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Rocky Mountain RNA Symposium

Friday, April 14
2023

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Featuring Rising Stars in RNA Research

Siggy Nachtergaele, PhD
Yale

Susan Shao, PhD
Harvard

Derrick Morton, PhD
USC

Ariel Bazzini, PhD
Stowers Institute

Chloe Barrington, Doctoral Student, Rissland Lab, CU – Anschutz

Thomas Forman, MD/PhD Student, Fantauzzo Lab, CU – Anschutz

Dylan Parker, PhD, Postdoctoral Fellow, CU – Boulder

Maddie Sherlock, PhD, Postdoctoral Fellow, Kieft Lab, CU – Anschutz

Naly Torres, Doctoral Student, Osborne Nishimura Lab, CSU

scan me for the symposium guide *aka* abstracts

agenda

Friday
April 14, 2023

8:00	am	Check In/Poster Set Up
8:55	am	Introductory Remarks
9:00	am	***Sigrid Nachtergaele, PhD***; Yale University
9:45	am	Dylan Parker, PhD; University of Colorado – Boulder
10:05	am	Naly Torres; Colorado State University
10:30	am	BREAK
11:00	am	Chole Barrington; University of Colorado – Anschutz
11:20	am	***Ariel Bazzini, PhD***; Stowers Institute
12:05	pm	LUNCH
1:30	pm	***Derrick Morton, PhD***; University of Southern California
2:15	pm	Thomas Forman; University of Colorado – Anschutz
2:45	pm	POSTER SESSION
3:55	pm	Madeline Sherlock, PhD; University of Colorado – Anschutz
4:15	pm	***Sichen Shao, PhD***; Harvard University
5:00	pm	POSTER Awards and Closing Remarks
6:00	pm	Networking Social, Stanley Beer Hall

COLORADO RNA CLUB

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Speaker Abstracts

In Order of Presentation

Sigrid Nachtergaele, PhD
Assistant Professor, Yale University

Gene expression regulation by RNA modifications

Chemical modification of RNA is a critical mechanism of gene expression regulation, controlling RNA processing, stability, and, in the case of mRNA, translation. The chemical diversity of RNA modifications suggests that there is extensive biology yet to be uncovered. A recent surge in this field has been driven by rapid advances in high throughput sequencing methods that allow us to map these marks on a transcriptome-wide scale. However, many of these studies remain correlative and are not able to reveal the molecular mechanisms of RNA modification function or regulation. The information we glean from such studies often represents an average across all transcripts in the cell, and does not take into account the spatial organization or temporal control of RNA transcription, processing, and trafficking. Moreover, the majority of work on mRNA modifications has focused on a single modification, N6-methyladenosine (m6A), which represents only one of over one hundred modifications annotated to date. Our work seeks to address fundamental questions about mRNA modifications, to expand beyond m6A-based studies, and to develop the tools to study a broader diversity of RNA modifications and their functions.

Dylan Parker, PhD
Postdoctoral Fellow, Parker Lab, University of Colorado – Boulder

G3BP1 functions as an RNA condenser by forming RNA-RNA interactions in stress granules

RNP granules are a well-studied class of biomolecular condensates requiring both RNA and proteins for their assembly. However, the cellular mechanisms and molecular interactions driving separation of RNP granules from the bulk cytoplasm remains incompletely understood. Stress granules are RNP granules that form upon stress induced increases in non-translating mRNPs, and due to their reversibility are a tractable model for understanding RNP granule assembly. The G3BP proteins are critical factors in stress granule assembly. A variety of mechanisms have been postulated for stress granule organization focusing primarily on the multivalent interactions of G3BP1 and G3BP2 with other stress granule proteins and RNA to form mesoscale RNP granules. We now demonstrate an additional role for G3BP1 in triggering RNP granule assembly by promoting the formation of intermolecular RNA::RNA interactions. We show that after the initial formation of RNP granules, G3BP1 is dispensable both in vitro and in vivo for persistence of the RNA component of granules suggesting that G3BP1 is only required after initial stress granule formation to counteract RNA decondensing machinery such as the ATP-driven DEAD-box proteins.[DP1] These results identify a general mechanism by which RNP granule-specific RNA binding proteins promote RNP granule assembly through "RNA condenser" activity by catalyzing the formation of intermolecular RNA interactions. Moreover, the stability of RNA-only granules in the absence of RNA binding proteins highlights the need for active mechanisms to limit condensate stability and lifetime. This finding has significant implications for diseases related to excessive formation and runaway aggregation of RNP granules.

Naly Torres
Doctoral Student, Osborne Nishimura Lab, Colorado State University

Drugging the embryo: How eggshell permeabilization helps us investigate the cytoskeletal impact on mRNA movement

Translation typically occurs in the cytoplasm or the endoplasmic reticulum. However, erm-1 (ezrin/radixin/moesin) mRNA concentrates at the plasma membrane where the protein it encodes connects the plasma membrane to the actin cytoskeleton. This molecular event serves to coordinate cell shape changes. Indeed, other transcripts that encode membrane-associated proteins also concentrate at the plasma membrane, but neither the mechanisms directing their localization nor the reasons for their local translation are understood. Previously, we determined that erm-1 mRNA localization to the membrane is translation-dependent and directed by its encoded N-terminal FERM-domain. Here, we test whether erm-1/ERM-1 localization occurs through active

or passive mechanisms, and we explore which cytoskeletal components are required for their transport. Therefore, we developed an eggshell permeabilization strategy, followed by drug treatment and then gentle smFISH. Using this approach, we tested the effect of Nocodazole (microtubule loss) or Cytochalasin D (actin loss) on mRNA localization. Using C. elegans, we can further investigate whether motor proteins are involved in mRNA transport process using both smFISH and live mRNA imaging techniques. Our data suggests that dynein, but not kinesin, perturbs RNA localization, though still unclear whether this effect is direct or indirect. This work is relevant because impaired mRNA localization in neurons and other cell types causes disease but studying mRNA localization in disease-specific models is challenging. Furthermore, the process of directing translation-dependent localization of mRNA to plasma membranes is a distinct and novel method of performing local translation, one whose mechanisms and impacts we aim to better understand.

Chloe Barrington
Doctoral Student, Rissland Lab, University of Colorado – Anschutz Medical Campus

Potent regulation of gene expression by the open reading frame

Non-optimal synonymous codon usage hinders gene expression, but the mechanisms by which this occurs are poorly understood. We and others have previously shown that non-optimal codons slow translation elongation speeds and thereby trigger mRNA degradation. Nevertheless, transcript levels are often insufficient to explain protein levels, suggesting there are additional mechanisms by which codon usage regulates gene expression. Using reporter assays in human and Drosophila cells, we found that transcript levels account for less than half of the variation in protein abundance. This discrepancy is explained by translational differences. With bulk and single-molecule imaging assays, we show that non-optimal transcripts are bound by fewer ribosomes, and reduced translation initiation is responsible. Non-optimal transcripts are also less bound by the key translation initiation factors eIF4E and eIF4G, providing a mechanistic explanation for their reduced initiation rates. Importantly, repression can occur in the absence of mRNA decay and doesn't depend on the non-optimality sensor, CNOT3. Our results reveal a potent new mechanism of regulation by codon usage, where non-optimal codons repress further rounds of translation.

Ariel Bazzini, PhD
Associate Professor, Stowers Institute for Medical Research

Post-transcriptional gene regulation in vertebrates

The Bazzini-lab studies how genes are regulated at the post-transcriptional level in vertebrates. Basically, what molecular mechanism dictate the stability and the level of translation of mRNA in human cells and zebrafish embryos? And how these regulations affect development (zebrafish embryos) and human diseases? Specifically, we are looking for brilliant students interesting in working in translation regulation mediated by small ORF. Our group demonstrated that translation affect mRNA stability in a codon dependent manner in zebrafish embryos and human cells, a mechanism called codon optimality. Then, we discovered that translation of small ORF in the 3'UTR (dORF) enhances translation of the canonical main ORF in zebrafish and human cells. And recently, we found the first method to trigger mRNA knockdowns in zebrafish embryos based on a Cas13d system. These are the three main research avenues of the lab.

Derrick Morton, PhD
Assistant Professor, University of Southern California

Investigating RNA dysregulation in complex brain disorders

The complexity of the transcriptome poses a challenge to cells because they must manage RNA processing events - degrading surplus and defective RNAs - without destroying functional RNA molecules. Remarkably, a multi-subunit post-transcriptional regulatory machine, the RNA exosome, performs these two divergent RNA processing roles: functional maturation and quality-control driven destruction. The RNA exosome complex is an essential and ubiquitous ribonuclease that is critical for proper processing and degradation of a variety of cellular RNAs. How the RNA exosome mediates precise processing of some RNA targets, yet complete destruction of others, is not understood. A currently favored theory is that distinct RNA exosome co-factors recruit specific RNAs to the complex. However, it is not known whether the same cofactors also guide RNA exosome activity

on recruited RNAs. The recent discovery that recessive mutations in genes encoding distinct structural RNA exosome subunits cause a variety of neurodevelopmental disorders makes defining the role of the RNA exosome in neurodevelopment critically important to understand the basis of these diseases. The primary research goal of my lab is to study the fundamental mechanisms of post-transcriptional regulation of gene expression with an emphasis on RNA processing factors mutated in complex brain disorders. Specifically, we are interested in the post-transcriptional activities of the RNA exosome in human neurodevelopment and disease. Thus, we have taken the strategy of coupling in vivo Drosophila genetics with in vitro human iPSC-derived brain organoid models to understand how defects in subunits of the ubiquitous RNA exosome complex cause tissue-specific consequences.

Thomas Forman
MD/PhD Student, Fantauzzo Lab, University of Colorado – Anschutz Medical Campus

Alternative RNA splicing downstream of PDGFRα signaling in craniofacial development

Signaling through the platelet-derived growth factor receptor alpha (PDGFRα) 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 PDGFRα 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 PDGFRα 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 midline facial clefting, due to defects in proliferation and survival of cranial neural crest cells, and over a thousand differential alternative RNA splicing events. Here, we demonstrate via enhanced crosslinking and immunoprecipitation (eCLIP)-seq analysis of MEPM cells that PDGF-AA stimulation leads to preferential binding of Srsf3 to exons. Further, an unbiased motif enrichment analysis of Srsf3 binding sites revealed a loss of binding to canonical Srsf3 CA-rich motifs in stimulated samples, which could be due to changes in ribonucleoprotein composition or loss of RNA-binding due to electrostatic repulsion. Through analysis of complementary RNA-seq data, we show that the subset of transcripts that are bound by Srsf3 and undergo alternative splicing upon PDGFRα signaling commonly encode regulators of Wnt signaling, a pathway known to be critical for mammalian craniofacial development. Taken together, these findings provide considerable insight into the mechanisms underlying gene expression regulation during craniofacial development.

Madeline Sherlock, PhD
Postdoctoral Fellow, Kieft Lab, University of Colorado – Anschutz Medical Campus

Structural basis for translation termination-reinitiation at overlapping open reading frames in viruses

The paradigm for eukaryotic translation dictates that each mRNA contains a single open reading frame (ORF) encoding one protein, but growing evidence suggests translation frequently occurs outside the main coding region. Under certain circumstances, failed ribosome recycling events lead to translation of a downstream ORF through reinitiation. Certain viruses, including norovirus and influenza B virus, use RNA structures embedded in the upstream coding region to induce reinitiation events and express essential viral proteins. These reinitiation-promoting viral RNA structures contain sequences that allow direct interactions with the ribosome following termination. The structure of reinitiation-promoting RNA representatives from different viruses as well as their mechanism of action have remained largely uncharacterized. By performing homology searches we found this RNA motif in viruses beyond those previously described. The highly-conserved base paired portions and the location of the ribosome binding nucleotides of multiple representatives of this viral RNA motif are supported by chemical probing data. Translation assays in lysate using dual luciferase reporters reveal that RNAs from different viruses reinitiate with variable efficiency due to differences in the RNA structure, relative affinity for the 40S subunit, and the stop codon context. Using single-particle cryoEM analysis, we determined the structure of a reinitiation-promoting viral RNA in complex with a mammalian 80S ribosome, confirming the binding site of the RNA on the 40S subunit. This structure lays the groundwork to understand how this class of viral RNA can prevent complete ribosome recycling and promote a reinitiation event at a specific downstream start codon.

Susan (Sichen) Shao, PhD
Associate Professor, Harvard Medical School

Mechanisms of small molecule translation inhibitors

Translation termination is an essential cellular process that is also of therapeutic interest for diseases that manifest from nonsense mutations. In eukaryotes, translation termination requires eRF1, which recognizes stop codons, catalyzes the release of nascent proteins from ribosomes, and facilitates ribosome recycling. Several small molecules have been reported to trigger eRF1 degradation, which in turns improves the translational readthrough of premature stop codons. However, the mechanism of action of these small molecules is poorly understood. Here, we report cryogenic electron microscopy (cryo-EM) structures showing that one such molecule glues the N domain of eRF1 involved in stop codon recognition to the ribosomal subunit interface near the decoding center. Aberrant stabilization of eRF1 on ribosomes leads to eRF1 ubiquitylation and a higher frequency of translation termination at near-cognate stop codons. Our findings raise new considerations for pharmacologically targeting translation termination.

posters

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Poster Abstracts

In Alphabetical Order by Presenting Author (in bold)

01

Improving the design of single-mRNA nascent chain tracking experiments by integrating mechanistic models and machine learning

Luis U. Aguilera, William Raymond, Tatsuya Morisaki, Timothy J Stasevich, and Brian Munsky

Colorado State University, Fort Collins, Colorado

Nascent Chain Tracking (NCT) is a powerful tool for studying the dynamics of single mRNA transcripts during translation. NCT has been proven to be effective in measuring crucial biophysical parameters such as elongation rates, initiation rates, and ribosomal densities. Furthermore, NCT has been used to investigate both canonical and non-canonical ribosomal translation processes. Despite its numerous advantages, NCT has limitations due to the limited number of signals most microscopes can detect, making it challenging to study multiple distinct RNA signals simultaneously. To overcome this challenge, we have developed a computational pipeline that integrates mechanistic models with machine learning algorithms to classify mRNA species based on their changing fluorescence intensity signals. Our proposed pipeline can guide the design of experiments to enable the discrimination of different types of translating mRNA in the same cell. Overall, this innovative approach promises to expand the use and applicability of NCT, providing new avenues for the investigation of mRNA translation and its regulation.

02

Sexual RNA replication among picornaviruses

Brian J Kempf[1], Elizabeth Jaworski[2], Andrew L Routh[2], Olve B Peersen[3] and **David J Barton**[1]

[1]Department of Immunology and Microbiology, University of Colorado School of Medicine
[2]Department of Biochemistry and Molecular Biology, University of Texas Medical Branch
[3]Department of Biochemistry and Molecular Biology, Colorado State University

Picornaviruses have both asexual and sexual replication strategies. Asexual RNA replication, with one parental template, is fast and efficient, producing vast amounts of virus. Sexual RNA replication (aka recombination), with two parental templates, is infrequent and inefficient; however, it counteracts error catastrophe and mediates genetic exchange between related viral (sub)species members. We hypothesize that sexual RNA replication involves an extended primer grip near the active site of the polymerase (Kempf et al. 2020). Crystal structures of $3D^{pol}$-RNA elongation complexes from the Peersen lab show six base pairs of dsRNA make direct contacts with the polymerase, and residues L420, M392 and K375/R376 constitute an extended primer grip that interacts with the viral RNA product as it exits the active site. An L420A polymerase mutation in the extended primer grip specifically inhibits sexual RNA replication but has no effect on asexual RNA replication. In humans, poliovirus recombines with related group C enteroviruses, especially subspecies C2 and C3 viruses (Brouwer et al. 2020; Smura et al. 2014). Co-infection of HeLa cells with poliovirus (subspecies C3) and Coxsackievirus A21 (subspecies C2) produces recombinant virion RNA. ClickSeq deep sequencing, in conjunction with ViReMa pipelines, reveals the frequency and location of crossover sites between the two viral genomes. Remarkably, recombination occurs predominantly in the P3 region of the viral genomes, where polymorphisms between CVA21 and poliovirus are predominantly silent, wobble position changes spaced 3, 6 or 9 bases apart. These data suggest that viral replication machinery can discriminate between homologous and nonhomologous partners during recombination.

03

Systematic identification of downstream ORFs and their biological functions

Cameron Berry, Ariel Bazzini

Stowers Institute for Medical Research, Kansas City, MO

Thousands of translated small open reading frames (ORFs) have been identified in untranslated regions (UTRs) of mRNAs. Some of these small ORFs encode small peptides that function in development and other biological processes, however small ORFs have also been shown, simply through the act of their translation, to have profound regulatory effects on gene expression. We and others have identified small translated ORFs located within 3'UTR sequences called downstream ORFs (dORFs), experimentally supported by ribosome profiling and reporter assays. We have recently demonstrated that dORFs enhance translation of the main ORF and emerge as a strong and uncharacterized regulatory mechanism across vertebrates. Due to technical limitations, we still don't know how many dORFs exist and to what extent dORFs are expressed and functional in a cell-specific manner. We are developing a new technique to identify dORF activity in 3'UTRs using a massively parallel reporter assay with fluorescent readout of dORF activity, which can be used in multiple human cell types. I am also exploring what cellular functions are associated with dORF activity through genome editing to introduce mutations in dORFs. We aim to further uncover the mechanisms by which dORFs enhance translation as well as discover functional roles for dORFs in cellular processes.

04

Identifying factors involved in the codon optimality mechanism in human cells

María José Blanco Salazar, Qiushuang Wu, Ariel Bazzini

Stowers Institute for Medical Research, Kansas City, MO

Codon composition strongly impacts mRNA stability and translation efficiency from yeast to human cells and zebrafish embryos. Some codons enhance translation efficiency and stability of mRNAs (optimal codons) and others have the opposite effect (non-optimal codons). This phenomenon, known as codon optimality, is one of the strongest mechanisms regulating gene expression post-transcriptionally. Although translational levels and the tRNA supply have been shown to be important determinants of codon optimality in humans, the molecular mechanism remains a mystery. To identify factors involved in the molecular mechanism of codon optimality in humans, we performed a genome wide CRISPRi screening. We found that the top hit in the screening binds to elongating ribosomes independently of the nascent peptide or the mRNA, by analyzing previously published polysome profiling, CLIP and Mass Spectrometry data. To validate the identified factors, inducible shRNA lines were used to, first, analyze reporter gene expression. And second, to analyze the stability of endogenous mRNA. The shRNA knockdown of one of our top candidates, reduced the expression of reporters enriched in non-optimal codons, without affecting those enriched in optimal codons. In summary, our work shows that transcripts mostly enriched in non-optimal codons become stabilized in the absence of our top candidate factor from a CRISPRi screening, and we describe a factor influencing codon optimality for the first time in humans.

05

A small RNA regulates expression of DbpA, VlsE and BpuR in the Lyme disease spirochete

Kevin Brandt, Julia Hilliard, Taylor Van Gundy, Brittany Armstrong, Melissa J. Caimano, Justin D. Radolf, Meghan Lybecker

Centers for Disease Control and Prevention, Fort Collins, CO

Many pathogenic bacteria utilize small RNAs (sRNAs) to regulate virulence gene expression. Over 1000 sRNAs have been identified in *B. burgdorferi* via transcriptomic analyses, yet few have been extensively characterized. Herein we identify and characterize a small RNA, termed CrtA. CrtA levels are modulated by major regulators Rrp1, $RelA_{Bbu}$ and PlzA. A *B. burgdorferi* strain lacking CrtA mis-regulates expression of DbpA, BpuR and VlsE; DbpA and VlsE levels are down-regulated by CrtA, while BpuR expression is stimulated. The *crtA* deletion strain traverses the tick mouse infectious cycle and isn't necessary for mammalian infectivity or tick acquisition.

06

Expansion of the SR-related protein family via analysis of Ser/Arg-rich low-complexity domains

Sean M. Cascarina and Eric D. Ross

Colorado State University, Fort Collins, CO

Proteins with serine/arginine-rich low-complexity domains ("RS domains") are abundant in eukaryotes and are often involved in RNA processing. The canonical family of "SR proteins" has strictly defined criteria for domain composition and domain ordering. However, additional proteins – the "SR-related proteins" – have remarkably similar domain features and molecular functions but, as a protein family, remain poorly defined. A hallmark feature of both protein families is the RS domain. Here, we systematically identify new proteins that contain RS domains using the LCD-Composer algorithm. These newly identified proteins exhibit remarkable similarity to known SR/SR-related proteins with respect to localization, post-translational regulation, domain composition, and protein functions. These features suggest that our newly identified proteins are legitimate members of the SR-related protein family. Finally, while our focus was on characterizing human SR-related proteins, we also apply the same methodology to explore the prevalence and functions of SR/SR-related proteins in all known reference proteomes, including viruses and non-eukaryotic organisms.

07

Codon-dependent regulation of gene expression during dengue virus infection

Luciana A. Castellano, Ryan McNamara, Diego E. Alvarez and Ariel A. Bazzini

Stowers Institute for Medical Research, Kansas City, MO

Control of translation is crucial during virus-host interaction because viral mRNA translation is strictly dependent on the host cell machinery. Recent work indicates that translation can affect mRNA stability in a codon-dependent manner in different species, through a phenomenon known as codon optimality. 'Optimal' codons enhance mRNA stability, and 'non-optimal' codons decrease it. However, whether this codon-mediated regulation is exploited during viral infections remains unknown. We focused our work on dengue infection, the most prevalent mosquito-borne viral disease in the world. To study whether dengue virus uses optimal or non-optimal codons relative to its hosts, we first defined the codon optimality code in mosquito cells through blocking transcription and studying the correlation between stability and codon composition of endogenous genes. Surprisingly, analysis of codon choice in thousands of worldwide isolated dengue genomes revealed that dengue uses non-optimal codons more frequently than most human and mosquito genes, suggesting the existence of an evolutionary pressure to select non-optimal codons. This preference for non-optimal codons is conserved in over 300 human viruses, including Zika and SARS-CoV-2. Thus, we hypothesized that dengue infection affects host gene expression in a codon-dependent manner. RNA-seq analysis indicated that human genes upregulated during infection are enriched in codons preferred by dengue. Interestingly, analysis of the fitness effect of mutations in the dengue genome revealed that mutations toward dengue's preferred codons tend to be beneficial. Altogether, our findings uncover a novel mechanism underlying virus-host adaptation and underscore codon optimality as a driving force of virus evolution.

08

Inducible depletion of Luc7 leads to global suppression of pre-mRNA splicing and mitochondrial dysfunction in the budding yeast

Subbaiah Chalivendra, Xueni Li, Mathew Taliaferro, Joseph Giovinazzo, John Rossi, Rui Zhao

University of Colorado Anschutz Medical Campus, Aurora, CO

U1 snRNP, 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 (CC1). To better understand the molecular mechanisms underlying 5'-SS recognition, we are using yeast as a model. Although the basic mechanism of 5'-SS recognition is conserved, the composition of the yeast U1 snRNP (**yU1**) is more complex than the human U1. To humanize the yU1, we modified U1C and Luc7, two proteins critical for the U1 snRNA and 5'-SS duplex formation. We replaced the first zinc-finger domain of yU1C with its human counterpart and introduced an inducible degradation system for yLuc7. Humanizing U1C alone did not change the growth phenotype of the strain. Accordingly, there was only a limited perturbation of pre-mRNA splicing in the strain, as revealed by RNA-seq analysis. In contrast, Luc7 depletion in the humanized U1C background led to a significant growth retardation and mitochondrial dysfunction as revealed by the release of Fe^{2+} into the growth medium and galactose toxicity. This was correlated with a global suppression of pre-mRNA splicing as well as repression of transcript abundance in multiple pathways, particularly those governing mitochondrial integrity and iron

homeostasis. We also found that Luc7-depleted yU1 was unstable and deficient in PRP40 and Snu71, two other essential proteins for U1 function. Our data reveals the requirement of Luc7 in the association of PRP40 and Snu71 with yU1 as well as for a stable and fully functional yU1.

09

Investigating the RNA modification N1-methyladenosine (m1A) in mRNA

Dorthy Fang, Li-Tao Guo, Olga Fedorova, Han Wan, Anna Pyle, Sigrid Nachtergaele

Yale University, New Haven, CT

RNA chemical modifications are changes to RNA structure which are responsible for widespread control of RNA metabolism. For example, regulation by modifications of RNA stability, translation, and splicing, have been shown to affect development, differentiation, and disease. N1-methyladenosine (m1A) is a dynamic modification that has only recently been found on mRNA transcripts, with potential implications for mRNA metabolism and regulation of downstream pathways of modified RNAs. However, the mapping of m1A across the transcriptome has been hindered by technical challenges and poor reproducibility, leading to discrepancies across previous studies. We are developing novel mutation signature-based m1A mapping methods using a recently characterized reverse transcriptase, MarathonRT, as well as secondary validation methods including m1A-immunoprecipitation, demethylase treatment, and mass spectrometry. Using these tools, our work explores the landscape of and rigorously interrogates the identity of m1A-modified RNAs. Through further characterization of these individual m1A-modified sites and their context-specific regulation, we are investigating the functional modulation of mRNA metabolism by m1A, especially under conditions of stress and disease.

10

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

Brenna J.C. Dennison, Eric D. Larson, **Katherine A. Fantauzzo**

University of Colorado Anschutz Medical Campus, Aurora, CO

Craniofacial development is a complex morphogenetic process, disruptions in which result in highly prevalent human birth defects. 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 performed sequencing of maxillary process mesenchyme RNA from E11.5 embryos that lack *Pdgfra*, *Pdgfrb* or both in the neural crest lineage to examine the transcriptional output in each context. DESeq2 analysis identified 23, 20 and 25 genes that were differentially expressed between $Pdgfra^{fl/fl;}Wnt1\text{-}Cre^{+/Tg}$, $Pdgfrb^{fl/fl};Wnt1\text{-}Cre^{+/Tg}$ and $Pdgfra^{fl/fl};Pdgfrb^{fl/fl};Wnt1\text{-}Cre^{+/Tg}$ 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 categories revealed an enrichment for protein serine/threonine kinase activity. For approximately one third of events from this GO term unique to a single genotype, the same transcript was subject to AS in one or more of the remaining genotypes at a different exon. This finding indicates that signaling downstream of the various PDGFR dimers targets an overlapping set of transcripts encoding protein serine/threonine kinases for AS, and that this regulation may occur through utilization of non-overlapping RNA-binding proteins. Together, our results demonstrate that AS is the predominant mechanism of gene expression regulation downstream of PDGFR signaling in the facial mesenchyme.

11

Hormone-mediated disruption of RNA polymerase III at tRNA genes in breast cancer

Jessica Finlay-Schultz, Kiran V. Paul, Laura K. White, Jay R. Hesselberth, Deborah L. Johnson, David Bentley, Peter Kabos, and Carol A. Sartorius

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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 hormones regulate Pol III in mammalian cells. 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, CUT&RUN, and genetic manipulation of Maf1. We found that P-induced PR binding at tRNA genes corresponds with a reduction of POLR3A and the TFIIIB component Brf1. 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 independent of Maf1. We propose that PR recruitment disrupts the Pol III complex by disrupting or displacing Brf1 and the TFIIIB preinitiation complex. 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.

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Visualizing light-inducible translation shut off at single-molecule resolution in live cells

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All protein-involving processes require the transition from genetic DNA to protein peptide chains. Translation, the conversion of mRNA to protein, involves many regulatory factors that are not well understood. Because of this, translational tracking has been explored for years across the field of biochemistry and only recently, through nascent chain tracking (NCT), have we been able to track single mRNA sites in live cells. Coupled with MS2 tagging systems, we can kinetically analyze translational pathways of various proteins in real time and in live cells. Despite this, there are still limitations with spatially and temporally tracking the recruitment of these translational effectors to translation sites. With the incorporation of optogenetic tools involving the cryptochrome 2 protein (cry2), we can generate biomolecular condensates comprised of GFP-tagged proteins at our reporter mRNAs. Two systems designed using Cry2, LARIAT and Cry2Olig, generate differing condensates allowing for kinetic comparison. Using this technology, I will recruit GFP-tagged ribosome quality control (RQC) proteins, such as GIGYF and 4EHP, and track their effects on translation signals within the condensates. It has already been seen that GFP-tagged GIGYF and 4EHP will recruit to the condensates, so further exploration on translation can begin. We are now using NCT along with optogenetics to visualize the translational control our quality control proteins have. We hope this will be an excellent tool to investigate translational kinetics in future studies.

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Dissecting the mechanism of dORF translation in human cells

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Downstream Open Reading Frames (dORFs) are small ORF translated in the 3' untranslated region (UTR) of a mRNA. In contrast to upstream ORFs (uORFs), dORF translation have been found to play an important role by enhancing translation of their corresponding canonical ORF in human cells and zebrafish embryos. However, the mechanisms by which dORFs are translated and the factors involved in this process remain unclear yet. To identify novel factors involved in the translation of dORFs we have performed a genome-wide CRISPR-I screening in human cells. To validate the top candidates, we are producing mRNA knockdowns with inducible shRNAs and assessing whether dORF translation is affected or not with a double reporter system encoding fluorescent mCherry followed by an internal UTR (iUTR) and a dORF fused to GFP. We propose that dORFs represent a new, strong, and universal translational regulatory mechanism in vertebrates. Understanding the

mechanisms underlying dORF translation will provide a foundation for future studies aimed at characterizing the functional role of dORFs and their implications in post-transcriptional gene regulation and human disease.

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Pin-point base editing system: A versatile editing platform driving cell therapies

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Horizon's modular Pin-point™ base editing (PnP BE) system uses a nickase Cas9 with an aptameric guide RNA to recruit a deaminase to the site of interest, facilitating highly efficient and precise nucleotide conversion. We optimized design and delivery conditions of chemically modified synthetic guide RNAs and enzymatic mRNA to apply multiplex base editing with the PnP BE system to the development of engineered CAR-T cells. We target a set of therapeutically relevant loci and achieve greater than 70% knockout efficiency and high purity at all sites simultaneously. Compared to Cas9-mediated knockout, base editing with the PnP BE system enables higher cell viability, less guide RNA-dependent off-target editing and significantly reduced frequency of chromosomal translocations. Importantly, edited T cells retain their proliferative and cytotoxic ability in vitro. An additional advantage of PnP BE technology is that the modular recruitment of deaminase via an aptamer enables robust simultaneous knock-in and knock-out in the same cell. We also show that the PnP BE system can be applied to other therapeutically relevant cell types, such as hiPSCs. The ability to edit multiple cell types efficiently and precisely will allow for a variety of therapeutic applications and a potential to treat monogenic and polygenic disorders, opening the door to more sophisticated cell and gene therapies.

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Toward a molecular understanding of tissue-specific phenotypes in RNA exosome-linked neurogenetic disorders: A Drosophila model

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The RNA exosome is an evolutionarily conserved, ubiquitously expressed 10-subunit ribonuclease complex that facilitates the precise processing of a variety of cellular RNAs. Recent studies have linked recessive missense mutations in genes encoding structural subunits of the RNA exosome to distinct tissue-specific disorders with shared neurological features. Intriguingly, these mutations cause a range of clinical manifestations that do not appear to correlate with the type of structural gene that is mutated. For example, RNA exosome cap subunit genes EXOSC2 and EXOSC3, and core subunit gene EXOSC9 are linked to distinct neurological defects. To begin to define RNA exosome function in cells of the nervous system in vivo, we generated an allelic series of disease-linked mutations via CRISPR/Cas9 technology in several genes that encode distinct subunits of the RNA exosome in Drosophila. Thus, I will test the hypothesis that RNA exosome subunits mediate critical yet distinct activities of the RNA exosome in post-transcriptional regulation of gene expression in cells of the nervous system in vivo. My preliminary data show that recessive heterozygous and homozygous genotypes observed in patients are viable when modeled in flies. Pathogenic RNA exosome mutations engineered in these flies cause reduced viability and produce neuronal-specific behavioral phenotypes. Accordingly, we will utilize molecular techniques and multi-omic approaches to systematically investigate and compare the cell type-specificity of each RNA exosome variant in the fly brain. This comparative analysis will provide insight into how the RNA exosome functions in post-transcriptional regulation of RNA in cells of the nervous system in Drosophila.

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Thiomorpholino oligonucleotides (TMOs) as a versatile tool for gene expression regulation

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Thiomorpholino oligonucleotides (TMOs) are nucleotide analogues developed in our laboratory, where when compared to DNA, the sugar unit is replaced by a morpholine ring and the internucleotide phosphodiester linkage is replaced by a thio-phosphoroamidate linkage. Based on our numerous collaborations with external laboratories, TMO modification shows great potential for gene expression regulation in splicing modulating approach (fully modified TMOs) and RNase H based approach (TMO/PS-DNA chimeras). During the study of the origin of their high activity at low concentrations, we were able to find out that TMO modified sequences have increased potency to be transported into the cell nucleus. The increasing number of positive results in cell lines prompted us to start animal studies along with initial toxicology studies, in which they are achieving exciting results. We further began to study the possibilities of combining TMOs with other modifications (RNA, 2´-MOE, LNA, etc.), as well as study of the rational design of the modification's distribution in these chimeras (alternating design, cap-gap design, and their combination). We managed to develop a protocol for the preparation of TMOs and their chimeras with any type of phosphorothioate modification, which is compatible with the phosphoramidite method of oligonucleotide synthesis on a solid support. With this portfolio of available TMO chimeras and a robust protocol for their preparation even in large-scale, we also turned our interest to study the potential for their application in newly rising internuclear approaches, such as targeting regulatory RNAs, or targeting the early phase of transcription to suppress haploinsufficiency.

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Uncovering the mechanism of localization of ASPM RNA to the centrosome

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The localization of RNA to different subcellular compartments is necessary for basic cell function. Proper RNA localization is critical for proper translation or scaffolding, which allows for the cell to respond rapidly to a stimulus. This is particularly important for highly orchestrated process of cell division, where local protein concentrations are paramount. ASPM, a gene that accounts for up to 60% of cases of primary microcephaly, has been shown to be required at the centrosome. Its RNA is known to localize to the centrosome, where it is translated to ASPM protein. However, the effects of mislocalizing the RNA transcript is completely unknown. And while we know that the localization of ASPM RNA is co-translational, the localization element required to localize the transcript is also unknown. I propose a series of experiments to carefully characterize ASPM RNA and protein at the centrosome, identify how the transcript localizes to the centrosome, and investigate the effects of mis-localization on cell cycle progression.

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The RNA binding protein hnRNPA2/B1 inhibits RNA localization to the neurite

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RNA localization is a post-transcriptional regulation mechanism that cells use to spatially regulate gene expression, especially in cells that are highly polarized with distinct morphology like neurons. RNA binding proteins are one of the regulators of this process, recognizing and binding specific sequences within target RNAs. hnRNPA2/B1 is a mediator of RNA localization to the neurite via plus end directed transport by binding sequences within the 3′UTRs of its target RNAs. However, the identity of these RNAs and the sequences bound by hnRNPA2/B1 remain understudied. Here, we show how hnRNPA2/B1 effects neurite localization of target RNAs and identify some of the sequence features that may be important in promoting localization activity. In addition, we select target RNAs for further study to narrow down specific sequence elements that may be important for driving localization to the neurite.

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c-di-GMP regulates RNA chaperone activity of PlzA in the Lyme disease spirochete

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PlzA is a c-di-GMP-binding protein crucial for adaptation of the Lyme disease spirochete *Borrelia (Borreliella) burgdorferi* during its enzootic life cycle. Unliganded *apo*-PlzA is important for vertebrate infection, while liganded *holo*-PlzA is important for tick acquisition; however, the biological function of PlzA has remained enigmatic. Here we report that PlzA has RNA chaperone activity that is inhibited by c-di-GMP binding. *Holo*- and *apo*-PlzA bind RNA and accelerate RNA annealing, while only *apo*-PlzA can strand displace and unwind double-stranded RNA. Guided by the crystal structure of PlzA, we identified several key aromatic amino acids protruding from the N- and C-terminal domains that are required for RNA binding and unwinding activity. Our findings illuminate c-di-GMP as a switch controlling the RNA chaperone activity of PlzA and we propose that complex RNA-mediated modulatory mechanisms allow PlzA to regulate gene expression during both the vector and host phases of the *B. burgdorferi* life cycle.

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Nsp15 is a manganese-dependent endoribonuclease that specifically targets pyrimidine-purine dinucleotide sequences

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The SARS-CoV-2 virus has recently spurred a pandemic of nearly unprecedented scale, largely due in part to the prolonged asymptomatic period that occurs during the early stages of viral infection. During this time, hosts are infectious, but due to the ability of SARS-CoV-2 to initially repress the response of the innate immune system, no observable physical symptoms immediately arise. Responsible for this immune repression is Non-Structural Protein 15 (Nsp15) of SARS-CoV-2, historically called an 'EndoU' nuclease due to its propensity to cleave in an endonucleolytic fashion at uridine bases. Recent in vivo data showed that Nsp15 achieves this effect by specifically targeting conserved pyrimidine-purine dinucleotide sequences of SARS' (+)-sense genomic RNA (gRNA), as well as a conserved CACA repeat found immediately adjacent to the poly-uridine (polyU) tail of the (-)-sense gRNA strand. We tested these findings by expressing recombinant Nsp15 and testing its reaction with in vitro transcribed RNAs that mimic one of the conserved cleavage sites of the (+)-strand gRNA. We transcribed 16 RNAs that differed in the identity of their 2 central nucleotides, generating all dinucleotide combinations. This allowed us to observe Nsp15's preference for different dinucleotide substrates in vitro, using a dPAGE gel-based readout. We also gathered kinetic data using a fluorescence-based RNA reporter, which qualitatively shows the effects that different metals have on the activity of Nsp15. Overall, our experiments reveal known features of Nsp15 activity, demonstrate its dependence on manganese, and raise new questions regarding the enzyme's capacity to recognize and process dsRNA.

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Iterative Screening of Repressor and Epigenetic Effector Domains Identifies a Novel CRISPR Interference Effector for Functional Gene Characterization

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While CRISPR interference (CRISPRi) systems have been widely implemented in pooled lentiviral screening, there has been limited use with synthetic guide RNAs for the complex phenotypic readouts enabled by experiments in arrayed format. To identify a more potent CRISPRi system amenable to use with synthetic sgRNAs, we performed an arrayed screen of repressor and epigenetic effector domains. We found domains from Sal-like protein 1 (SALL1) and Sin3a corepressor complex component (SDS3) to be the most effective repressors tested, and we used these domains to engineer a bipartite repressor dCas9 fusion, dCas9-SALL1-SDS3, which produces greater target gene repression than first or second generation CRISPRi systems when used with chemically modified synthetic single guide RNAs (sgRNAs). An unbiased protein interaction screen showed that dCas9-SALL1-SDS3 interacts with key members of the histone deacetylase and Swi-independent 3 complexes, which are the endogenous functional effectors of SALL1 and SDS3. We demonstrate that dCas9-

SALL1-SDS3 can be used orthogonally to siRNA for functional gene characterization in arrayed experiments with readouts not amenable to pooled screening. Finally, we show that dCas9-SALL1-SDS3 can be delivered alongside sgRNAs via a single lentiviral vector to mediate the sustained target repression needed for long-term loss-of-function studies.

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Role of prion-like domains in the assembly of biocondesates

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The cellular environment organizes its organelles into either membrane-bound or membrane-less organelles (MLOs). MLOs carry out various functions within the cell, including organizing and sequestering specific molecules. MLOs such as stress granules (SGs) and processing bodies (P-bodies) form in response to stress, allowing eukaryotic cells to adapt to rapidly changing environmental conditions. SGs and P-bodies, which are suggested to play a role in post-transcription regulation, form via condensation of untranslated mRNAs, ribosomal components, and RNA-binding proteins (RBPs). Many RBPs found in MLOs contain prion-like domains (PrLDs) which are thought to be involved in MLO assembly. PrLDs are a type of intrinsically disordered domains (IDRs) that are compositionally similar to yeast prion domains. Mutations within PrLDs that associate with SGs have been associated with neurodegenerative diseases, as they lead to the formation of solid irreversible aggregates that can be toxic. While mechanisms of MLO assembly are unclear, understanding how they assemble and maintain specificity in the cellular environment would provide insights into the regulation process of their assembly and disassembly. We are particularly interested in defining the role of PrLD assembly in MLOs. To answer this question, we use yeast SGs as a model to study the sequence features that promote PrLD recruitment to SGs. Additionally, to determine how specificity is achieved between different MLOs, we will use yeast P-bodies as a model to identify the sequence features that promote P-body assembly, and how they differ from SGs.

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Uncovering the molecular determinants of RNA localization

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RNA localization is critical for a diverse set of biological processes. The localization of an RNA depends on cis-elements, features inherent to the transcript, and trans-factors, effectors independent of the target transcript, which are often RNA binding proteins. Cis-elements that regulate RNA localization are often found in the 3′ untranslated regions (UTRs) of transcripts. However, for more than 99% of the known localized RNAs, the cis-elements that regulate their localization are unknown. Previous work from Taliaferro lab identified several 260 nucleotide RNA sequences within the 3′ UTRs of neurite-localized RNAs that were necessary and sufficient for neurite RNA transport from a pool of roughly 10,000 RNA sequences derived from endogenous 3′ UTRs. However, well characterized RNA binding protein consensus binding motifs are typically single-stranded stretches RNA between 4 and 6 nucleotides long, suggesting that localization activity is conferred by a subset of the 260 nucleotide sequences. To further understand the minimal elements needed to drive RNA localization to neurites, deletions within the 260 nucleotide RNA sequences were made. Windows between 5, 10, 20, 50, 100, 150, and 200 nucleotides were deleted across the each of the 260 nucleotide sequences, generating approximately 1,000 unique sequences per 3′ UTR. When the localization activity of the deletion derived sequences was assayed in cells, it was revealed that the sequences necessary for full activity range from 10 nucleotides to 250 nucleotides.

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Characterizing the dynamics of Z-DNA/Z-RNA adoption

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The left-handed Z-conformation of nucleic acids can be adopted by both DNA and RNA when bound by Zα domains found within a variety of innate immune response proteins. Zα domains stabilize this higher-energy conformation by making specific interactions with the unique geometry of Z-DNA/Z-RNA. However, the mechanism by which a typical A-form or B-form right-handed helix contorts to become left-handed, and the intermediate steps involved, is poorly understood. Using Nuclear Magnetic Resonance, we have determined that chemically modified B-DNA and A-RNA under low-salt conditions dynamically flip to the Z-conformation on a very slow timescale. We also show that DNA and RNA duplexes transiently sample an unfolded state at higher temperatures, with duplex melting being correlated with Z-form adoption rates in the presence Zα. Our results support our prior observations that Z-conformations are more likely to be adopted by double-stranded DNA and RNA regions flanked by less stable regions. Such regions would be more likely to transiently unfold allowing for Zα binding and Z-conformation stabilization. This process may be promoted by helicase activity or helical torsion which would promote duplex melting and increase the population of transient Z-conformations.

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Mechanistic analysis of small-molecule translation inhibitors

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Translational control is essential to cancer transformation, progression, and metastasis. Small molecule translation inhibitors are promising therapeutic modalities for the treatment of certain cancers. However, the exact mechanisms of translation inhibitors in normal and cancer cells remain largely unknown. In this work, I aim to characterize translation elongation arrest by different translation inhibitors and to dissect and reconstitute translational control pathways that contribute to their anti-cancer activities. A combination of structural biology, functional assays, in vitro reconstitution, and ribosome footprint profiling will be used. The goal of this study is to gain mechanistic insights into translation inhibitors, and the outcome may provide new strategies for designing and screening translation inhibitors for more effective cancer therapies.

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Mechanisms of the m6A RNA modification in meditating cell state switches, from breast cancer to pathogenic yeast

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How do cells switch states? How do commensals become pathogens? My research addresses these questions and suggests that the RNA modification N6-methyladenosine (m6A) plays a role in mediating these dynamic processes. In breast cancer, high expression of the long noncoding RNA (lncRNA) HOTAIR causes increased metastasis and poor outcomes for patients. We have identified a specific m6A site that mediates these affects by interacting with the m6A reader YTHDC1 and inducing chromatin association and gene repression of HOTAIR's targets. In the pathogenic yeast *Candida albicans*, we have found a role for the m6A methyltransferase *IME4* in facilitating temperature-induced opaque-to-white phenotypic switching and preventing mating. In both cases, m6A mediates the switch from a healthy or commensal state to a more pathogenic one, providing major clinical implications for potential treatments for these devastating diseases.

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Cardiac myocyte-specific loss of the lncRNA Malat-1 protects against pathological hypertrophy and induction of pro-apoptotic genes

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Reprogramming of the cardiomyocyte (CM) transcriptome underlies maladaptive pathological remodeling in cardiac hypertrophy and heart failure. The long non-coding RNA (lncRNA) Malat-1 is highly abundant in the heart

and is of particular interest as it is involved in regulation of mRNA splicing, ERK/MAPK signaling, histone methylation and miR-133 activity – processes established as contributing to heart failure pathophysiology. Results: Profiling of the transcriptome in human CMs identified a dramatic increase in Malat-1 expression in the failing heart compared with healthy controls, which was also observed in rodent pathology. Pressure-overload induced pathological remodeling of the heart invoked by transverse aortic constriction (TAC) was attenuated in CM-restricted Malat-1 KO (Malat-1 CM-KO) mice compared with control mice. Probing the CM transcriptome revealed downregulation of pro-apoptotic genes in Malat-1 CM-KO mice. Notably, the initiator caspases 8 and 9 were downregulated in Malat-1 KO-CMs, genes which were otherwise upregulated in CMs with TAC in control mice. CM death is associated with pathological hypertrophy and heart failure, which was reflected by a drop in the fraction of CM nuclei measured during flow sorting in control mice with TAC, that was not seen in TAC Malat-1 CM-KO mice. Suppression of CM apoptosis induced by TAC in Malat-1 KO mice was validated using the TUNEL assay. Conclusion: Loss of Malat-1 in CMs protects against maladaptive cardiac hypertrophy through attenuation of apoptosis.

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Experimental methods and mathematical modeling to quantify DUSP1 mRNA expression dynamics in response to dexamethasone

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Overactivation of mitogen-activated protein kinase (MAPK) signaling pathways is key to multiple inflammatory responses, and synthetic glucocorticoids (GC), such as Dexamethasone (Dex), have long been used to treat inflammatory pathologies. Upon cell entry, Dex binds to the glucocorticoid receptor (GR), which initiates translocation to the nucleus. Nuclear GR then 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 the protein mitogen-activated protein kinase phosphatase 1 (MKP-1), which regulates the cell's anti-inflammatory response through dephosphorylation of the P38 and JUN N-terminal kinase (JNK) MAPK pathways. In this presentation, we combine single-cell measurements and discrete stochastic models to elucidate the spatiotemporal mechanisms by which Dex modulates GR localization and DUSP1 transcription. Using single-molecule inexpensive fluorescent in situ hybridization, we quantify the nascent transcription, spatial localization, and cellular heterogeneity of DUSP1 mRNA for hundreds of individual HeLa cells at 20 different time points following application of continuous 100nM Dex stimulation. We observe a rapid increase in DUSP1 transcription sites (TS's) as early as 10-20 minutes, followed by a four-fold increase in expression of mature DUSP1 mRNA that peaks at 75-90 minutes and a temporary decrease in DUSP1 expression at 120-150 minutes post stimulation. Finally, we use these experimental data to infer parameters and mechanisms for a discrete stochastic model that quantitatively reproduces the time-varying probability distributions for single-cell DUSP1 expression at all time points following Dex stimulation.

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The evolution and specificity of polyadenylation signals in protists

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Cleavage and polyadenylation are important steps of eukaryotic mRNA 3' end formation. In humans and other model organisms the most important element is the poly(A) signal - AAUAAA hexamer. We recently discovered that deeply branching eukaryote – Giardia lamblia, uses different, but well-defined signal, AGURAA. To better characterize when this evolutionary shift in the poly(A) signal occurred, we performed direct RNA sequencing of four protists within the Metamonada supergroup and two outgroup protists. Both outgroup protists and the non-Giardia Metamonada species use AAUAAA poly(A) signal, indicating it is the ancestral signal. In contrast, all Giardia species use AGURAA poly(A) signal, indicating it is derived within Giardia or Fornicata. The change in this ubiquitous regulatory element raises questions about sequence features that specify genuine poly(A) sites and how to avoid premature cleavage in coding sequence. Therefore, we used a sequence classifier, a gapped k-mer support vector machine, that was able to discriminate between AGURAA sites in 3'UTRs and those in the

CDS with no evidence of cleavage (F1 = 0.97). We found that nucleotides directly flanking the poly(A) signal are crucial for its recognition. We also identified U-rich sequences enriched surrounding the 3'UTR poly(A) signals. Finally, we identified amino acid changes in G lamblia ortholog of CPSF30 that may explain difference in poly(A) signal specificity. These results identify unique features of the pathogen G. lamblia that could be targets for drugs and highlight the diversity and evolution of mRNA 3' end formation in eukaryotes.

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Functional implications of structural heterogeneity in HCV-like Internal Ribsome Entry Sites

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Internal Ribosome Entry Sites (IRESs) are structured RNA motifs that facilitate non-canonical translation initiation in viruses. Broadly, there are several types of IRESs classified by the mechanism of translation initiation, with each class varying in structure and required initiation factors. Hepatitis C virus (HCV)-like IRESs are a class of IRES found in the Picornaviridae order of viruses. While the HCV IRES and a few other representative examples from this class have been extensively studied and structurally characterized, the full scope of variation in this class of IRESs in terms of structure and how this might alter the mechanism of action had not yet been fully explored. Using bioinformatic homology search methods, we have identified previously uncharacterized HCV-like IRESs with a range of expansions and truncations to the "model" secondary structure of the HCV IRES. Regions that make specific contacts with the ribosome are highly conserved while areas that bind other initiation factors, such as eIF3, are more variable. These putative IRESs are found in a variety of viruses, with host species ranging from mammals to fish. Structural characterization of select IRESs individually via chemical probing confirm secondary structure variability. Further in vitro and in vivo characterization of a subset of structurally distinct IRESs showed a range of translational efficiency, suggesting that structural variation in IRESs may be tied to host-cell context or otherwise tuning viral protein expression. These studies will further the understanding of the structure-function relationship of HCV-like IRESs and potentially expand diversity of IRES mechanism.

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Real-time single-molecule investigation of dynamic biological processes: from structure to function

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Essential biological processes ensuring proper cellular metabolism, imperative to life, are performed by proteins interacting with and processing DNA and RNA amongst other biological matter. In fact, detailed insights into these processes provide essential information for understanding the molecular basis of life and the pathological conditions that develop when such processes go awry. The next scientific breakthrough consists in the actual, direct, real-time observations and measurements of the individual mechanisms involved, in order to validate and complete the current biological models. To this end, structural and functional information, which are often indivisible, need to be studied together in real time and at the molecular level. In this complex context, single-molecule technologies offer an exciting opportunity to meet these challenges. Here, we present our efforts in further enabling discoveries in the field of biology and biophysics using a fully integrated optical tweezers with single-molecule fluorescence microscopy technology. We show the latest applications of these technologies that can enhance our understanding not only in the field of DNA/RNA structure and their interactions with proteins but also in the fields of molecular motors, protein folding/unfolding, cell membranes and genome structure and organization. These experiments show that the technological advances in hybrid single-molecule methods can be turned into an easy-to-use and stable instrument with the ability to open new venues in many research areas.

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SomaScan© Platform Confirmation and Performance Validation

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The SomaScan® Platform for proteomic profiling uses 7288 (7k) SOMAmer® reagents, single-stranded DNA aptamers, to 6596 unique Human Protein Targets. The modified aptamer binding reagents1, SomaScan assay2, its performance characteristic for 5k3 and 7k4 content sets, and specificity5,6,7 to human targets have been previously described. We combine profiles of validation and performance metrics with orthogonal confirmation of specificity from published literature to provide a comprehensive view of the specificity and utility of the SomaScan Platform. Validation of SOMAmer reagents results in a set of metrics that profile performance of the reagents to the protein standards used for discovery. These include linear ranges and affinity measures: dose-response 50 (apparent KD), and solution KD. Validation of the SomaScan Platform includes replicates of individual and pooled samples over 15 assay runs in both plasma and serum. Population ranges and performance are generated from matched plasma and serum drawn from more than 1,000 U.S. normal volunteers. Reproducibility and signaling metrics are summarized and reported. Production use of the SomaScan Platform includes replicates to monitor the accuracy and precision of the assay over time. Results from more than 3,000 replicates are aggregated and reported. Secondary confirmation of specificity is explored using published outcomes from alternative proteomic or genomic profiling methods. Results are extracted from the literature and assembled by reagent identifier. Alternative experiments that confirm protein targets are described and reported separately. The SomaScan® Platform for proteomic profiling relies on a deep validation workflow for reagents and for the platform. Transparency in the methods and results is critical to help users interpret platform results.

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Metaphors for making (sense of) RNA

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Where DNA is routinely described through genetic text metaphors, RNA is described through metaphors that emphasize textual or informational features, but also structure, function, lineage, location, behavior, and evolutionary relationships. As an interdisciplinary group based in humanities that studies microbial biotechnologies, we argue that these metaphors are important because language shapes scientific work just as physical tools do. We argue that attending to the metaphors that are currently employed for making sense of RNA, and to the metaphors that may be necessary to make synthetic RNA, will be important for both technical and broader societal reasons. Among other things, metaphorical heterogeneity suggests the possibility that scientific groups working through different metaphors may construct divergent visions and versions of RNA, potentially leading to further linguistic, conceptual, and technical rifts. Consequently, this heterogeneity may be especially significant for RNA synthetic biology, as bioengineers rebuild RNA in the image of their expectations about it. Genetic text metaphors employed for designing and building with DNA are proving insufficient for working with RNA, so that alternative metaphors employed across other subfields of RNA biology may suggest a wider set of relationships that need to be sustained in bioengineering—bringing more cellular context into a field typically focused on the genetic pathway or circuit. At the same time, the challenges of engineering RNA suggest new ways to rethink conventional genetic text metaphors that are also becoming limiting for working with DNA, and for societal conversations that still tend to suggest scientifically unsupportable genetic determinism.

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RNA localization to the midbody

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TBD

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Identification of RNA chaperones by gradient profiling in the Lyme disease spirochete, Borrelia burgdorferi

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RNA chaperones are a class of RBPs that modulate RNA configurations by disrupting RNA structure (unwinding, unfolding, strand-displacing) and/or accelerating base-pairing of RNAs (annealing). “Matchmaker” RNA chaperones facilitate base-pairing between trans-acting small regulatory RNAs (sRNAs) and their RNA targets. Mutation of RNA chaperones in bacteria results in pleiotropic phenotypes including reduced virulence, impaired growth, reduced ability to respond to and survive environmental stresses. B. burgdorferi harbors several RNA binding proteins that regulate posttranscriptional gene expression, but their RNA chaperone activity and mechanisms of regulation have not been extensively studied. Here, we use gradient profiling coupled with mass spectrometry to identify proteins that are resolved in the same fractions as sRNAs. Proteins co-fractionated with sRNAs were tested for RNA chaperone activity using in vitro RNA annealing and displacement assays. In vivo RNA unwinding activity was also assayed in a heterologous system. Finally, we show that several of these novel B. burgdorferi RNA chaperones bind Borrelia RNA in vitro. In conclusion, we identified several proteins (BosR, CheY2, Bb0713 and Bb0749) with RNA chaperone activity that are required or may be required for B. burgdorferi infectivity.

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Profiling the translatome to identify novel autoimmune beta cell antigens

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Type 1 Diabetes Mellitus (T1D) is an autoimmune disease characterized by the destruction of insulin producing βcells in the pancreas. βcells are targeted by autoreactive T cells that recognize self-reactive antigens presented by β ells. Most of these antigens are derived from canonical open reading frames (ORFs) of protein coding genes. However, a defective ribosomal product (DRiP) was recently identified from the insulin mRNA (INS-DRiP) that is the result of an alternative translation start site and is known to produce autoreactive antigens. Here, we applied cell-type specific ribosome profiling as an unbiased approach to discover novel autoreactive βcell antigens. Bulk ribosome profiling would not be sufficient since the process of differentiation to stem cell derived βcells (sBCs) leads to a mixed population of cells. We found that bulk ribosome profiling and IP-ribosome profiling of cells expressing RPL22-FLAG were virtually identical. To identify the sBC translatome, we performed IP-ribosome profiling of differentiated sBCs expressing the FLAG-tagged RPL22 protein driven by an insulin promoter. Since the data exhibited very high 3-nucleotide periodicity, we were able to use this pattern to identify 15,519 translated ORFs, of which 813 were non-canonical ORFs. We also identified >140 of the non-canonical ORFs in βcell mass-spectrometry data. Moreover, we found matches for at least 15 peptides that are known to be presented on βcells from immunopeptidomics. These data and approach will be invaluable in the search of autoreactive antigens in T1D and will provide unique insights into the impact of translational regulation in βcell biology.

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A conserved RNA identified in piconaviridiae competitively inhibits RNase L

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RNase L is a mammalian endonuclease that cleaves single-stranded RNA following viral infection. Endonuclease activity is regulated by antiviral interferons and requires activation of oligoadenylate synthetases (OAS) to generate 2'-5' linked oligoadenylates (2-5A). 2-5A is required for RNase L dimerization, which, along with ATP, creates the active endoribonuclease. Poliovirus is a single-stranded positive sense RNA that contains a sequence that competitively inhibits endonuclease activity of RNase L. While this sequence is in a protein coding region of the poliovirus genome, it has a predicted secondary and tertiary structure. It is known that this competitive inhibitory RNA (ciRNA) binds to the RNase domain of the protein, however the mechanism of inhibition remains elusive.

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