Welcome

to the

4th Annual

Rocky Mountain RNA Symposium

Thursday, May 22, 2025

CU Anschutz
Aurora, CO

hosted by

Colorado RNA Club

in partnership with

RNA Bioscience Initiative

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(Alphabetical Order by Last Name)

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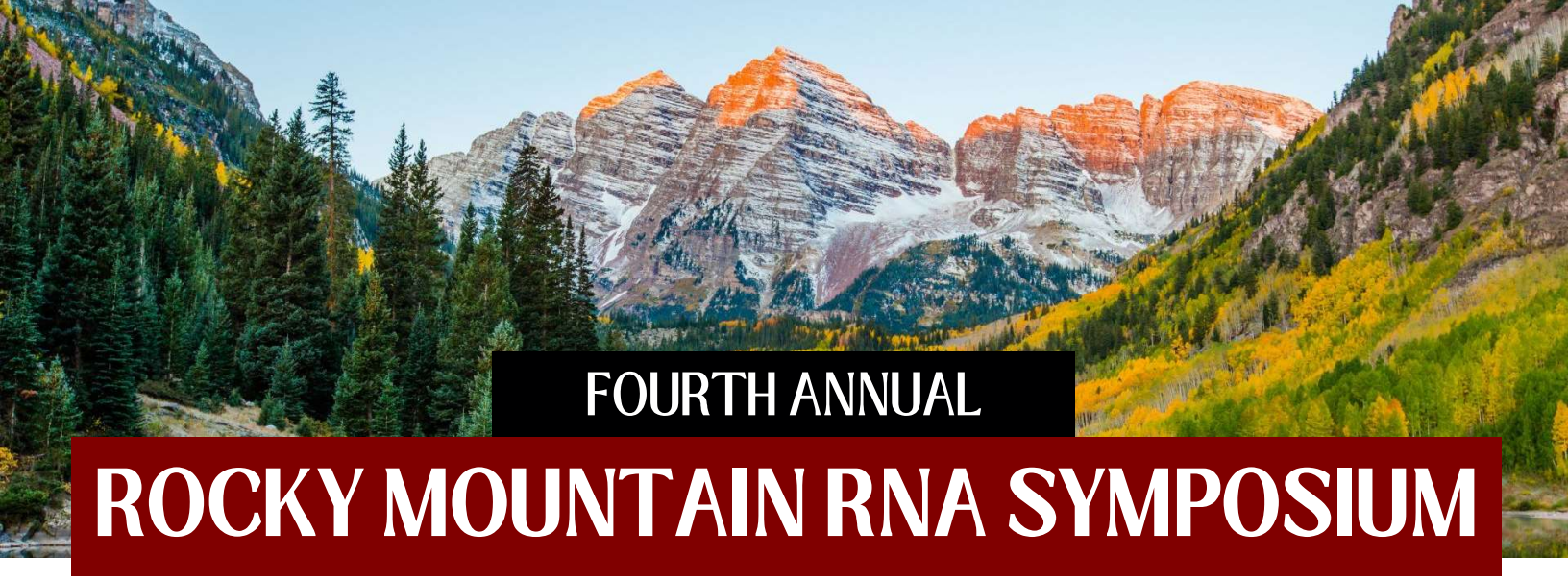
Edgardo Linares	Johnson Lab	University of Colorado – Anschutz
Federico Martinez-Seidel, PhD	Rissland Lab	University of Colorado – Anschutz
Katie Vaeth	Taliaferro Lab	University of Colorado – Anschutz



FOURTH ANNUAL

ROCKY MOUNTAIN RNA SYMPOSIUM

Thursday, May 22, 2025

Featuring Talks From:

Sara Rouhanifard, PhD
Northeastern University

Nicole Martinez, PhD
Stanford University

Aaron Hoskins, PhD
University of Wisconsin Madison

Joshua Arribere, PhD
University of California Santa Cruz

Join us for a fun day of science & networking!

Evening Social • Poster Session (with prizes!) • Food & Beverages

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Program Schedule

ROCKY MOUNTAIN RNA SYMPOSIUM

Thursday, May 22, 2025

Elliman Conference Center, AHSB, CU Anschutz

8:30 AM	Check-In / Poster Set-Up / Breakfast
9:00 AM	Introductory Remarks
9:10 AM	**Dr. Sara Rouhanifard** - Northeastern University
10:00 AM	**Charlie Moffatt** - CU Anschutz
10:20 AM	Morning Poster Session
11:20 AM	**Dr. Nicole Martinez** - Stanford University
12:10 PM	Lunch
1:00 PM	**Jacob Stanley** - CU Boulder
1:20 PM	**Dr. Aaron Hoskins** - University of Wisconsin Madison
2:10 PM	**Jill Bilodeaux** - CU Anschutz
2:30 PM	Afternoon Poster Session
3:40 PM	**Dr. Chen-Shan Julia Woodcock** - National Jewish Health
4:00 PM	**Dr. Joshua Arribere** - UC Santa Cruz
4:50 PM	Poster Awards & Closing Remarks
5:00 PM	Networking Social, Tstreet Kitchen (AHSB 1st floor)

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Poster #1: Ella Tommer

Title: Regulation of DNMT1 by RNA and by its N-terminal low-complexity domain

Authors: Ella Tommer, Jessica Song, Eliza Lee, Paul Rothman, Vignesh Kasinath, Tom Cech

Abstract: DNMT1 recapitulates 5-methylcytosine patterns from parental DNA strands to daughter strands following DNA replication, maintaining epigenetic gene regulation. Here, we aim to elucidate how human DNMT1's activity is regulated by its N-terminal domains and by RNA. We find that N-terminal truncations greatly reduce the affinity of DNMT1 for hemi-methylated DNA and diminish its catalytic activity. In vitro methylation assays suggest that the low-complexity N-terminus is involved in tethering DNMT1 to DNA, while the RFTS domain is involved in binding and/or translocating to the next CpG site. To understand these mechanisms in detail, we have developed nanopore sequencing of in vitro reaction products with single nucleotide resolution of modified bases. We previously showed that DNMT1 binds many RNAs including pUG folds, non-canonical G-quadruplex structures. This binding is higher affinity than that of hemi-methylated DNA and inhibits methyl transfer. We have used cryo-EM to determine a 2.8 Å – 8 Å structure of DNMT1 bound to pUG-fold RNA, identified the amino acids important for interactions with the RNA, and used this information to design and test RNA binding-defective mutants. We are utilizing a DNA-to-RNA tethering system in cells with Halo-tagged DNMT1 knocked-in at the endogenous locus to study pUG RNA:DNMT1 binding in vivo. We suggest that RNA binding may prevent DNMT1 from methylating highly transcribed genes, thereby inhibiting inappropriate epigenetic silencing.

Poster #2: Johnathon Schafer

Title: Spliceosome Mutations in Myelodysplastic Syndrome Impair Host Defense

Authors: Johnathon Schafer, Natalia J Gurule, Chelsea Harris, Scott Alper

Abstract: Myelodysplastic Syndrome (MDS) is a hematopoietic stem cell disorder that has the potential for progression to Acute Myeloid Leukemia (AML). 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 patients with MDS. Our lab uses mouse models expressing MDS-associated spliceosome mutations and myeloid cells isolated from peripheral blood from MDS patients to investigate why MDS patients are susceptible to infection. We previously found that the MDS-associated spliceosome mutation U2AF1-S34F induces significant immunodeficiency by compromising neutrophil migration. I have observed that two other mouse models of MDS associated spliceosome mutations, SF3B1-K700E and SRSF2-P95H, also are immunodeficient. SF3B1-K700E mutant mice exhibit defects in neutrophil migration and moderate inability to control E. coli infection. SRSF2-P95H mice likewise appear to be more susceptible to bacterial infection. I have also determined that human peripheral blood monocytes from MDS patients with or without spliceosome mutations have reduced ability to differentiate into macrophages. Those macrophages that do differentiate have phagocytosis and antibacterial defects. These studies highlight multiple mechanisms by which MDS mutations reduce innate immune function, contributing to the increased susceptibility to infection in these patients.

Poster #3: Kelleen McEntee

Title: Personalized antisense oligonucleotides target biallelic intronic mutations to restore SDCCAG8 expression and ciliogenesis

Authors: Kelleen McEntee, Bailey L McCurdy, Austin Larson, Emily A McCourt, Michael L Kaufman, Amy E Campbell, Chad G Pearson, Scott Demarest, Jay R Hesselberth, Sujatha Jagannathan

Abstract: Bardet Biedl Syndrome (BBS) is a ciliopathy that often results in progressive blindness, obesity, and other symptoms. A patient presenting with BBS was discovered to have two mutations within 55bp of each other in intron 7 of SDCCAG8 (BBS16). One of the biallelic mutations, c.740+356C>T, causes inclusion of cryptic exon(s) while c.740+301G>A has not been characterized. We hypothesized that ASOs complementary to the patient's mutations would correct the splicing of SDCCAG8 between exons 7 and 8 to prevent the inclusion of cryptic exons and restore SDCCAG8 expression to potentially prevent further vision loss. We screened 20nt-long ASOs placed every 2bp across each mutation in patient-derived fibroblasts using RT-PCR assays to assess exon 7 and 8 splicing. We identified one ASO for each mutation that restored the splicing pattern observed in an unaffected cell line. These lead ASOs were further investigated through RT-qPCR, RNA sequencing, and western blotting to confirm ASO-mediated restoration of wild-type transcript and protein. Functional restoration of SDCCAG8 was tested by the induction of ciliogenesis in patient-derived fibroblasts which revealed increased cilia production under ASO treatment. These ASOs may lead to the treatment of BBS-related retinal dystrophy for this patient and others that share these mutations.

Poster #4: Katie Vaeth

Title: RNA localization to the midbody

Abstract: 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.

Poster #5: Samuel Hunter

Title: Transcriptional Consequences of the RNA Binding Affinity of Estrogen Receptor alpha

Abstract: Estrogen receptor alpha (ERα) is a nuclear hormone receptor which mediates the cellular response to estrogens by regulating target gene transcription. In addition to its well-established role as a transcription factor, ERα has recently been identified as an RNA-binding protein. However, the functional significance of this RNA-binding activity remains unclear. To investigate this, we analyzed the binding kinetics and transcriptional activity of ERα mutants with and without the ability to bind RNA. We found that RNA-binding affinity correlates with both the receptor's efficiency in locating its genomic target sites and its transcriptional activity. These results suggest that RNA binding enhances ERα function by facilitating its recruitment to target genes, thereby promoting transcriptional activation.

Poster #6: Sam Klink

Title: Near Native HIV-1 Frameshifting Dynamics in Live Cells

Authors: Sam Klink, Ryan Hasbrook, Tatsuya Morisaki, Ryan Jeep, Chaoping Chen, Timothy Stasevich

Abstract: During HIV-1 infection, a programmed -1 frameshift between Gag and Gag-Pol genes occurs at a regular ratio of 19:1 Gag to Gag-Pol. Maintenance of this ratio is critical to viral fitness and disturbing this ratio up or down significantly impacts HIV-1's replication cycle. However, how this ratio is maintained at the single-mRNA level is largely unknown. To get at this question, we probe HIV-1 frameshifting events using dual tagged, fully infectious HIV-1 virions in live cells. By quantifying translation of both Gag and Pol from individual viral mRNAs in fixed and live cells, we show frameshifting is well characterized by a bursty model, wherein frameshifting RNAs are more likely to continue to frameshift. The stability of the frameshifting stimulatory sequence and ribosome elongation rates are implicated in this model, though their direct contribution to frameshifting remains unknown.

Poster #7: Angie Liu

Abstract: Transcription factors (TFs) are canonically understood to interact with DNA and regulate downstream genetic activities. However, certain TFs have also been shown to bind RNA, which may add an additional layer of transcriptional control. Recent in cell crosslinking experiments reinforce this notion, and further suggest that TF-RNA binding is a ubiquitous phenomenon. However, the governing principles behind such interactions remain poorly understood, preventing a full understanding of transcriptional regulation.

Bioinformatic analyses reveal that many TFs contain the well-established RNA binding ARM (Arginine Rich Motif) domain. In order to determine whether and how TFs use their ARM to interact with RNA, the established RNA binding TF estrogen receptor alpha (ERα) will be used as a model system.

In vitro binding data suggest that ERα-ARM recognizes the terminal loop of RNA hairpins with structural specificity. However, a more comprehensive screen of RNA structures (e.g., internal loops, internal bulges, and multi-helix junctions) is needed to understand the full range of RNA targets recognized by ERα-ARM. To this end, I will use in vitro binding assays to understand ERα-ARM's preference for various RNA structures. These experiments will elucidate whether ERα-ARM promiscuously interacts with all loop-containing RNA targets or displays structural plasticity to differentially engage with unique structural features.

In addition, I will perform X-ray crystallography to obtain a crystal structure of an ERα-RNA complex. I will survey different RNA secondary structures and condition spaces to pursue crystallization. These insights will help reveal the governing principles behind TF-RNA interactions, adding to our understanding of transcriptional regulation and proposing a novel framework for therapeutic intervention.

Poster #8: Federico Martinez-Seidel

Title: Translational control of collagen: A new paradigm of ribosome stalling

Authors: Federico Martinez-Seidel, Addison Rains, Olivia Rissland

Abstract: Collagen is the most abundant protein in our body, but it is very difficult to produce, which raises the question of how cells manage to mass produce such a demanding protein. How is cellular metabolism coordinated to meet the translation requirements? And what are the molecular consequences of such a commitment? Our results show that collagen synthesis has significant effects on the translation machinery that must be optimally tuned to ensure steady collagen production.

Poster #9: Evan Brooks

Title: Differential Srsf3 protein interactions upon PDGFRa signaling in mouse embryonic palatal mesenchyme

Authors: Evan C. Brooks[1], Thomas E. Forman[1], Katherine A. Fantauzzo[1,2]
[1]Department of Craniofacial Biology, University of Colorado Anschutz Medical Campus, Aurora, CO; [2]RNA Bioscience Initiative, University of Colorado Anschutz Medical Campus, Aurora, CO

Abstract: Craniofacial development is a complex morphogenetic process, disruptions in which result in highly prevalent human birth differences such as cleft lip/palate. Signaling through the receptor tyrosine kinase platelet-derived growth factor receptor alpha (PDGFRα) plays critical roles in this process in humans and mice. We previously identified the RNA-binding protein Srsf3 as an effector of PDGFRα signaling in mouse embryonic palatal mesenchyme (MEPM) cells that regulates alternative RNA splicing following phosphorylation-dependent translocation into the nucleus. To identify proteins that differentially interact with Srsf3 depending on its phosphorylation in response to PDGF-AA ligand stimulation, MEPM cells were treated with PDGF-AA ligand for 0, 15 or 60 min. Srsf3-interacting proteins were isolated via immunoprecipitation and analyzed by mass spectrometry. Our screen identified 52 unique proteins, 20 and 32 of which had increased and decreased spectral counts upon PDGF-AA ligand treatment, respectively. These proteins included 17 that have previously been implicated in RNA binding, including the RNA-induced silencing complex protein Ago2. Variants in human AGO2 cause Lessel-Kreienkamp syndrome, with some patients exhibiting dysmorphic facial features. Consistent with the mass spectrometry results, we have biochemically confirmed the increased interaction of Srsf3 with Ago2 upon PDGF-AA ligand stimulation of MEPM cells via immunoprecipitation and western blotting. Future studies will focus on whether these protein-protein interactions require RNA and affect Srsf3 subcellular localization and/or Srsf3-mediated alternative RNA splicing.

Poster #10: Edgardo Linares

Title: m6A marks influence long noncoding RNA-mediated silencing

Authors: Edgardo Linares and Dr. Aaron Johnson

Abstract: Gene regulation is essential for cellular identity and function, with long non-coding RNAs (lncRNAs) playing a key role in modulating chromatin states. The lncRNA HOTAIR facilitates gene repression by recruiting chromatin-modifying complexes. Our lab identified a critical N6-methyladenosine (m6A) modification at position 783 (m6A783), which, when recognized by the nuclear m6A reader YTHDC1, is required for silencing tumor suppressor genes. Mutation of A783s decreased doubling time and increased matrigel invasion phenotypes in triple-negative breast cancer cells. However, YTHDC1 does not directly execute repression, suggesting additional factors are involved. Notably, thirteen additional m6A sites were identified and mutated to uracil resulting in decreased HOTAIR expression. Some of these sites disappear in the absence of m6A783. My work aims to identify factors involved in HOTAIR m6A783-mediated silencing and to determine how other m6A sites influence HOTAIR expression, stability, methylation, and protein interactions. To achieve this, I will conduct a CRISPR-inhibition screen in a cell line containing a luciferase reporter regulated by HOTAIR repression, identifying genes that disrupt silencing with increasing luciferase expression. Additionally, we will map each m6A site's interactome by using proximity labeling of nearby proteins for mass spectrometry identification. Expression and stability will be analyzed for individual HOTAIR A-to-U mutants at each of the thirteen identified m6A sites. These findings will provide insight into how different m6A patterns can influence lncRNA biology.

Poster #11: Luis Aguilera

Title: Mining PDB database to generate a novel tagging system for tracking translation live

Authors: Luis U. Aguilera, Ashlyn Chen, Jake Yarbro, Rhiannon Sears, Jacob DeRoo, Hunter Ogg, Brian Geiss, Chris Snow, Timothy Stasevich, Ning Zhao

Abstract: Live-cell imaging and functional intracellular antibody fragments (intrabodies) tagging systems, including SunTag, AlfaTag, and HA Frankenbody, have facilitated single-molecule resolution and real-time visualization of translation events. To increase the limited number of available imaging systems, we introduce a novel strategy utilizing structural data from the Protein Data Bank (PDB) to rationally design and engineer a new genetically encoded probe, termed UTag. UTag has been optimized for enhanced intracellular folding, binding specificity, and photostability. This study systematically compares our newly developed UTag system against the well-established SunTag and AlfaTag systems under various experimental conditions. We validate UTag's capability for live-cell imaging using the reporter gene KDM5B, encoding a nuclear protein. We demonstrate rapid intracellular diffusion of the anti-UTag probe using fluorescence recovery after photobleaching (FRAP) and confirm efficient cytosol-to-nucleus translocation of the reporter construct. Western blot analysis confirms the expression of the full-length protein, indicating that UTag does not affect protein production. Treatment with translation inhibitors harringtonine and puromycin resulted in the disappearance of translation spots, confirming that observed signals represent actively translating mRNAs. Finally, we integrated long-term live-cell imaging, autocorrelation function analyses, and a Total Asymmetric Exclusion Process (TASEP) mathematical model to precisely estimate translation initiation (~ 0.04 sec-1) and elongation rates (~4.5 aa/sec).

Poster #12: Chloe Wohlenberg

Title: Disruptions to polyamine transport and eIF5A biogenesis compromise the cellular response to ribosomal stalling

Authors: Chloe Wohlenberg[1], Parissa Monem[1], and Joshua Arribere[1]
[1]University of California, Santa Cruz, Department of Molecular, Cell, and Developmental Biology, Santa Cruz, CA, USA

Abstract: Translation elongation management is critical to ensure proper protein production. Translation elongation slows when a ribosome pauses on an mRNA. If the pause persists, ribosomes will stall, collide, and trigger No-Go mRNA Decay (NGD), which degrades the stall-inducing mRNA. While it is understood that there are diverse possible outcomes of ribosome pausing and stalling, the distinguishing characteristics that commit a translational complex to one outcome or another remain poorly defined. In a C. elegans forward genetic screen utilizing an NGD reporter, we uniquely identified a polyamine transporter CATP-6 (human PARK9/ATP13A2) to be required for NGD. Following up on this lead, we determined that cellular polyamine levels and the translation elongation factor eIF5A, with its critical post-translational polyamine-dependent hypusine modification, are also required for NGD. This requirement of hypusinated eIF5A in NGD is particularly surprising based on the in vitro biochemical understanding of eIF5A's cellular role. Our current research is dedicated to determining the specific molecular mechanisms behind how eIF5A signals translational stress responses like NGD. This work reveals an unexpected role for the highly-conserved eIF5A factor in NGD which lays the foundation to explore how the cell modulates diverse outcomes of translation elongation events.

Poster #13: Grace Gustafson

Title: A developmental buffering mechanism of initiation codon mutations.

Authors: Grace E. Gustafson, Raisa Bailón-Zambrano, Abigail Mumme-Monheit, Juliana Sucharov, James T. Nichols

Abstract: Deleterious mutations can display a range of severity among individuals. This phenomenon is likely due to differences in buffering: lessening the effects of genetic perturbation. In recent years, the study of transcriptional adaptation (TA) through nonsense-mediated decay has increased. In TA, compensatory paralog upregulation protects against premature termination codon mutations. This mechanism, however, is confined to nonsense mutations. Mutations of other character exist that do not undergo TA, but still present phenotypes that are variably less severe than expected. Here we study one such allele with a mutation in the initiating methionine of the zebrafish transcription factor encoding gene mef2ca. This gene has a key role in craniofacial development, and mef2ca mutants display a range of phenotypes. This mutant was predicted to produce no protein, yet it unexpectedly produces a very mild phenotype. Here we propose a novel mechanism of buffering against start codon mutations functioning at both the protein and mRNA levels. We discovered that this initiating AUG to CUG mutation produces Mef2ca protein and a downstream transcriptional target, dlx5a, is expressed in mutants. We identified the presence of in-frame downstream AUGs, that could provide alternative start sites for translation of a protein if it cannot initiate off the mutant CUG at the canonical transcriptional start. Surprisingly, this mutant also displays higher relative mRNA abundance than wild-type siblings, either due to increased transcript stability or higher expression levels. We predict that this mutant uses both increased mutant mRNA stability and alternative translational start site usage to buffer the severity of the resultant phenotype, creating a highly expressed, partially functional protein that confers a near wild-type phenotype. This novel mechanism is likely applicable across biological systems with similar start codon mutations.
Funded by NIDCR: 5R01DE029193 and NSF GRFP: 2439026

Poster #14: Wendy Trieu

Title: Pseudouridines and PUS7 are regulated during neurodevelopment

Authors: Wendy Trieu and Nicole M. Martinez

Abstract: PUS7 is a pseudouridine synthase that isomerizes uridine to pseudouridine in pre-mRNA and mediates prevalent changes in alternative splicing, 3' end processing and mRNA levels. Patients with mutations in the pseudouridine synthase PUS7 exhibit neurodevelopmental phenotypes including microcephaly, aggression, intellectual disability, and speech delay. However, the molecular mechanisms by which pseudouridines and PUS7 might impact neurodevelopment are unknown. We used human embryonic stem cell (hESC) differentiation into neurons by inducible neurogenin-2 expression as a model to elucidate the role of PUS7 in neurodevelopment. Using this system, we found that PUS7 mRNA and protein levels decrease during differentiation, and a highly neuron specific microexon is included in the mRNA, which alters the PUS7 protein coding sequence. To map pseudouridines that are regulated during neurodevelopment we performed direct RNA Nanopore sequencing in hESCs and induced neurons. We discovered that hundreds of pseudouridines are dynamically regulated during neurodevelopment. Pseudouridines that are downregulated during neurodevelopment are enriched for the canonical sequence motif recognized by PUS7, coincident with the downregulation of PUS7 protein levels. Finally, depletion of PUS7 disrupts the neurodevelopmental gene expression program as demonstrated by RNA-seq. Together, our results reveal that pseudouridines and PUS7 are neurodevelopmentally regulated, and that PUS7 has an important function in gene regulation during neuronal differentiation. Ongoing work is interrogating the mechanisms underlying these molecular phenotypes and uncovering cellular phenotypes mediated by PUS7 in neurodevelopment. This work will identify biological functions of Ψ sites in mRNAs and the molecular and cellular underpinnings of PUS7's role in neurodevelopment.

Poster #15: Meaghan Courvan

Title: Mechanisms of Mediator Kinase Inhibition in Inhibiting Leukemia Proliferation

Authors: Meaghan Courvan, Kira Cozzolino, Zachary Poss, Dylan Taatjes, Robin Dowell

Abstract: Leukemia patients face a difficult path, with fewer than 50% of patients surviving 5-years after diagnosis. Leveraging our understanding of transcriptional biochemistry may unlock more effective leukemia treatments. We observe that the inhibition of a key transcriptional complex, known as the Mediator complex, is selectively lethal to a subset of leukemias. The Mediator complex is essential for coordinating transcription in response to regulatory factors, non-coding regulatory elements, and external signals. Central to its function is its kinase module, which contains either of the paralogue kinases CDK8 or CDK19. Although Mediator is present in all cells, CDK8 and CDK19 kinase inhibition is only lethal to specific myeloid, mixed-lineage, and megakaryoblastic leukemia cell lines. Could drugs inhibiting CDK8/19 kinase be a potent and specific way to treat leukemia? A multi-omics approach – including transcriptomics, nascent sequencing, phospho-proteomics, and metabolomics – combined with the CDK8/19 kinase-inhibiting drug cortistatin A reveals how transcriptional networks are rewired shortly after drug-treatment (1-6 hours), and how this alters cell state and ultimately leads to cell death (6-48 hours).

Poster #16: Bailey Lubash

Title: RNA Polymerase III subunit Polr3a is required for craniofacial cartilage and bone development

Authors: Bailey Lubash, Roxana Gutierrez, Emma Heiny, Kristin Watt

Abstract: 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, including POLR3A, lead to a variety of phenotypes including hypomyelination in the central nervous system, hypodontia, and other rare anomalies of the head and skull. Given the perturbed development of cranial neural crest cell (NCC)-derived tissues in humans with pathogenic variants in POLR3A, we hypothesized that Pol III-mediated transcription is required for craniofacial development through the regulation of ribosome biogenesis and translation in NCCs. To test this, we established polr3a mutant zebrafish and examined craniofacial development. These mutant zebrafish display hypoplasia of NCC-derived craniofacial cartilage and bone by 5 days post fertilization (dpf), with a more significant disruption in bone development. To determine how these changes arise in polr3a mutants, we assessed the NCC population, proliferation, and cell death from 1.5 - 5 dpf. Surprisingly, no significant changes were observed in NCC development, proliferation, or cell death prior to 2 dpf. However, at 3 dpf and beyond, both increased cell death and reduced proliferation were observed throughout the head. Interestingly, markers of cartilage and bone development were not changed at this stage, suggesting that cartilage and bone hypoplasia at 5 dpf could be the result of changes in cell proliferation and survival. Quantitative RT-PCR demonstrated reduced transcription of tRNAs and increased levels of tp53 at 3 dpf, and we also observed diminished ribosome assembly and transcription of tRNAs at 5 dpf. Altogether, this indicates that mutations in polr3a disrupt ribosome biogenesis and tRNA transcription, leading to increased cell death and reduced proliferation in cranial tissues, and result in hypoplasia of the craniofacial cartilage and bone at 5 dpf. Current and future work aims to understand the tissue-specific changes in Pol III-mediated transcription during craniofacial bone and cartilage differentiation.

Poster #17: Cassie Minne

Title: Alternative RNA splicing of transcripts encoding protein serine/threonine kinases downstream of PDGFR signaling in the facial mesenchyme

Authors: Cassandra B. Minne[1], Brenna J.C. Dennison[1], Eric D. Larson[2,3], Katherine A. Fantauzzo[1,4]
[1]Department of Craniofacial Biology, University of Colorado Anschutz Medical Campus, Aurora, CO; [2]Department of Otolaryngology – Head and Neck Surgery, University of Colorado Anschutz Medical Campus, Aurora, CO; [3]Basic and Translational Sciences, Penn Dental Medicine, University of Pennsylvania, Philadelphia, PA; [4]RNA Bioscience Initiative, University of Colorado Anschutz Medical Campus, Aurora, CO

Abstract: Craniofacial development is a complex morphogenetic process, disruptions in which result in highly prevalent human birth differences. Signaling through the platelet-derived growth factor receptors (PDGFRs) plays critical roles in this process in humans and mice. However, the gene expression changes that mediate cellular activity downstream of PDGFRα and/or PDGFRβ signaling are incompletely understood. Here, we sequenced maxillary process mesenchyme RNA from E11.5 mouse embryos that lack Pdgfra, Pdgfrb or both in the neural crest lineage. DESeq2 analysis identified 23, 20 and 25 genes that were differentially expressed between Pdgfrafl/fl;Wnt1-Cre+/Tg, Pdgfrbfl/fl;Wnt1-Cre+/Tg and Pdgfrafl/fl;Pdgfrbfl/fl;Wnt1-Cre+/Tg samples as compared to wild-type, respectively. In contrast, rMATS analysis detected over 5,000 differential alternative RNA splicing (AS) events per genotype compared to wild-type samples, with the majority of events involving skipped exons. Gene ontology (GO) analysis of the genes encoding the transcripts in the skipped exon category of each genotype revealed an enrichment for protein serine/threonine (S/T) kinase activity functioning within the PI3K and/or MAPK signaling pathways. Alternatively-spliced transcript Rps6ka3 encodes a protein S/T kinase (Rsk2) required for proper craniofacial development in humans and mice. We predict that the AS event detected in all experimental genotypes will generate an upstream open reading frame that represses translation of the main coding sequence. Together, our results demonstrate that AS is the predominant mechanism of gene expression regulation downstream of PDGFR activity in the facial mesenchyme, serving to regulate intracellular signaling.

Poster #18: Usman Hyder

Abstract: Cellular transformation, the process of turning normal cells into cancer cells, is highly dependent on gene expression changes that includes transcription and post-transcriptional regulation. Given the fundamental nature of these steps for both normal cells and cancer cells, factors that regulate individual steps have not presented therapeutic potential. However, the transcription elongation rate, or the speed by which RNA Polymerase II (Pol II) travels in gene bodies of actively transcribing genes, has been unexplored as a therapeutic modality in cancer. Here we report that elongation rate is globally increased in normal breast cells upon cellular transformation by oncogenic RAS signaling, suggesting that blocking factors that control elongation rate may blunt the transformation process. Additionally, the histone chaperone complex FACT has been known to play context-dependent roles in gene regulation in cancer cells when compared to normal cells and has recently emerged as a critical regulator of elongation rate, suggesting that FACT transcriptional control may present selective therapeutic vulnerabilities. We suggest that dosing elongation rate at the level of normal cells will block gene expression changes critical for cellular transformation that may lead to novel therapeutic approaches.

Poster #19: Keqing Nian

Title: inCu-click: DNA-enhanced ligand enables live-cell, intracellular click chemistry reaction

Authors: Keqing Nian[1], Yifang Liu[1], Yuchen Qiu[1], Zhuoyu Zhang[1], Laura Brigandi[1], Meni Wanunu[1,2] and Sara H. Rouhanifard[1,#]
[1]Dept. of Bioengineering, Northeastern University, Boston, MA
[2]Dept. of Physics, Northeastern University, Boston, MA
[#]Corresponding author.

Abstract: Of the various conjugation strategies for cellular biomolecules, Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) is the preferred click chemistry approach due to its fast reaction rate and the commercial availability of a wide range of conjugates. While extracellular labeling of biomolecules using CuAAC has been widely adopted, intracellular labeling in live cells has been challenging as the high copper concentrations required for CuAAC reaction is toxic to biological systems. As a critical first step towards CuAAC-mediated intracellular labeling, an ultrasensitive CuAAC ligand is needed to reduce cytosolic copper concentrations while maintaining fast reaction kinetics. Here, we developed inCu-click (intracellular Cu-catalyzed click), a modified CuAAC reaction enabled by BTT-DNA, a new DNA oligomer-conjugated ligand. The DNA oligo attachment serves several purposes, including: 1. Increased localization of copper atoms near the ligand, which enables ligation of azide tags with much lower copper concentrations than commercially available CuAAC ligands and without the addition of exogenous copper salt; 2. Allows nucleic acid template-driven proximity ligation by choosing the attached DNA sequence, 3. Enables the liposome encapsulation and delivery of the ligand into live cells, and 4. Facilitates intracellular labeling of nascent phospholipids in live cells. We demonstrate that the inCu click reaction has little to no effect on the overall cell health.

Poster #20: Addison Rains

Title: Impacts of UV irradiation on collagen post-transcriptional gene regulation

Authors: Addison B. Rains, Federico Martinez-Seidel, and Oliva S. Rissland

Abstract: Ultra-violet (UV) radiation from the sun is a common environmental exposure that causes damage to DNA, RNA, and proteins, leading to cell senescence or cell death. Chronic UV exposure to the skin leads to premature aging because of UV damage to collagen I fibers in the extra-cellular matrix (ECM), and loss of dermal fibroblasts, that produce and maintain the collagen-rich ECM. Recently, UV irradiation was found to disrupt protein translation through the initiation of the ribotoxic stress response (Vind et al., 2024, Sinha et al., 2024). UV irradiation has also been shown to act as a form of endoplasmic reticulum (ER) stress, causing proteins to accumulate in the ER and leading to activation of the unfolded protein response (Komori, et al., 2012). As collagen I is the most abundant protein in the skin, I hypothesize its biosynthesis is disrupted by UV exposure, specifically its translation and protein folding. Here, I use cultured human dermal fibroblasts and a collagen induction paradigm of TGF and vitamin C treatment to evaluate the effect of UV exposure on collagen post-transcriptional regulation.

Poster #21: Madeline Kugler

Title: Identifying the Thermodynamic Basis for DNA and RNA Binding of the Estrogen Receptor Alpha.

Authors: Madeline Kugler, Halley Steiner, Deborah Wuttke

Abstract: The estrogen receptor alpha (ERα) is a ligand-activated transcription factor that binds DNA in response to hormone treatment to regulate gene expression of processes including reproductive fertility and physiology and metabolic homeostasis. ERα drives the growth of many cancers including about 70% of breast cancers (Arnesen et al., 2021). Given its biomedical importance, an abundance of information is available on the roles of the DNA binding domain (DBD) and ligand binding domain (LBD) of Erα. Excitingly, recent evidence has confirmed the existence of a region which interacts with RNA in vitro (Steiner et al, 2022). This region, known as the hinge region, is highly basic and lies just C-terminal of the DBD. Sequence analysis comparing the ERα to other nuclear hormone receptors, as well as ERα in other organisms, has revealed certain conserved basic residues and RG motifs in the respective hinge regions of these proteins, two features commonly found in RNA-binding proteins (RBPs).

As many mutations in ERα have been directly or indirectly linked to breast cancer proliferation, among other diseases, it is important to understand how the mutations in essential, conserved regions of the protein affect nucleic acid interactions and possibly contribute to irregular cell behavior. Utilizing the sequence analysis data and new understanding of RNA-DNA interactions for ERα, this project investigates which residues of the ERα hinge region are required for DNA and RNA binding using alanine scan mutagenesis. The effects of mutations were quantified using fluorescence anisotropy to measure the tightness of interaction between protein and nucleic acid as a KD (dissociation constant). These dissociation constants were then compared to the wild type interactions to classify the mutant behavior and note any significant changes to binding. While some mutants acted similarly to wild type ERα, others demonstrated reduced binding ability, suggesting the presence of a defined RNA binding domain.

Poster #22: Ambika Basu

Title: Exploring mRNA localization and Translation Dynamics of Nuclear Periphery Localization

Authors: Ambika Basu, Erin Osborne Nishimura.

Abstract: Local translation is an understudied aspect of gene expression, cell biology, and development. Regulatory processes like mRNA degradation or appropriate binding partner interactions are known to regulate local proteome in the early embryonic stages. During Caenorhabditis elegans embryogenesis, the maternally inherited mRNA imb-2 (Importin beta-2) concentrates around the nuclear periphery together with its encoded nuclear import protein. Current fixed cell imaging systems have been able to give us information about subcellular transcriptomics and proteomics and abundance, but we need live imaging to help us understand the specific spatiotemporal control of gene expression of these maternally inherited transcripts. Live imaging in Caenorhabditis elegans comes with a lot of challenges – like mRNA decay, or aberrant sequestration of mRNAs in nucleus. Especially since imb-2 is nuclear periphery localized and may be associated with mRNA decay machinery, it becomes particularly difficult.Hence, I plan to use the existing live imaging techniques of Nascent Chain Tracking system to image spatial translation of tnpo-1 (imb-2 homolog) in human cells to determine how localization relates to ongoing translation. I would then extrapolate this information to C elegans. I studied tnpo-1 localization in cancer cell lines using single molecule fluorescent in-situ hybridization (smFISH). I observed that tnpo-1 has an increased abundance around the nucleus but is not localized like in C elegans embryo. The protein, TNPO1, however localizes to the nuclear periphery like in C elegans embryo. This is a weird observation because IMB-2 and TNPO-1 are functional homologs and both proteins show nuclear periphery accumulation. We next want to ask if tnpo-1 is localizing close to a particular cellular organelle and what is the specific spatiotemporal translation pattern observed in this transcript.

Poster #23: Steven Graham

Title: Developing Hybridization Chain Reaction for RNA in situ hybridization in Caenorhabditis elegans embryos.

Authors: Steven Graham, Dr. Erin Osborne Nishimura

Abstract: Hybridization Chain Reaction (HCR) is a technique for RNA fluorescence in situ hybridization (RNA FISH) which is brighter and lower background than traditional RNA FISH methods. The fundamental difference between HCR and legacy FISH methods is the application of fluorescently labeled metastable hairpins which polymerize off the target RNA molecule, amplifying signal. Signal amplification also enables the visualization of small RNA targets, which is not feasible with traditional FISH methods. Here, we describe methods for applying HCR to Caenorhabditis elegans embryos, an important model for studying the mechanisms of mRNA localization in early development. We compare both HCR RNA FISH and traditional RNA FISH for mRNAs with distinct localization patterns in C. elegans embryogenesis. Going forward, we will test whether HCR can be used as a tool to visualize small RNAs in C. elegans embryos, and study small RNA pathways in *C. elegans* embryogenesis.

Poster #24: Camille Goo

Title: RNA polymerase I and III subunit Polr1c in neuronal development and disease

Authors: Camille Goo, Lauren Sands, Laura White, Kristin Watt

Abstract: Ribosome biogenesis is crucial for organismal development and cellular homeostasis and is initiated by RNA Polymerases I and III (Pol I and Pol III). Pol I transcribes ribosomal RNAs (rRNAs) while RNA Pol III transcribes transfer RNAs (tRNAs), non-coding RNAs (ncRNAs), and 5S rRNA. Pathogenic variants in POLR1C, a shared subunit of Pol I and III, have been implicated in RNA Pol I-related Treacher Collins Syndrome (TCS), resulting in craniofacial differences, and RNA Pol III-related hypomyelinating leukodystrophy (HLD), characterized by reduced myelination in the brain. However, the mechanisms of how perturbations in ribosome biogenesis result in tissue-specific diseases remain unclear. To understand the developmental and molecular consequences of Polr1c disruption, we utilized a previously established polr1c mutant zebrafish model. These mutant zebrafish exhibit phenotypes consistent with TCS and HLD by 5 days post fertilization (dpf). Using in situ hybridization, we observed diminished neuronal and glial development as early as 36 hours post fertilization in polr1c-/- zebrafish. We next assessed rRNA and pre-tRNA expression levels by quantitative PCR to understand if reduced neuronal development was due to changes in Pol I and III. This revealed downregulation of pre-rRNA and some pre-tRNAs at 3 dpf. Surprisingly, analysis of tRNA sequencing data revealed no significant differences in mature tRNA abundance at 3 dpf. Together, our studies provide insights into the mechanisms of how mutations in polr1c lead to developmental diseases including TCS and HLD.

Poster #25: Charles Griffin

Title: Characterizing the role of Akt-mediated phosphorylation of Srsf3 during mouse craniofacial development

Authors: Charles W. Griffin[1], Thomas E. Forman[1], and Katherine A. Fantauzzo[1,2]
[1]Department of Craniofacial Biology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA; [2]RNA Bioscience Initiative, University of Colorado Anschutz Medical Campus, Aurora, CO, USA

Abstract: Signaling through the platelet-derived growth factor receptor alpha (PDGFRα) is critical for craniofacial development in humans and mice. Mutations in PDGFRA are associated with cleft lip/palate in humans and Pdgfra mutant mouse models similarly display midline facial clefting phenotypes. Phosphatidylinositol 3-kinase (PI3K) is the primary effector of PDGFRα signaling during skeletal development in the mouse, leading to the activation of the serine/threonine kinase Akt. We previously showed that Akt phosphorylates the RNA-binding protein serine/arginine-rich splicing factor 3 (Srsf3) downstream of PI3K-mediated PDGFRα signaling in mouse embryonic palatal mesenchyme (MEPM) cells, resulting in its nuclear translocation. We further demonstrated that ablation of Srsf3 in the mouse neural crest cell (NCC) lineage leads to severe midline facial clefting, due to defective cranial NCC proliferation and survival. Here, we identified proteins that differentially interact with Srsf3 in response to PDGF-AA ligand stimulation of MEPM cells via immunoprecipitation followed by mass spectrometry. Of the 52 proteins identified, 18 have been implicated in mitochondrial translation, including Ptcd3. We biochemically confirmed the decreased interaction of Srsf3 with Ptcd3 upon PDGF-AA ligand stimulation of MEPM cells. Further, we generated an Srsf3 phosphomutant knock-in allele (Srsf3A7) by replacing the terminal serine residue in the seven Akt consensus motifs in Srsf3 with an alanine residue. We have not recovered any Srsf3A7/A7 embryos from E8.5 through birth. To circumvent this embryonic lethality, we generated transheterozygous Srsf3A7/fl;Wnt1-Cre+/Tg embryos in which the Srsf3A7 allele is the only Srsf3 allele expressed in the NCC lineage. These embryos phenocopied Srsf3 NCC conditional knock-out embryos, with facial process hypoplasia and severe midline facial clefting at mid-gestation. Further, introduction of a ROSA26mTmG allele to examine NCC distribution revealed reduced GFP intensity in the facial processes of transheterozygous embryos at E9.5. Together, our findings provide insight into the requirement for RNA-binding protein post-translational modification during craniofacial development.

Poster #26: Hope Townsend

Title: Improving confidence of differential transcription calls in transcribed regulatory elements

Authors: Hope Townsend and Robin Dowell
Molecular Cellular Developmental Biology CU-Boulder, Computer Science CU-Boulder

Abstract: Recent studies highlight that the vast majority of disease-associated genetic variants reside in non-coding, unannotated regulatory RNAs, yet their linkage to drug targets remains limited. Transcribed regulatory elements (tREs) responsive to disease-relevant perturbations provide critical insights into gene regulation. However, current tools 1) inadequately capture their dynamic transcriptional changes and 2) cannot estimate full tRE RNA lengths for downstream annotation. These limitations result in the loss of substantial transcriptional data for downstream analysis characterizing tREs and linking variants to disease-relevant regulatory networks.
To address these challenges, we present two novel approaches. First, we adapt a Bayesian statistical model LIET to accurately predicting full tRE transcript lengths. Given this method is computationally intensive, we also develop a rapid algorithm that accurately captures tRE transcription levels while accounting for length variation and neighboring transcription. Second, we leverage the ranking of differential signals combined with motif enrichment to prioritize tREs contributing to significant TF enrichment. This TF-based method simultaneously facilitates the identification of perturbation responsive tREs and their upstream regulators.
Through these complementary methodologies, we demonstrate improved identification of perturbation-responsive tREs and enhanced mapping of regulatory networks in disease contexts. With the improved pipelines for differential tRE response analysis, we determine that many previously identified cell-type specific tREs are actually universal but were called cell-type specific because of false negatives. Additionally, we showed that our tools can allow cleaner signal assessment within datasets with technical noise breaking the assumptions of classical differential expression and TF-prediction statistical models. Overall, our approaches provide a robust framework for studying disease-relevant regulatory mechanisms and linking non-coding variants to actionable therapeutic targets across various biological systems.

Poster #27: Lily Beck

Title: Inhibition of Polycomb Repressive Complex 2 by R-loops is Rescued by SOSSB1

Authors: Lily Beck, Maggie Balas, Aaron Johnson

Abstract: The Polycomb Repressive Complex 2 (PRC2) plays an important role in epigenetic regulation of transcriptional repression. The catalytic component, EZH2, carries out trimethylation of lysine 27 on the histone H3 tail (H3K27Me3), serving as a signal to start epigenetic repression of transcription via heterochromatin. The nascent RNA produced by RNA polymerase on chromatin is an intrinsic inhibitor of PRC2 activity, because PRC2 has a higher affinity for RNAs with G-tracts than for chromatin. However, the binding of PRC2 to nascent RNA contributes to localization of PRC2 to target genes. In recent years, it has been shown that R-loops contribute to PRC2-mediated transcriptional silencing and recruits PRC2 to these sites. We initially hypothesized that formation of an R-loop with inhibitory RNA could leave PRC2 free to bind the linker DNA between nucleosomes and be catalytically active. However, our preliminary data revealed that the unpaired DNA strand in an R-loop was sufficient to inhibit PRC2. Therefore single-stranded DNA inhibition must be relieved to restore the activity of PRC2. Human Nucleic Acid Binding Protein 1 (hSSB1/SOSSB1) was discovered a decade ago as a novel single-stranded DNA binding protein. It makes up part of a heterotrimeric complex called the Sensor of Single-Stranded DNA complex 1 (SOSS1). SOSS1 forms a stable interaction with the transcription regulator Integrator-PP2A (INTAC), which localizes to R-loops to prevent genome instability. SOSSB1 preferentially binds ssDNA exposed in R-loops, therefore it is plausible that it could also serve as an activator for PRC2.

Poster #28: Georgia Barone

Title: All good things come to an end: Identifying and analyzing sites of Pol II termination.

Authors: Georgia E.F. Barone, Jacob Stanley, Hope A. Townsend, Rutendo F. Sigauke, Mary A. Allen, Robin D. Dowell

Abstract: RNA Polymerase II (Pol II) transcribes all protein-coding and many non-coding genes in the genome. Pol II transcriptional termination is crucial for proper mRNA maturation, as improper termination can lead to inefficient mRNA processing, mRNA export problems, and potentially cell death. Termination involves two intertwined processes: pre-mRNA cleavage, and Pol II release from the DNA (disassociation). Despite its importance, the exact mechanism underlying Pol II disassociation from the DNA remain poorly understood. Moreover, under certain cellular stress conditions, Pol II disassociates downstream of where it disengages from the DNA in control conditions—a phenomenon known as transcriptional run-on. The functional consequences of this shift remain unclear.

We performed the first-ever systematic analysis of Pol II termination across cell types and species and provide novel insights into the mechanism of disassociation. The position of Pol II disassociation was captured by the LIET (Loading, Initiation, Elongation, Termination [1]) model using read distributions from nascent RNA sequencing data. To understand how the position of disassociation varied across species and cell types, I looked across all high-quality control PRO-seq samples nascent sequencing samples in the DBNascent repository [2] and found the termination point is very consistent within but not between species and likely involves a sequence bias near the dissociation site. Additionally, I discovered that perturbation changes the Pol II disassociation site to be independent of sequence.

References:
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Poster #29: Katherine Matlin

Title: eIF3d and eIF3e regulate a selective translation program required for the hypoxic response

Authors: Kate Matlin, Connor Purdy, Amber Baldwin, Chris Alderman, Rui Zhao, Heide Ford, Neel Mukherjee
University of Colorado Anschutz Medical Campus

Abstract: Cancer cells are well-equipped to respond to environmental stress, including hypoxia, through the tight control of gene expression. In hypoxia, gene expression changes enable cells to survive, gain an invasive phenotype, and metastasize more efficiently. While the transcriptional response to hypoxia has been well-defined, the translational response to hypoxia is less understood but likely contributes to metastasis. In many cellular stresses, canonical translation is diminished, and to mount a proper stress response, cells rely upon specialized non-canonical translation mechanisms. The human eukaryotic initiation factor (eIF)3 complex consists of 13 proteins essential for canonical translation initiation. Recently, one of the complex members, eIF3d, was found to promote translational initiation on selective mRNAs, particularly under cellular stress, through its function as an alternative mRNA cap-binding protein. While canonical translation is attenuated in hypoxia, the identity and impact of specialized non-canonical translation is poorly understood.

We hypothesized that eIF3d and its binding partner eIF3e may drive non-canonical translation in hypoxia. To test this model in an unbiased manner, we measured transcriptome-wide changes in translation using ribosome profiling and RNA-seq in normoxic and hypoxic conditions in a metastatic breast cancer cell line (MCF7-SIX1) with or without depletion of eIF3d or eIF3e. Specifically, we calculated changes in translational efficiency (TE ~ the per transcript ratio of ribosome-protected footprints vs RNA levels) to account for mRNA expression changes across conditions (e.g. normoxia vs hypoxia, siCtrl vs si3e or si3d). We observed hundreds of changes in TE after acute 1% hypoxia (1 hr) almost completely driven by changes in ribosome occupancy and nearly no changes in RNA levels. Remarkably, depletion of eIF3e or eIF3d ablated the acute hypoxic translational changes. We also found that eIF3e and eIF3d regulated the translation of more mRNAs in hypoxia than in normoxia.

We next explored the role of eIF3e and eIF3d in hypoxia and invasion phenotypes. We found that eIF3e and eIF3d are required for upregulating HIF1alpha in 2D cell culture. Further, depletion of eIF3e or eIF3d suppressed the formation of the hypoxic core in tumorspheres and decreased invasion. To query the clinical relevance of targeting

eIF3e or eIF3d in breast cancer, we generated signatures for RNAs differentially expressed in our control cells versus eIF3e/eIF3d depleted cells. Using RNA microarray and RNA-seq data from breast cancer patients in METABRIC and TCGA patient cohorts, we calculated the enrichment of eIF3e/d signatures in patient samples and how enrichment correlated with patient survival. We found that high enrichment of the eIF3e signature correlated with worse survival rates, while the eIF3d signature did not predict survival. Finally, we identified a compound that binds eIF3e, which also ablated the acute hypoxic translational response, attenuated HIF1alpha expression, and reduced the formation of the hypoxic core in tumorspheres. Our findings suggest that inhibiting non-canonical translation through eIF3e/d could be a means to target metastasis in a cancer-cell-specific manner.

Poster #30: Megan Pockalny

Title: Investigating the mechanisms of nutrient-sensitive mRNA localization in the intestinal epithelia

Authors: Megan C. Pockalny, Raeann Goering PhD, Nathaly Limon de la Rosa, Peter Dempsey PhD, J. Matthew Taliaferro PhD

Abstract: 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, 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. We further show that the 5' TOP motif is sufficient for this mTOR-dependent localization. These findings suggest that mTOR may control nutrient-dependent dynamic RNA localization through phosphorylation of LARP1.

Poster #31: Adriana Ivich

Abstract: Accurate deconvolution of bulk RNA-sequencing data depends critically on the quality of cell-type reference profiles. However, the inherent limitations of obtaining comprehensive single-cell RNA-sequencing (scRNA-seq) data across all cell types have prompted the need for integrating single-nucleus RNA-sequencing (snRNA-seq) data as surrogates. In this study, we introduce a systematic framework that simulates missing cell types in scRNA-seq data by deliberately holding out a cell type and replacing it with transformed snRNA-seq profiles. We compare multiple transformation strategies—including PCA-based shifts, differential expression gene (DEG) filtering, and scVI-based methods (both conditional and non-conditional, including a novel local latent space shift)—to harmonize the snRNA-seq data with scRNA-seq profiles. The generated references are then evaluated using BayesPrism deconvolution of synthetic pseudobulk mixtures and by measuring their proximity to real bulk data in low-dimensional spaces. Our results indicate that the scVI-based local latent space transformation yields references that more closely resemble the true scRNA-seq profiles, as reflected by improved correlation metrics and reduced error rates. This work offers a robust, versatile approach for enhancing the accuracy of bulk deconvolution analyses through innovative reference transformation techniques.

Poster #32: Lukasz Olenginski

Title: Designing small molecules that target a cryptic RNA binding site via base displacement

Authors: Lukasz T. Olenginski, Aleksandra J. Wierzba, Shawn P. Laursen, and Robert T. Batey

Abstract: The rational design of small molecules that selectively target RNA is a longstanding problem in chemical biology but has the potential to develop chemical probes to study RNA function and therapeutics to treat RNA-mediated disease. One of the main challenges to this field is the unfavorable properties of both interacting partners. Targeting RNA is complicated by its limited chemical diversity and druggable features. RNA-binding small molecules have limited solubility, weak affinity, and/or lack of specificity, restricting the medicinal chemistry required for lead compound discovery. However, only through a robust understanding of the underlying principles governing RNA-ligand interactions can we expect to rationally design RNA-targeting small molecules in a reproducible and scalable way. Central to this understanding are strategies that enable access to privileged RNA chemical space that can be interrogated with a wide range of experimental techniques that are commonplace in developing protein-targeting small molecules. While there have been extensive efforts to meet this high standard, most attempts are narrowly focused on ligand-based approaches devoid of structural information or simply take structural “snap shots” without an emphasize on mechanism.
Our work aims to address this knowledge gap by providing a robust chemical and structural characterization of RNA-targeting ligands. We designed a library of small molecule “guests” conjugated to a cobalamin (Cbl) “host” and investigated how these ligands interact with a Cbl riboswitch. This bifunctional host-guest strategy not only solubilized ligands that are intractable to chemical analysis on their own but delivered them site-specifically to a structured RNA binding pocket. This enabled a systematic exploration of the chemical features that promote high affinity guest-target interactions through a diverse set of experimental approaches. Combining in vitro binding, cell-based assays, chemoinformatic modeling, and structure-based design, we unmasked a cryptic binding site within the riboswitch that was exploited to discover lead compounds that have affinity exceeding the native ligand and antagonize riboswitch function – and even those divorced from the Cbl host. Our results uncover new structural insights into how privileged chemical scaffolds interact with RNA, which can be leveraged for the rational design of new probes and therapeutics.

Poster #33: Kathryn Walters

Title: Determining the Mechanism of MSI2 Mediated RNA Regulation

Authors: Kathryn Walters, Amber Baldwin, Neel Mukherjee

Abstract: RNA-binding proteins (RBPs) play essential roles in post-transcriptional gene regulation, influencing mRNA stability, localization, and translation. Musashi-2 (MSI2) has been implicated in neural development, stem cell maintenance, and cancer, yet its regulatory mechanism remains unknown. Here, we investigate the role of MSI2 alternative splicing in translational regulation and identify isoform-specific functions that contribute to its diverse regulatory effects. Using a tethering assay, we demonstrate that MSI2-201 promotes translation, a function that depends on its PABP-binding domain (PBD). In contrast, we characterize MSI2-203, a previously unexamined isoform, which acts as a translational repressor. Mass spectrometry-based proteomic analysis reveals distinct MSI2 protein interactors, suggesting that MSI2-201 and MSI2-203 exert their effects through interactions with different cofactors. Finally, we validate these findings across multiple cell lines and demonstrate that while MSI2-201 consistently promotes translation, MSI2-203 exhibits cell-type specific repression. Our results highlight the role of alternative splicing in MSI2-mediated gene regulation and suggest that isoform switching may contribute to the context-dependent regulatory effects observed in MSI2-related biological processes and disease states. These findings provide new insights into the molecular mechanism governing MSI2 function and highlight the need for isoform-specific analyses in studies of MSI2-mediated regulation.

Poster #34: Erin Richards

Title: Improvement of Riboglow, a fluorescent RNA tag for live-cell imaging

Authors: Erin Richards, Ola Wierzba, Shelby Lennon, Rob Batey, and Amy Palmer

Abstract: The proper localization of an RNA in the cell and the proper timing of this process are fundamental for that RNA's function, its downstream protein's function, overall cellular function, organism development, and organism health. Over the last 30 years, many tools have been developed to track RNA in living cells. The Palmer and Batey labs at CU Boulder collaborated to create Riboglow which is comprised of two parts: a robustly folding cobalamin riboswitch and a modular cobalamin-containing fluorescent probe. In the first generation of the Riboglow probe, a simple linker connects cobalamin to the fluorophore. In the second generation, the nonfunctional linker is replaced with a peptide nucleic acid (PNA) sequence designed to hybridize with a ssRNA region of the cobalamin riboswitch. This new linker increases binding affinity by a sequence-specific interaction and drastically increases performance and usability of Riboglow in live-cell assays. Current work aims to decrease the repetitiveness of the array of riboswitches linked in series necessary to see target RNA above background signal in live-cell imaging.

Poster #35: Frank Lee

Title: LPS exposure decreases SRSF1 expression to modulate MyD88 alternative splicing

Authors: Frank Lee[1,2] and Scott Alper[1,2]
[1]National Jewish Health Department of Immunology and Genomic Medicine and The Center for Genes, Environment, and Health
[2]University of Colorado Department of Immunology and Microbiology

Abstract: Toll-like receptor (TLR) signaling is required to fight infection, but chronic TLR signaling can contribute to inflammatory disease. In the presence of lipopolysaccharide (LPS), a bacterial cell wall component, TLR4 transduces signals through an adaptor protein, MyD88, to drive the immune response. MyD88 pre-mRNA can be alternatively spliced into two isoforms: (1) the canonical 5 exon long mRNA (MyD88-L) which promotes immune signaling and (2) a shorter splice form (MyD88-S) in which exon 2 is skipped, and which inhibits immune signaling. Expression of the inhibitory MyD88-S isoform can be induced by LPS exposure; thus LPS-induced production of MyD88-S is thought to form a negative feedback loop that limits chronic signaling and prevents inflammatory disease. We previously demonstrated that the RNA binding protein SRSF1 binds to MyD88 pre-mRNA exon 2 and that the binding of SRSF1 promotes exon 2 inclusion in the mature mRNA (production of the MyD88-L isoform). Moreover, we found that LPS inhibited the binding of SRSF1 to MyD88 pre-mRNA, likely because LPS decreased SRSF1 mRNA and protein levels. We have now found that LPS has opposing effects on SRSF1 mRNA levels, both decreasing SRSF1 promoter activity but increasing SRSF1 mRNA stability. These opposing effects of LPS on SRSF1 mRNA levels lead to an overall net decrease in SRSF1 production, which we hypothesize drives the LPS-induced production of MyD88-S. We are currently investigating the mechanisms used by LPS to mediate decreased SRSF1 expression.

Poster #36: Jessica Finlay-Schultz

Title: Maf1 Cooperates with Progesterone Receptor to Repress RNA Polymerase III Transcription of tRNAs

Authors: Jessica Finlay-Schultz, Kiran V. Paul, Benjamin Erickson, Lynsey M. Fettig, Benjamin S Hastings, Deborah L. Johnson, David Bentley, Peter Kabos, and Carol A. Sartorius.

Abstract: 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 demonstrated that PR is associated with the Pol III complex at tRNA genes and that progestins downregulate tRNA transcripts in breast tumor models. To further elucidate the mechanism of PR-mediated regulation of Pol III, we studied the interplay between PR, the Pol III repressor Maf1, and TFIIIB, a core transcription component. ChIP-seq was performed for PR, the Pol III subunit POLR3A, the TFIIIB component Brf1, and Maf1 in breast cancer cells with or without progestin treatment. Upon progestin exposure, PR localized to approximately half of POLR3A-occupied tRNA genes, with Maf1 co-recruited to many of these PR-POLR3A sites. While progestin treatment did not significantly alter the number of tRNA genes occupied by Pol III or Brf1, Brf1 occupancy was stabilized, as indicated by increased peak amplitudes. Analysis of nascent tRNA transcription revealed a specific progestin-induced downregulation of approximately one-third of highly expressed tRNA genes. This repression was significantly reduced by Maf1 knockdown, indicating that Maf1 is necessary for PR-mediated tRNA transcription downregulation. Overall, these findings demonstrate a ligand-dependent PR-mediated repression of tRNA transcription through Maf1. 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.

Poster #37: Junqiao Zhu

Title: Mechanism and consequence of RNA duplex formation within the exit channel of RNA polymerases

Authors: Junqiao Zhu, Michael Palo, Robert Landick, Aaron Hoskins

Abstract: During the co-transcriptional folding of nascent RNA, the regulatory interplay between RNA polymerase (RNAP) and nascent RNA structure is a “two-way street.” RNA structures forming within the RNA exit channel interact with the polymerase to influence RNA synthesis. Conversely, RNAP itself can impact RNA structure folding within the channel. Understanding this interplay enhances our knowledge of RNAP as a potential RNA-folding chaperone and the connection between transcription, RNA folding, and processing. To unravel this interplay, we utilized single-molecule and biochemical bulk experiments to study RNA duplex formation within the exit channel of bacterial (E. coli) and eukaryotic (S. cerevisiae) RNAPs and its functional consequences on transcription.
Single-molecule experiments showed that RNA exit channel impacts RNA structure formation kinetics, depending on the structure’s thermodynamic stability and the complementary RNA sequence’s position. Distinct behaviors were observed between E. coli and S. cerevisiae RNA exit channels. Biochemical bulk experiments revealed that RNA exit-channel duplex formation affects transcription pausing in opposite ways in E. coli versus S. cerevisiae RNAPs. In E. coli, duplex formation stabilizes the swiveling module, facilitating pausing, while in S. cerevisiae, it inhibits pausing, potentially by preventing backtracking.

Poster #38: Aileen Button

Title: MEG3 Regulates Activation of Fibroblasts in IPF

Authors: Aileen C. Button*1, Ivana V. Yang1, David A. Schwartz1; 1Department of Medicine, University of Colorado, Aurora, CO, United States

Abstract:
Introduction:
Idiopathic pulmonary fibrosis (IPF) is a chronic progressive fatal fibrosing interstitial pneumonia, characterized by the relentless progression of respiratory insufficiency. IPF is characterized by patterns of usual interstitial pneumonia and hallmark lesions of fibroblastic foci and honeycomb cysts. These lesions represent transitional and end-stage disease, respectively. Despite the progress from extensive work on the protein-coding genes in IPF, there is no clear explanation for the molecular heterogeneity of IPF or the low penetrance of risk variants in this rare disease. We are working to bridge this gap by investigating the non-coding transcriptome of IPF and the regulatory pathways of the disease. As a result of this work, we have found that a long non-coding RNA, MEG3, is highly overexpressed in the fibroblastic foci of IPF. MEG3 has been implicated in cellular senescence and upregulates p53. We have found that MEG3 regulates fibroblasts in IPF by controlling the expression of p53.
Methods:
Published fibroblast scRNA-seq datasets were re-analyzed to identify dysregulated long non-coding RNA (lncRNA) between IPF and control lungs. These results were validated by RNAscope in IPF and control lung tissues. Primary fibroblasts were isolated from control and explanted IPF lungs or IPF biopsy tissue and used for in vitro assays to determine the mechanisms of MEG3 overexpression and control of p53 in IPF.
Results:
MEG3 is highly overexpressed within the fibroblastic foci. This result was validated across multiple forms RNAseq (scRNAseq - GSE136831, unpublished CosMx from Schwartz/Yang lab, bulk RNAseq of micro-dissected fibroblastic foci - GSE169500) and RNAscope of FFPE samples from transitional regions of IPF lungs. Fibroblasts expressing MEG3 show co-expression of markers of senescence (including p21) by scRNAseq and western blot. Induction of MEG3 can be recapitulated in primary IPF fibroblasts by inducing senescence via serum starvation or DNA damage with doxorubicin or bleomycin. We have found that GapmeR-based knockdown of MEG3 alters primary IPF fibroblasts' phenotype and modulates p53 activity.
Conclusions:
We have identified the lncRNA MEG3 as a significant player in the pathophysiology of IPF, particularly within the fibroblastic foci characteristic of the disease. The

dysregulation of MEG3 in these regions appears to influence the fibroblast response to p53, a critical regulator of cellular senescence and apoptosis. This interplay suggests that MEG3 not only contributes to the altered cellular dynamics observed in IPF but may also play a pivotal role in the transition from early-stage fibrosis to more advanced, irreversible forms of the disease.

The ability of MEG3 to modulate p53 activity highlights its potential as a therapeutic target for IPF. Targeting MEG3 may provide a novel approach to address the molecular heterogeneity of the disease and enhance treatment efficacy. Our findings underscore the importance of non-coding RNAs in the complex regulatory networks of fibrotic diseases.

Poster #39: Elizabeth Spear

Title: The structural basis for coordination between tandem exonuclease resistant RNAs (xrRNAs) in Dengue Virus

Authors: Elizabeth Spear, Zoe O'Donoghue, Steve Bonilla, Samantha Zangari, Kate Segar, Madeline Sherlock, Andrea MacFadden, & Jeffrey S. Kieft

Abstract: Flaviviruses are positive-sense single-stranded RNA viruses whose genomes contain functionally important structured RNA elements. These include exoribonuclease-resistant RNAs (xrRNAs), structures that reside at the beginning of the 3' untranslated region (UTR) where they prevent degradation of the downstream sequence by host 5' to 3' exoribonucleases such as Xrn1. This incomplete degradation results in accumulation of infection important non-coding subgenomic flavivirus RNAs (sfRNAs). Many flaviviruses contain two tandem xrRNAs, and multiple studies reveal that in dengue virus serotype 2 (DV2) the function of the tandem xrRNAs are mysteriously coupled. We hypothesized that there are important molecular interactions taking place between the two xrRNA structures that facilitate specific sfRNA biogenesis patterns during infection. We therefore tested the effect of mutations that altered (1) the order of the xrRNAs, (2) the spacing between them, or (3) the sequence of a short 'single-stranded' intervening linker, using both infections and a novel reporter system in mammalian and insect cells. These studies suggested that an A-rich linker region between the tandem xrRNAs is necessary for coupling the activities of the two xrRNAs, and therefore for determining the patterns of sfRNA formation. Also, the coupling occurs outside of authentic infection – it is an inherent feature of the RNA structure itself. Further exploration of these mutant tandem xrRNA structures with chemical probing and small angle X-ray scattering revealed that increasing the local flexibility of the A-rich linker propagates to affect the global shape of the tandem xrRNAs, and cryo-electron microscopy reveals that this linker may act to favor a specific 3-D orientation of the two xrRNAs. In summary, these studies are now the first explanation for how different structural elements in the DV2 3' UTR can communicate and points the way to further exploration of how the global architecture of flavivirus 3' UTRs influence infection.

Poster #40: Clarence Mills

Title: Tight temporal control over CRISPR activation through dual transcriptional and post-translational regulation of dCas9-VPR expression

Authors: Clarence Mills[1], Brian Ziemba[1], Johanna De Castro Arce[1], Allyson Malloy[1], Andrew Riching[1], Emily Anderson[1], Josien Levenga[1]
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Abstract: CRISPR-Cas9 has been widely adapted for use in transcriptional modulation and epigenetic engineering with deactivated Cas9 (dCas9) systems to enable CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) applications. However, few of these systems provide researchers with the ability to control the timing of CRISPR-mediated transcriptional modulation. Here we describe a novel small molecule-inducible system for potent, stringently regulated CRISPR activation. The dCas9-VPR CRISPRa effector is fused to an FKBP12-derived destabilizing domain and expressed from a Tet-inducible promoter to prevent transcription and stable translation of dCas9-VPR in the absence of the doxycycline and Shield1 ligand. We show that in combination, the Tet-On system and the FKBP12-derived destabilizing domain minimize basal target gene activation (leakiness) while maintaining robust induction across gene targets and cell types. Furthermore, the inclusion of the destabilizing domain enables a degree of temporal control that is not possible with the Tet-On system alone. We then demonstrate how this dual regulated dCas9-VPR can be applied in human induced pluripotent stem cells (hiPSCs) to overexpress key proneural transcription factors. In addition, we present a proof-of-concept model where siRNA and CRISPRa are simultaneously used to suppress or enhance target genes governing drug resistance in populations of drug-resistant cancer cell lines. By strategically combining a selection of these siRNA and CRISPRa resistance targets, we observe a synergistic effect, further restoring drug sensitivity in these resistant cell lines and underscoring how siRNA and CRISPRa can be paired within a single experiment for pathway-based screening and drug target identification.

Poster #41: Jeffrey Krall

Title: Biophysical Characterization of the Nucleic Acid Binding Domains of Z-DNA Binding Protein 1 (ZBP1)

Authors: Jeffrey B. Krall, Lily G. Beck, Parker J. Nichols, Quentin Vicens, Morkos A. Henen, Beat Vögeli

Abstract: Z-DNA Binding Protein 1 (ZBP1) is a critical pattern recognition receptor within the innate immune response to viral infection. ZBP1 senses foreign nucleic acids in the unusual, left-handed Z-conformation via binding through its N-terminal Zα1 and Zα2 domains and activates downstream pro-necroptotic and -apoptotic pathways to initiate cell death and allow for viral clearance. Both dsDNA and dsRNA can adopt the Z-conformation, however, the conformational change is energetically expensive, especially for dsRNA, and requires chemical modifications or protein binding to induce a right-to-left-handed conversion and stabilization. ZBP1 has been previously shown to bind and convert B-DNA to the Z-conformation and was assumed to be able to convert A-RNA as well, despite the lack of experimental validation. Here, we use a variety of NMR and other biophysical experiments to characterize the Z-DNA and Z-RNA binding properties of ZBP1's Zα1 and Zα2 domains. While ZBP1's Zα domains are able to convert and stabilize unmodified dsDNA in the Z-conformation, both domains are incapable of flipping unmodified A-conformation dsRNA. We show that ZBP1's Zα domains require dsRNAs with Z-promoting chemical modification in order for them to bind and stabilize the Z-conformation. These results contrast with the Zα domain from Adenosine Deaminase Acting on RNA 1 (ADAR1), which can bind and flip both dsDNA and dsRNA into the Z-conformation, potentially indicating finely tuned competition between ADAR1 and ZBP1 for pro-survival and pro-death outcomes, respectively. This works highlights the functional variability of Zα domains and narrows down the potential physiological substrates of ZBP1 in infection and disease.

Poster #42: Liyi Cheng

Title: rG4 & DHX36: Stress Granule Modulators?

Authors: Li Yi Cheng, Nina Ripin, Thomas R. Cech, Roy Parker

Abstract: Stress granules are RNA-protein condensates that form in response to an increase in untranslating mRNPs. Stress granules form by the condensation of mRNPs through a combination of protein-protein, protein-RNA, and RNA-RNA interactions. Several reports have suggested that RNA sequences capable of forming G-quadruplexes promote stress granule formation. Here, we provide three observations arguing that rG4 motifs do not promote mRNAs partitioning into stress granules in human osteosarcoma cells. First, we observed no difference in the accumulation in stress granules of reporter mRNAs with and without rG4-forming sequences in their 3' UTRs. Second, in U-2 OS cell lines with reduced DHX36 expression, which is thought to unwind G-quadruplexes, the partitioning of endogenous mRNAs was independent of their rG4-forming potential. Third, while mRNAs in stress granules initially appeared to have a higher probability of forming rG4s than bulk mRNAs, this effect disappeared when the abundance of rG4s was standardized by mRNA length. However, we observe that in a G3BP1/2 double knockout cell line, reducing DHX36 expression rescued stress granule-like foci formation. This indicates that DHX36 can limit stress granule formation, not by limiting RNA partitioning, but potentially by unwinding trans rG4s, or limiting other intermolecular RNA-RNA interactions that promote stress granule formation.

Poster #43: Daniella Ugay

Title: Transcription factor GATA1 binds RNA through its DNA-binding domain

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

Abstract: A growing number of 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, which play essential roles in hematopoiesis and hematopoietic disorders, including high mutation rates in Down syndrome-associated leukemias, are previously unrecognized RNA-binding proteins, suggesting this TF family has a potential RNA-dependent gene regulation function. The scope, specificity, and precise functional relevance of RNA interactions with GATA-family TFs are yet to be determined. To address this issue, we have set out to investigate RNA binding of GATA1 TF. and have discovered that GATA1 is capable of binding small RNAs with diverse structural features in vitro through its DNA-binding domain (DBD). Moreover, GATA1 binds DNA and RNA competitively through a shared surface that spans the zinc-finger motifs and an arginine-rich motif (ARM) like domain. Although this ARM-like domain has previously been proposed to function as independent noncanonical RNA-binding domain (RBD), we found that ARM-like domain of GATA1 contributes to both DNA and RNA binding in distinct modes: high-affinity and high-specificity binding mode for DNA and plastic recognition of RNA driven by electrostatics. This competitive binding ability suggests malleable functionality of DBDs in nucleic acid recognition, expanding molecular mechanisms of protein-RNA interactions beyond canonical RBDs. Functionally, competitive DNA/RNA binding by GATA1 may provide riboregulatory mechanisms for GATA1-mediated gene expression during hematopoiesis and account for disease-related phenotypes that cannot be attributed to abnormal DNA- and/or protein-associated malfunctions.

Poster #44: Rhiannon Sears

Title: Visualizing Co-Translational Folding at Single mRNA Resolution Live

Authors: Rhiannon M Sears, Luis Aguilera De Lira, Jake Yarbro, and Ning Zhao

Abstract: Proteins are essential molecules of life that maintain proper function through their proper folding. The folding of many proteins occurs as early as co-translationally and involves the orchestration of numerous cellular factors, including a large set of ribosome-associated proteins and chaperones. In co-translational folding, the folding is intimately coupled to translation. If the ribosome elongates too fast or stalls for too long at the wrong place and time, folding kinetics can be severely perturbed, leading to misfolding and/or aggregation. In extreme cases, this can lead to diseases. A better understanding of protein co-translational folding kinetics in the native context of translation is therefore critical to human health. The major challenge in the field is the lack of spatiotemporal resolution needed to track and quantify co-translational folding in a living intracellular environment. To address this challenge, we have developed a novel technology that enables directly visualizing co-translational folding with single mRNA resolution in living cells. With this technology, we will investigate co-translational folding kinetics in living cells and further investigate an ongoing central question in the field – how alterations in translation elongation rate affect folding. The study will help us to further understand co-translational folding process and investigate its regulatory mechanism, which has the potential to lead to new therapeutics that have never been explored before for protein misfolding-related diseases.

Poster #45: Subbaiah Chalivendra

Title: Yeast PRP40 may regulate transcription elongation, besides its role in splicing

Authors: Subbaiah Chalivendra, Xueni Li, Shasha Shi, John Rossi, Matthew Taliaferro, Rui Zhao
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Abstract: Although pre-mRNA splicing is depicted as a post-transcriptional event, studies have shown that splicing and transcription are interdependent and concurrent processes. For example, in yeast and other eukaryotes, RNA pol II (RNAPII) is shown to stall until splicing of a proximal intron is completed before further elongation. PRP40, an essential component of yeast U1 snRNP (U1), is predicted to play a role in the regulation of RNAPII activity in the elongation phase. To test this proposal, we generated a yeast strain where PRP40 is depleted using an inducible system (PRP40D) and are analyzing the effects on U1 composition, splicing, transcript profiles and interactions with known transcription elongation/termination factors. In addition to growth defects, PRP40 depletion led to the loss of U1 snRNP integrity, recapturing the effect of Luc7 loss reported earlier. U1 preparation from PRP40 depleted strain also lost Luc7 and Snu71, suggesting that these three proteins associate with the U1 only as a trimer but not individually. We are currently confirming this by In vitro reconstitution studies. PRP40 depletion also led to a poor association of RNAPII with U1, which was correlated with hyperphosphorylation of the RPB1 C-terminal domain (CTD). RT-PCR studies show that these changes in RNAPII were further associated with transcriptional readthrough in addition to splicing defects. We are analyzing the extent of these defects by RNA-seq studies. NPL3, known to co-transcriptionally recruit U1 and regulate transcription elongation by RNAPII via its interaction with the phospho-CTD, also showed decreased association with U1 in PRP40D. A mutant defective in the dephosphorylation of NPL3 was shown to poorly bind phospho-CTD, resulting in an extensive transcription readthrough. Both CTD and NPL3 are known to be dephosphorylated by GLC7, a protein phosphatase that is required for transcription termination. We speculate that the recruitment of GLC7 to the transcription elongation complex in PRP40D is impaired and leads to hyperphosphorylation of both CTD and NPL3 affecting of transcription termination.