among individuals. Differences in severity are likely due to differences in overcoming or buffering against the mutation. This phenomenon of buffering has been accepted as a biological concept for decades. Recently, a molecular mechanism underlying buffering was discovered. Individuals with the same mutation may vary in severity, assumedly due to their own unique genetic backgrounds. Transcriptional adaptation through nonsense-mediated decay has recently become a growing topic of study due to the molecular mechanism of compensatory gene upregulation to protect against premature termination codons introduced in a single gene. This mechanism involves recognition and digestion of an mRNA containing an early stop codon, to prevent a more severe phenotype. This mechanism, however, is confined to nonsense mutations; mutations of other character exist that do not undergo transcriptional adaptation but do present less severe phenotypes than expected. Here we show one such allele with a mutation in the initiating methionine that was predicted to produce no protein, yet it unexpectedly produces a very mild phenotype. We propose a novel mechanism of buffering against initiating methionine mutant alleles. We discovered that this initiating AUG to CUG mutation produces protein. We also identified in-frame downstream AUGs, that could provide alternative start sites for translation of a protein. We seek to determine if this protein is from downstream AUG usage, or from the CUG, which has been described in other systems. We also discovered that the mutant produces higher relative mRNA abundance than wild-type siblings, either due to increased stability or higher expression levels. Thus, this allele may be buffered by a new alternative mechanism, upregulating mRNA, in contrast to downregulation of mutant mRNA by nonsense mediated decay. Buffering has historically been understood only by characterizing phenotypic severity, however we now are able to investigate mechanisms on a molecular level. This novel mechanism is likely applicable across biological systems with similar mutations.

39 - Kaitlyn Armijo

Stress-responsive membraneless organelles orchestrate the immune response in macrophages

Kaitlyn S. Armijo1*, Sikander Azam1, Chi G. Weindel1, Morgan J. Chapman1, Alice Devigne2, Shinichi Nakagawa3, Tetsuro Hirose4, Susan Carpenter2, Robert O. Watson1, Kristin L. Patrick1

1Department of Microbial Pathogenesis and Immunology, Texas A&M University, School of Medicine, Bryan, TX 77807 USA.
2Department of Molecular, Cell, & Developmental Biology, University of California, Santa Cruz, Santa Cruz, CA 95064 USA.
3RNA Biology Laboratory, Hokkaido University, Sapporo 060-0812 Japan.
4RNA Biofunction Laboratory, Graduate School of Frontier Biosciences, Osaka University, Osaka 565-0871 Japan.

Through sequestration and compartmentalization of proteins and nucleic acids, membraneless organelles (MLOs) play a pivotal role in organizing the cytoplasm and nucleus of cells. Nuclear paraspeckles are stress-responsive MLOs that form on the arcRNA Neat1. Although we know that paraspeckles aggregate in response to stresses like changes in osmolarity, temperature, and mechanical stress, their ability to respond to more physiologically relevant environmental cues remains understudied. Macrophages are innate immune cells that have evolved to sense and respond to a variety of microbes. We hypothesized that the paraspeckle may help the macrophage reorganize its nucleus upon pathogen sensing to upregulate inflammatory gene expression. In a recent study, we reported that macrophage paraspeckles rapidly aggregate (0.5 h poststimulation) and disaggregate (2 h poststimulation) in repones to lipopolysaccharide (LPS), a component of the outer membrane of gram-negative bacteria. We also found that macrophages lacking paraspeckles (Neat1 KO) fail to properly express a large cohort of proinflammatory cytokines, chemokines, and antimicrobial mediators, implicating Neat1 and the paraspeckle in orchestrating the immune response. Curiously, we found that macrophage paraspeckles do not respond uniformly to different pathogen associated molecular patterns (PAMPs), suggesting that macrophage paraspeckles may calibrate distinct gene expression programs, depending on the nature of the detected threat. We also found that other nuclear MLOs, notably the nucleolus, exhibit altered morphology in response to certain PAMPs. Together, our results argue that strategic sub-nuclear reorganization via MLOs aggregation and dissolution is a general characteristic of the macrophage response to pathogens.

40 - Kristin Watt

RNA Polymerase III transcription in cranial neural crest cell development

Kristin Watt1, Di Wu2, Paul Trainor2,3

1Department of Craniofacial Biology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA; 2Stowers Institute for Medical Research, Kansas City, USA; 3 Department of Anatomy & Cell Biology, University of Kansas Medical Center, Kansas City, USA

Ribosome biogenesis and protein translation are essential processes required in all cells, yet disruptions in this process lead to tissue-specific human phenotypes which frequently affect craniofacial development. Pathogenic variants in genes encoding subunits of RNA Polymerase (Pol) III lead to a variety of developmental disorders which may include neurological disorders and anomalies of the head and skull. Pol III is a 17-subunit complex which transcribes 5S ribosomal RNA (rRNA), a critical component of the ribosome, as well as other noncoding RNAs including tRNAs. Given the perturbed development of cranial neural crest cell (NCC)-derived tissues in humans with pathogenic variants in Pol III subunits POLR3A and POLR1C, we hypothesized that Pol III-mediated transcription is required for craniofacial development through the regulation of ribosome biogenesis and translation in NCCs. Consistent with this, zebrafish with mutations in polr1c and polr3a display hypoplasia of the craniofacial cartilage, bone, and pharyngeal teeth. The cranial NCC population is reduced in polr1c mutants as early as 36 hpf due to increased Tp53-dependent cell death, while polr3a mutants develop normally through 3 dpf and do not display an increase in cell death. This suggests that polr3a mutants are mechanistically distinct from polr1c mutants. To further explore the requirement for Pol III in craniofacial development, we performed quantitative RT-PCR and tRNA-sequencing. Preliminary results indicate changes in Pol I and III-mediated transcription in polr1c and polr3a mutant zebrafish that may underlie deficient proliferation. Together, these zebrafish models indicate that Pol III functions in the growth and development of craniofacial cartilage and bone. Current and future work aims to understand the tissue-specific changes in Pol III-mediated transcription during cranial NCC development and differentiation utilizing transcriptomic studies in these zebrafish models.

41 - Neel Mukherjee

Proteogenomic discovery of novel and regulatory open reading frames in human beta cells

Kathryn Walters1,2*, Roberto Castro Guiterrez4,5,6*, Soumyadeep Sakhar3, Amber Baldwin1,2, Ernesto S. Nakayasu3, Neelanjan Mukherjee1,2#, Holger A. Russ4,5,6#

1Department of Biochemistry and Molecular Genetics, University of Colorado-Anschutz Medical Campus, Aurora, CO, USA. 2RNA Bioscience Initiative, University of Colorado- Anschutz Medical Campus,Aurora, CO, USA 3Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA 99354, USA4Barbara Davis Center for Diabetes, University of Colorado- Anschutz Medical Campus, Aurora, CO 80045, USA5Diabetes Institute, University of Florida, Gainesville, FL 32610, USA6Department of Pharmacology and Therapeutics, College of Medicine, University of Florida, Gainesville, FL 32610, USA

*Co-first authors, #Co-corresponding authors

Ribosome profiling (Ribo-seq) provides a comprehensive view of translational regulation and reveals novel unannotated open reading frames (nuORFs) otherwise difficult to identify. Recent reports demonstrate that nuORFs regulate gene expression and immune recognition, highlighting their emerging biological roles. Insulin-producing pancreatic beta cells are critical for maintaining euglycemic conditions and beta-cell impairment contributes to diabetes development. nuORF and protein/peptide products have not been identified previously in human beta cells, but such knowledge might reveal novel mechanisms that regulate beta-cell function during homeostatic and disease conditions. Here, we applied a proteogenomics approach to human beta cells to comprehensively define previously unknown protein/peptide products. First, we applied cell-type-specific Ribo-seq to map the translatome of human stem cell-derived beta cells (sBCs) as a readily available human beta cell model system. Key pathways crucial for beta cell function and antigen presentation were translationally regulated. Importantly, we readily detected a recently described known immunogenic neoantigen, INS-DRiP, originating from an alternative start site in INS mRNA. Moreover, our analysis revealed 986 novel nuORFs in sBCs, with the majority showing protein-level support. Comparison with primary human islets further validated the nuORF translation and highlighted beta cell specificity of the identified nuORFs. Finally, we identified a novel and primate-specific regulatory upstream ORF within TYK2, a gene implicated in type 1 diabetes and crucial for beta cell function and interferon response. Our findings underscore the importance of translational regulation in beta cell function and provide an important resource to the wider diabetes research field.

42 - Brody Reynolds

Reactivity of PNPase towards oxidized RNA containing 7,8-dihydro-8oxoguanine.

Reactive oxygen species can be generated in biological systems through regular metabolic processing of oxygen; and have a role in the development/progression of several diseases, as well as aging. Guanine in RNA can be oxidized at the C8 position and give rise to 7,8-dihydro-8-oxoguanine (8-oxoG), which is commonly used as a biomarker of oxidative stress. Polynucleotide Phosphorylase (PNPase) is an endogenous exoribonuclease that specifically degrades oxidized RNA by successive digestion from the 3’ end. However, recent evidence in our laboratory indicates that the enzyme stalls at sites where an 8-oxoG is present. We set out to explore the effects of pH, divalent metal cation concentration, and sequence context, on this phenomenon. Oligonucleotides of RNA were obtained through solid phase synthesis, 32P radiolabeled at the 5’-position, and treated with PNPase under various conditions. Product distribution was then carried out via electrophoretic analysis (20% dPAGE). We used 17-nt long strands of RNA as a model; sequence: 5’-CAU GAA ACA AGG XXA GU-3’ (G = G or 8-oxoG, X = A,G,C,U). Interestingly, decreasing the pH or [Mg2+] led to increased stalling activity and sequence context did not induce a significant change. Further research into the implications of 8-oxoG causing stalling in PNPase is underway.

43 - Nadine Koertel

TRUB1-mediated RNA modification networks regulate aldosterone production

Nadine Koertel1, Marcin Sajek1, Amber Baldwin1, Laura White1, Jay Hesselberth1, Neel Mukherjee1

1Department of Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, Colorado 80045, USA

Aldosterone is the master regulator of blood pressure. However, our understanding of the fundamental molecular mechanism regulating hormone production remains incomplete. While the key signaling pathways, transcription factors, and steroidogenic enzymes necessary for adrenocortical steroidogenesis have been identified, very little is known about the contribution of post-transcriptional gene regulation of steroidogenesis by RNA-binding proteins (RBPs) or RNA modifications. Chemical modifications to RNA have been identified as important and dynamic regulators for post-transcriptional gene expression. Aldosterone is produced de novo in response to angiotensin II (AngII) stimulation, which makes precise temporal control of gene expression crucial to facilitate rapid activation and effective resolution of the AngII response. We previously identified RBPs that either promote or suppress aldosterone production. Here, we identified the pseudouridine synthase TRUB1 as an aldosterone repressor. TRUB1 isomerizes uridine to pseudouridine in both mRNA and tRNA. However, the mechanism of TRUB1-mediated aldosterone repression is unknown. We hypothesized that TRUB1-mediated pseudouridylation is a key regulator of aldosterone production by dynamically controlling translation. Indeed, we show that a catalytic mutant of TRUB1 was incapable of repressing AngII-stimulated aldosterone production. Since TRUB1 pseudouridylates tRNA and mRNA, it raises the key question of how does TRUB1 mediated pseudouridylation influence tRNA and/or mRNA regulation to repress aldosterone? To answer this question, we performed RNA-seq and Ribo-seq. We identified 92 mRNAs translationally upregulated, and 125 mRNAs translationally downregulated, including transcripts specifically encoding for steroidogenesis- promoting proteins. However, we do not know if the TRUB1-mediated effects are through tRNA or mRNA pseudouridylation. Therefore, we determined the modified RNA targets of TRUB1, we mapped the binding sites of TRUB1 using CLIP-seq, and the pseudouridylation sites using nanopore direct RNA sequencing, transcriptome-wide. Interestingly, we show evidence that TRUB1-mediated pseudouridylation precedes m1A modification, indicating a TRUB1-regulated sequential order to RNA modifications.

Currently, we are determining the mechanism by how this RNA modification network is regulating the translation and/or stability of steroidogenic mRNAs, which could be caused by altering the tRNA pool or through direct effects of mRNA pseudouridylation. Taken together, we provide the first evidence that aldosterone production is regulated by RNA modifications. Moreover, we show evidence for a TRUB1-regulated RNA modification network.

44 - Subbaiah Chalivendra

Humanizing the zinc-finger domain in U1C protein of the budding yeast alters RNA-binding and confers cold-sensitivity, splicing and global gene expression changes.

Chalivendra S, Shi S, Li X, Veith K, Taliaferro M, Zhao R et al.

Department of Biochemistry and Molecular Genetics, Anschutz Medical Campus, Aurora, CO 80045

U1 snRNP (U1, here after) a conserved ribonucleoprotein complex among eukaryotes, is critical for the recognition of 5’-splice site (5’ ss) and forming the first commitment complex of the splicing process. Of the U1 components, U1C protein plays a critical role in the stabilization of U1snRNA-5’ ss duplex, particularly when the 5’ ss is non-canonical. Unlike in other eukaryotes (including yeast), human 5’ ss sequences are highly divergent, which seems to allow extensive (in >90% of genes) alternative splicing and the expansion of human proteomic diversity. At the same time, many human genetic diseases are due to the failure of 5’ ss recognition by human U1 (hU1). Therefore, the role of hU1C protein in the recognition and stabilization of U1 interaction with 5’ ss needs a thorough investigation. To dissect structure-function relations of hU1C in 5’ ss recognition, we humanized yeast U1C (yU1C), by replacing the zinc-finger domain (ZnF; known to be sufficient for the stable binding of U1snRNA to 5’ ss) with its human counterpart. The resultant strain (h-yU1C, here after) showed cold-sensitivity and perturbation of pre-mRNA splicing as well as global gene expression, as revealed by RNA-seq analysis. yU1 from h-yU1C was not structurally different from the wildtype U1, as revealed by

cryo-EM analysis. The purified complex showed a significantly but moderately tighter binding to the canonical 5' ss of ACT1 pre-mRNA. However, the introduction of h-yU1C in PRP28-1 background rescued the growth phenotype of the latter, suggesting a weaker binding of modified U1 to 5' ss of most yeast pre-mRNAs. This correlated with the increased intron retention observed in h-yU1C. We found that replacing only two of the ZnF residues (T17S and K28R) was not sufficient to mimic the changes brought in by the 1-36 amino acid swap, highlighting the role of other residues (particularly a hydrophobic patch) within the ZnF of yU1C in RNA-binding. Previous work suggests that the region beyond the first 36 amino acids, that include a hydrophobic segment, may participate in RNA-binding. We are planning to extend the humanization of yU1C to the first 53 amino acids.

45 - Shea Siwik

Insights into "scaffolded" selection revealed from the crystal structure of an L-DOPA/dopamine binding aptamer

Shea H Siwik, David Stelzig, Simone D Hall, Briana Aboulache, Shelby R Lennon, and Robert T Batey

RNA aptamers have enormous potential to address a spectrum of diagnostic, therapeutic, and basic research application needs. The key strength of aptamers is that the appropriate selection strategy can result in an RNA that binds a target molecule with high affinity and selectivity. While several protein binding aptamers have achieved real-world application, small molecule binding aptamers have not been as successful. We have hypothesized that RNA folding may be a significant problem with in vitro selected aptamers and have proposed a strategy to address this issue using naturally occurring aptamers as scaffolds to host a randomized set of nucleotides. This approach seeks to develop novel aptamers that retain the overall tertiary structure of the parental scaffold to encourage robust folding and function. Novel neurotransmitter binding aptamers have been raised using this method (Porter et al., Nature Chem Biol, 2017), and the structure and biochemical properties of a serotonin aptamer scaffolded by a guanine riboswitch aptamer was analyzed as part of validating this approach.

To further validate this selection strategy, we have performed a detailed structural and biochemical analysis of a scaffolded dopamine aptamer. Crystal structures of the unbound and bound states confirm the global architecture of the parent purine riboswitch scaffold is preserved with no major differences between the two aptamers. The structure further reveals a binding pocket within the three-way junction, the site of the initial randomized region. Unexpectedly, the dopamine aptamer exhibits unintentional perturbations to its local secondary structure that occurred in nonrandomized regions which we have termed "scaffold shuffling." These alterations include an extended J1/2 and flipped out nucleotides in P3 and highlight the plasticity of RNA to alter its secondary structure during the selection process. Binding analysis indicates that this aptamer accomodates analogues of dopamine including L-DOPA, epinephrine, and norepinephrine. These compounds all contain the same catechol ring with different substituents extending off the ring at the 1-position. SHAPE chemical probing revealed no observable reactivity differences between the apo and bound states, suggesting a rigid aptamer with a preformed ligand binding pocket which is an unusual feature of small molecule binding aptamers. Comparison of three different aptamers raised by this strategy strongly supports robustness of scaffolded selection using biological RNAs as hosts of novel activities.

46 - Jessica Finlay-Schultz

Steroid hormone disruption of RNA polymerase III at tRNA genes in breast cancer.

Jessica Finlay-Schultz, Kiran V. Paul, Deborah L. Johnson, David Bentley, Peter Kabos, and Carol A. Sartorius.

RNA Polymerase III (Pol III) transcribes small molecules necessary for translation including all transfer RNAs (tRNAs). Pol III activity is tightly regulated by tumor suppressors, the nutrient-sensing mTOR pathway, and the transcriptional repressor Maf1. Little is known of how steroid hormones regulate Pol III in mammalian cells, particularly since most studies on Pol III are in organisms lacking steroid receptors. Using breast cancer cells abundant for progesterone receptors (PR) and estrogen receptors (ER), we discovered that PR are recruited to the Pol III complex at tRNA genes upon treatment with progesterone (P). P treatment rapidly reduced expression of target pre- and mature tRNAs. To better understand the dynamics of proteins involved in the recruitment and inhibition of the Pol III complex, we employed ChIP-seq for PR and Pol III complex components, genetic manipulation of Maf1 in breast cancer cells, and Bru-seq to identify global pre-tRNA changes. We found that PR recruitment to tRNA genes corresponds with recruitment of Maf1. Knockdown or

overexpression of Maf1 correlated with an increase and decrease in pre-tRNAs, respectively, but did not impact P repression of tRNAs. These data suggest that PR may repress Pol III tRNA transcription through mechanisms both including and independent of Maf1. We propose that PR recruitment disrupts the Pol III complex through recruitment of Maf1 and an additional unknown mechanism. Depletion of select tRNAs alters the mature tRNA pool and impacts selective translation. We suggest this is one mechanism by which P indirectly suppresses estrogen-driven growth in breast cancer cells and has wider implications for hormone regulation of cell growth and differentiation.

47 - Marcin Sajek

Evolutionary dynamics of polyadenylation signals and their recognition in protists

Marcin P Sajek1,2, Danielle Y Bilodeau1,2, Michael A Beer3,4, Yukiko Miyamoto5 Katrina B Velle6, Lars Eckmann5, Lillian Fritz-Laylin6, Olivia S Rissland1,2, Neelanjan Mukherjee1,2

1Department of Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora CO 80045, USA, 2RNA Bioscience Initiative, University of Colorado School of Medicine, Aurora CO 80045, USA, 3Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA, 4McKusick-Nathans Department of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA, 5Department of Medicine, University of California San Diego, La Jolla, CA 92093, USA, 6Department of Biology, University of Massachusetts, Amherst, MA 01003, USA

Cleavage and polyadenylation are the final steps of eukaryotic mRNA 3' end formation. The most critical element in humans and other model organisms is the poly(A) signal, an AAUAAA hexamer. We recently discovered that the deeply branching eukaryote – Giardia lamblia uses a different but well-defined poly(A) signal, AGURAA. To better characterize when this evolutionary shift in the poly(A) signal occurred, we performed direct RNA sequencing on four protists within the Metamonada supergroup and two outgroup protists. Both outgroup protists and the non-Giardia Metamonada species use the AAUAAA poly(A) signal, indicating it is the ancestral signal. In contrast, all Giardia species use the WGURAA poly(A) signal, indicating it is a derived feature within Giardia or Fornicata. The change in this ubiquitous regulatory element raises questions about the sequence features that specify genuine poly(A) sites and avoidance of premature cleavage in the coding sequence. We used a gapped k-mer support vector machine that was able to nearly perfectly discriminate between WGURAA sites in 3'UTRs and those in the CDS ($F1 \geq 0.95$). We found that Giardia lamblia uses nucleotides directly flanking the poly(A) signal for its recognition, with identity of the nucleotide just downstream of the the poly(A) signal being the most important. Poly(A) signals are flanked by pyrimidines, whereas WGURAA hexamers in coding sequences by purines. Interestingly, another member of the Giardia genus, Giardia muris, used a different strategy: almost complete depletion of WGURAA hexamers in coding sequences. The few remaining hexamers in the coding sequence were recognized as poly(A) signals and undergo premature cleavage. Taken together, we found that the WGURAA poly(A) signal evolved within the Fornicata phylum, and organisms utilizing this signal developed at least two different strategies for its proper recognition. Finally, these unique regulatory features of the Giardia pathogens that could be exploited for target therapy.

48 - Frank Lee

The SRSF1 and HNRNPU RNA Binding proteins regulate MyD88 Alternative Splicing

Frank Fang Yao Lee a b c, Chelsea Harris a b c, Scott Alper a b c

a Department of Immunology and Genomic Medicine, National Jewish Health, Denver, CO, 80206, USA, b Center for Genes, Environment and Health, National Jewish Health, Denver, CO, 80206, USA, c Department of Immunology and Microbiology, University of Colorado School of Medicine, Anschutz, CO, 80045, USA

Toll-like Receptors (TLRs) detect the presence of pathogens and transduce signals via the adaptor protein MyD88 to induce inflammation. While this response is essential to combat infection, chronic inflammation can damage tissues and contribute to many diseases. One mechanism that limits persistent inflammation is alternative splicing of TLR pathway genes including MyD88. The MyD88 gene can produce two isoforms, one full-length, long MyD88 isoform (MyD88-L) that promotes inflammation and a second shorter isoform in which exon 2 is skipped (MyD88-S) that inhibits inflammation. Importantly, production of MyD88-S is induced by inflammatory agonists such as lipopolysaccharide (LPS), and thus this alternative splicing mechanism

represents a negative feedback loop that limits inflammation. To understand the underlying mechanism that controls MyD88 alternative splicing, we employed siRNA screening and identified two RNA binding proteins, SRSF1 and HNRNPU, that regulate MyD88 alternative splicing. Knockdown of SRSF1 increases MyD88-S expression while silencing HNRNPU strongly reduced MyD88-S expression. Mechanistically, SRSF1 binds to an enhancer element located within MyD88 exon 2 in a manner inhibited by LPS. HNRNPU binds to a silencer region within MyD88 intron 1 in a manner enhanced by LPS. Thus, SRSF1 and HNRNPU may mediate the effects of LPS on MyD88 alternative pre-mRNA splicing.

49 - Hei-Yong Lo

Quantification of subcellular RNA localization through direct detection of RNA oxidation

Hei-Yong G. Lo, Raeann Goering, Agnese Kocere, Megan C. Pockalny, Laura K. White, Haydee Ramirez, Abraham Martinez, Seth Jacobson, Robert C. Spitale, Chad G. Pearson, Marino J. E. Resendiz, Christian Mosimann, and J. Matthew Taliaferro

Across cell types and organisms, thousands of RNAs display asymmetric subcellular distributions. The study of this process often requires quantifying abundances of specific RNAs at precise subcellular locations. To analyze subcellular transcriptomes, multiple proximity-based techniques have been developed in which RNAs near a localized bait protein are specifically labeled, facilitating their biotinylation and purification. However, these complex methods are often laborious and require expensive enrichment reagents. To streamline the analysis of localized RNA populations, we developed Oxidation-Induced Nucleotide Conversion sequencing (OINC-seq). In OINC-seq, RNAs near a genetically encoded, localized bait protein are specifically oxidized in a photo-controllable manner. These oxidation events are then directly detected and quantified using high-throughput sequencing and our software package, PIGPEN, without the need for biotin-mediated enrichment. We demonstrate that OINC-seq can induce and quantify RNA oxidation with high specificity in a dose- and light-dependent manner. We further show the spatial specificity of OINC-seq by using it to quantify subcellular transcriptomes associated with the cytoplasm, ER, and the inner and outer membranes of mitochondria. Finally, using transgenic zebrafish, we demonstrate that OINC-seq allows proximity-mediated RNA labeling in live animals. In sum, OINC-seq together with PIGPEN provide an accessible workflow for the analysis of localized RNAs across different biological systems.

50 - Adam Koch

Quantifying translational effects of SARS-CoV-2 UTRs with single molecule resolution in living cells

Adam Koch, Timothy Stasevich

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a large (~30kb) positive-sense RNA virus containing a highly structured 5’untranslated region (UTR), 14 open reading frames (in which the spike protein is located), and a structured 3’ UTR. The structural elements of the UTRs have been well defined; however, it is not fully understood what the functionality and roles of the UTRs are in the lifecycle of the virus. UTRs have long been known to be important for RNA structure, fidelity, and translation efficiency. Recent literature has indicated that the UTRs of SARS-CoV-2 may play a role in increased viral protein production and may mediate interactions with nonstructural proteins such as Nsp1 that are responsible for the shutdown of host translation. However, basic questions remain unanswered. For example, since Nsp1 has been implicated to downregulate host translation globally, how does the virus escape this inhibition? Do interactions with the viral UTRs somehow prevent inhibition, or are there other mechanisms at play? To begin to address these questions directly, we developed an array of single-mRNA biosensors with different combinations of 5' and 3'UTRs. This system allows us to directly image and quantify the relative contributions of each UTR to SARS-CoV-2 translation efficiency, localization, and mobility, both in the presence and absence of co-expressed Nsp1. We will present data showing these biosensors can be expressed in cells and tracked with single-mRNA precision, and the results of ongoing analyses that suggest different combinations of UTRs regulate translation in distinct ways.

51 - Sam Klink

HIV-1 Under the Lens: Real-time Fluorescent Imaging of Fully Infectious Viral Translation Dynamics

Human Immunodeficiency Virus-1 encodes its fifteen-protein genome onto a single RNA. Indeed, HIV-1 orchestrates a complex series of regulatory pathways to not only establish a productive infection, but also maintain genetic and structural stability. One key mechanism is a frameshifting site (FSS) between the structural Gag, and enzymatic Pol genes. A stable RNA stem-loop structure induces a -1 ribosomal frameshift during translation of the Gag polyprotein, resulting in translation of full-length Gag-(pro)Pol polyprotein. Curiously, the ratio of zero frame Gag and -1 frame Gag-Pol is strictly maintained at a 19:1 ratio, or about 5-10% Gag-Pol expression. Furthermore, the FSS alone is sufficient to maintain this programmed frequency. However, in the context of the HIV-1 lifecycle, it is unknown whether frameshifting is stochastic among the total population of viral genomic RNA, or if a specific subset of gRNAs are regulated differently. Do all gRNAs have an equivalent 5-10% chance to frameshift, or are 5-10% of gRNAs sequestered into a frameshifting pool? For the first time, I plan to answer this question using a novel, fully functional HIV-1 virion engineered with tags pre- and post-FSS in conjunction with single molecule fluorescent microscopy to view HIV-1 protein synthesis in real time, and in live cells. Preliminary results successfully establish infection in my cell cultures, and current efforts are focused on establishing a timeline from early to late-stage infection. Perturbation of this conserved 19:1 ratio significantly affects HIV-1 fitness, and greater characterization of this key regulatory mechanism lays the foundations for future drug development.

52 - Megan Pockalny

Role of mTOR in RP mRNA Localization

Megan Pockalny, Raeann Goering, Matthew Taliaferro

The function of enterocytes is to absorb nutrients from the intestinal lumen. The apical pole of enterocytes has a higher concentration of translation machinery and translational efficiency than the basal pole. mRNAs encoding ribosomal proteins (RPs) are basally localized in enterocytes through the interaction of the 5' TOP motif found on RP mRNAs and the RBP LARP1 in starvation conditions. Upon refeeding, these mRNAs become more apically localized. Though this pattern has been observed in both in vivo and in vitro models, the mechanisms regulating RP mRNA localization and translation have not yet been identified. The mTOR pathway is the major nutrient-sensing regulator of growth in cells and LARP1 has been identified as a downstream target of mTORC1. Using smFISH and high-throughput transcriptome-wide RNA profiling of subcellular fractions, we show that the localization of RP mRNAs is dependent on mTOR activity through the manipulation of feeding and starvation patterns as well as through pharmacological means. Specifically, RP mRNAs are basally localized in enterocytes when mTOR is inhibited and move apically when mTOR is activated. Neuronal cells show mTOR-sensitive localization of RP mRNAs as well, where activation of mTOR leads to localization of these transcripts to the soma and inhibition of mTOR results in localization to the neurite. We further show that the 5' TOP motif is sufficient for mTOR-dependent localization. These finding suggests that mTOR may control nutrient-dependent dynamic RNA localization through phosphorylation of LARP1.