6th Annual Symposium on RNA Science and Its Applications

The Language of RNA in Disease and Development
March 21-22, 2019

THE RNA INSTITUTE
UNIVERSITY AT ALBANY STATE UNIVERSITY OF NEW YORK
Front cover image credit:
Image courtesy of Kevin O'Keefe and Melinda Larsen.

Adult mouse gland multiplex immunostained to detect smooth muscle alpha actin (red), cytokeratin 5 (green), beta III tubulin (blue), retinoic acid receptor alpha (magenta), and vimentin (cyan) with DAPI (gray).
Welcome to Albany!

The RNA Institute at the University at Albany is delighted to welcome you to the 6th Annual Symposium “The Language of RNA in Disease and Development” being held March 21-22, 2019.

This day and a half symposium is unique in that it brings together an outstanding and diverse group of scientists to discuss various aspects of RNA. Our focus this year is on the role that RNA plays in disease and development. We hope that this meeting will serve to inspire up-and-coming young scientists, as well as stimulate collaboration and interdisciplinary research. Hopefully, our meeting interactions will also provide unique insights into the fascinating world of RNA and reveal ideas and new approaches for the next stages of your research.

We would like to thank all of you for joining us from near and far and hope you will enjoy everything this Symposium has to offer.

Warm regards,

Andy Berglund, PhD
Director
RNA Institute
Welcome,

The Director of the RNA Institute, Andy Berglund, the Associate Director Thomas Begley, and the organizing committee of Joan Curcio and Melinda Larsen (Co-Chairs), Jia Sheng, Bijan Dey, Jason Herschkowitz, and Prashanth Rangan, welcome you to the 6th Annual Symposium on RNA Science and its Applications – “The Language of RNA in Disease and Development”. The RNA Institute wants to thank all those who have traveled from near and far to participate in our scientific event. Our symposium brings together leading RNA researchers, academics, companies and students, and we look forward to an exciting meeting. We are honored to host our keynote speakers Craig Mello, Gisela Storz, Elçin Ünal, Eric Kool, Matt Disney, Eliezer Calo, Charles Thornton, Cara Pager and Wendy Gilbert. We would like to thank all the attendees who have traveled from Connecticut, Virginia, Florida, Maryland, Massachusetts, South Carolina, Illinois, New Jersey, Pennsylvania, California, Oregon, Rhode Island, Texas, and China to be with us in Albany, NY.

Vision

The RNA Institute is positioned to make significant contributions towards understanding the role of RNA in fundamental biological processes, developing RNA as a tool for science and harnessing this knowledge to improve human health. The Institute brings together teams of researchers from multiple Departments and Universities with expertise in Biology, Bioinformatics, Chemistry, Engineering, Genetics and Structural Biology to achieve our goals.

Mission

Training the next generation of RNA scientists with a commitment to improving the number of individuals from under-represented groups is a priority for the Institute. The driving force behind the RNA Institute is our active researchers from multiple Universities who share the common goals of providing outstanding mentoring to our trainees and using the advanced technologies in the Institute to make new discoveries centered around RNA Biology and Chemistry. The long term goal of the Institute is to translate our scientific discoveries into improving the human condition.

Here’s to a successful meeting!

The Symposium Organizing Committee
Thank you to the generous sponsors of the 6th Annual Symposium The Language of RNA in Disease and Development
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<td>Advisory Board Meeting</td>
<td>University Hall Room 306</td>
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<tr>
<td>8:30 AM</td>
<td>Workshops</td>
<td>Various locations and durations</td>
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<td>8:55 AM</td>
<td>Session 1: Regulatory RNAs</td>
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<td>1:00 PM</td>
<td>Session I: Lecture Center East</td>
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<td>2:00 PM</td>
<td>Session II: Lecture Center East</td>
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<td>Session 3: RNA Focus in Disease Research</td>
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<td>5:15 PM</td>
<td>Closing Reception</td>
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**Wifi Connection:** The UAlbany Guest network is available for users that do not have a UAlbany username and password. UAGuest will be a wifi connection option. To sign in, please use **UAGuest** as your username and **yankees** as the password to login.

**Shuttle bus schedule to the PACs on March 21st and 22nd**

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<tr>
<td>3/21/2019</td>
<td>7:45 AM</td>
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<tr>
<td>3/22/2019</td>
<td>7:00am</td>
<td>From the Hilton Garden Inn (Washington Ave) to the PAC</td>
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<td>3/22/2019</td>
<td>7:15am</td>
<td>From the Hampton Inn (Western Ave) to the PAC</td>
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<td>3/22/2019</td>
<td>7:15pm</td>
<td>From PAC to Hilton Garden Inn (Washington Ave) and Hampton Inn (Western Ave)</td>
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**Visitor Parking on UAlbany campus:**

Parking has been reserved for Symposium guests in the State Student Parking Lot, see map below for details and directions to relevant buildings.

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**Hilton Hotel**

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6th Annual Symposium on RNA Science and its Applications
The Language of RNA in Disease and Development
March 21 - 22, 2019

Thursday, March 21, 2019

9:30 am – 1:00 PM  Advisory Board Meeting
Scientific, Corporate and Faculty Advisory Boards
University Hall Room 306

Workshops

8:30 am – 3:00 pm  Nanoswitch-Mediated Detection of Micro-RNAs
Ken Halvorsen, RNA Institute, University at Albany
Life Sciences Research Building, Room 1079

10:30 am – 2:00 pm  Gradient Fractionation
Gabriele Fuchs, Department of Biological Sciences, University at Albany
Fuchs Lab: Life Sciences Research Building Room 2027

8:45 am – 2:00 pm  RNA Simulations
Alan Chen, Department of Chemistry, University at Albany
Paul Whitford, Department of Physics, Northeastern University
Massry Center for Business, BB 119

9:00 am – 2:00 pm  Epitranscriptomics
Dan Fabris, Department of Chemistry, University at Albany
Qishan Lin, Research, Associate Professor, University at Albany
Life Sciences Research Building, Room LSRB 1074

9:00 am – 2:00pm  Sequencing Technologies
Morgan Sammons, Department of Biological Sciences, University at Albany
Location: Life Sciences Research Building 1143

12 pm – 1:00pm  Workshop Lunch
Lunch provided for workshop participants Life Sciences Research Building 1144

Performing Arts Center (PAC) – Main Theater, University at Albany

11:00 – 5:00 pm  Registration
Performing Arts Center Lobby

1:00 – 3:00 pm  Light Refreshments
Lecture Center Hall – East Concourse

1:00 – 3:00 pm  Poster Sessions (Lecture Center – East Concourse)
(1:00 – 2:00 pm)  Poster Session I
(2:00 – 3:00 pm)  Poster Session II
3:00 – 3:10 pm  
Trainee Presentation Welcome Remarks  
Performing Arts Center, Main Theater  
Prash Rangan, University at Albany, SUNY

Trainee Presentation Session I

Chair: Justin Waldern, University at Albany

3:10 – 3:25 pm  
Selected Speaker, Hyaeyeong Kim, Weill Cornell Medical College  
"Imaging metabolite dynamics in live cells using corn, a fluorogenic homodimer RNA reporter"

3:25 – 3:40 pm  
Selected Speaker, Pooja Yadav, UT Health San Antonio  
"RNA demethylase ALKBH5 regulates osteosarcoma growth and chemosensitivity"

3:40 – 3:55 pm  
Selected Speaker, Patricia Garay, University of Michigan  
"Nascent RNA sequencing reveals neuronal activity-dependent transcription start sites"

3:55 - 4:10 pm  
Selected Speaker, Ying Tang, Rutgers NJ Medical School  
"Impact of microRNAs on renal disease induced calcification"

4:10 – 4:30 pm  
Coffee Break (Performing Arts Center Lounge)

Trainee Presentation Session II

Chair: Shane Breznak, University at Albany

4:30 – 4:45 pm  
Selected Speaker, Kimberly Harris, Yale University  
"Identification of large noncoding RNA-protein interactions and their effects on stress re- sponse in bacteria"

4:45 – 5:00 pm  
Selected Speaker, Chen Bao, University of Rochester  
"mRNA stem-loops can induce ribosome pausing by hindering A-site tRNA binding"

Distinguished Keynote Presentation

5:05 – 5:15 pm  
Welcome Remarks  
Elga Wulfert, Interim Provost and Vice President for Academic Affairs, University at Albany  
Patrick Blatt, Graduate Student, University at Albany

5:20 – 6:15 pm  
Distinguished Keynote  
Craig Mello, University of Massachusetts Medical School  
"RNA-guided inheritance in C. elegans"

7:00 – 8:30 pm  
Symposium Dinner  
Albany Pump Station, 19 Quackenbush Square, Albany NY 12207
6th Annual Symposium on RNA Science and its Applications
The Language of RNA in Disease and Development
March 21 - 22, 2019

Friday, March 22, 2019

Performing Arts Center, Main Theater, University at Albany

7:30 – 8:45 am  Registration: Performing Arts Center – Downstairs Lounge
Continental Breakfast: Performing Arts Center – Futterer Lounge
Poster Set-Up: Lecture Center – East Concourse

8:45 – 8:55 am  Opening Remarks
Andrew Berglund, Director, The RNA Institute

Session 1  Regulatory RNAs
Chair: Joan Curcio, Wadsworth Center, NYSDOH; University at Albany

8:55 – 9:25 am  Keynote: Gisela Storz, National Institutes of Health
“Astonishing diversity and redundancy in small RNA-mediated regulation”

9:25 – 9:55 am  Keynote: Elçin Ünal, University of California, Berkeley
“LUTI mRNAs: a fresh perspective on gene regulation”

9:55 – 10:10 am  Selected Speaker: Joseph Wade, Wadsworth Center, NYSDOH, University at Albany
“Nus factors prevent premature transcription termination of bacterial CRISPR arrays”

10:10 – 10:30 am  Coffee Break (Performing Arts Center – Futterer Lounge)

Session 2  RNA Chemistry
Chair: Jia Sheng, Department of Chemistry, University at Albany

10:30 – 11:00 am  Keynote: Eric Kool, Stanford University
“Small molecule reactive probes of RNA”

11:00 – 11:30 am  Keynote: Matt Disney, The Scripps Research Institute
“Translating RNA sequence into lead small molecule medicines”

11:30 – 11:45 am  Selected Speaker: Xiaohui Qu, Memorial Sloan Kettering Cancer Center
“A high-resolution in vitro single-molecule assay for eukaryotic cap-dependent initiation kinetics”

11:45 – 12:00 pm  Selected Speaker: Sara Rouhanifard, Northeastern University
“ClampFISH detects individual nucleic acid molecules using click chemistry-based amplification”
12:00 – 2:00 pm  Lunch Break (Lecture Center – East Concourse)

12:00 – 2:00 pm  Poster Sessions (Lecture Center – East Concourse)
(12:00 – 1:00 pm)  Poster Session I
(1:00 – 2:00 pm)  Poster Session II

Session 3  RNA Focus in Disease Research

Chair: Bijan Dey, The RNA Institute, University at Albany

2:00 – 2:30 pm  Keynote: Eliezer Calo, Massachusetts Institute of Technology
“DNA-PK leverages non-coding RNA binding to regulate nucleolar functions”

2:30 – 3:00 pm  Keynote: Charles Thornton, University of Rochester Medical Center
“RNA as perpetrator and victim in myotonic dystrophy”

3:00 – 3:15 pm  Selected Speaker: Jill Kreiling, Brown University
“The mir-465 family is upregulated with age and attenuates growth hormone signaling”

3:15 – 3:35 pm  Coffee Break
Futterer Lounge, PAC

Session 4  RNA Modifications and Machines

Chair: Jason Herschkowitz, Cancer Research Center, School of Public Health, University at Albany

3:35 – 4:05 pm  Keynote: Cara Pager, University at Albany
“RNA modifications fine-tune viral gene expression”

4:05 – 4:35 pm  Keynote: Wendy Gilbert, Yale Center for RNA Science and Medicine
“Thousand-fold regulation of translation initiation by transcript leader features”

4:35 – 4:50 pm  Selected Speaker: Maksim Royzen, University at Albany
“Bio-orthogonal chemistry-based method for labeling of cellular RNA”

4:50 – 5:05  Selected Speaker: Nilesh Banavali, Wadsworth Center, NYSDOH; University at Albany
“A Cryo-EM-derived Structural Ensemble of Zn-deficient Ribosomes Implicated in Mycobacterial Hibernation”

5:05 – 5:20 pm  Closing Remarks: Joan Curcio, Melinda Larsen and Tom Begley
Awards and prizes

5:20 – 7:00 pm  Closing Reception (Futterer Lounge)
Cocktails and hors d’oeuvres

Symposium Organizing Committee:
Joan Curcio and Melinda Larsen (Co-chairs), Tom Begley, Bijan Dey, Jason Herschkowitz, Prashanth Rangan, and Jia Sheng
Thursday March 21, 2019
Friday March 22, 2019
Performing Arts Center
Main Theater

Keynote Speaker Biographies
Distinguished Keynote Speaker

Craig Mello, Ph.D., University of Massachusetts Medical School

“RNA-guided inheritance in C. elegans”

Investigator, Howard Hughes Medical Institute
Blais University Chair in Molecular Medicine
Professor, RNA Therapeutics Institute and Program in Molecular Medicine
University of Massachusetts Medical School, Worcester, MA

Dr. Craig C. Mello is an Investigator of the Howard Hughes Medical Institute, the Blais University Chair in Molecular Medicine and Co-Founder of the RNA Therapeutics Institute at the University of Massachusetts Medical School.

Dr. Mello’s lab uses the nematode C. elegans as a model system to study embryogenesis and gene silencing. His collaborative work with Dr. Andrew Fire led to the discovery of RNA interference (RNAi), for which they shared the 2006 Nobel Prize in Physiology or Medicine. Together they showed that when C. elegans is exposed to double-stranded ribonucleic acid – dsRNA, a molecule that mimics a signature of viral infection, the worm mounts a sequence-specific silencing reaction that interferes with the expression of cognate cellular RNAs. For the layperson, RNAi is the cell’s search engine; the Google of the cell. Using readily produced short synthetic dsRNAs, researchers can now submit their own RNAi search queries to silence any gene in organisms as diverse as corn and humans. RNAi allows researchers to rapidly “knock out” the expression of specific genes and to thus define the biological functions of those genes. RNAi also provides a potential therapeutic avenue to silence genes that contribute to disease.

Before the Nobel Prize, Dr. Mello’s work on RNAi was recognized with several awards including the National Academy of Sciences Molecular Biology Award, the Canadian Gairdner International Award, the Paul Ehrlich-and Ludwig Darmstaedter Award, and the Dr. Paul Janssen Award for Biomedical Research. He is a member of the National Academy of Sciences, the American Academy of Arts and Sciences, and the American Philosophical Society.
Keynote Speaker

Gisela Storz, Ph.D., National Institutes of Health

“Astonishing diversity and redundancy in small RNA-mediated regulation”

Dr. Gisela Storz is the Associate Scientific Director of the Division of Molecular and Cellular Biology of NICHD. She received her Ph.D. from the University of California, Berkeley, and then carried out postdoctoral work at the National Cancer Institute and Harvard Medical School. For many years, a major focus of her group was the study of the bacterial and fungal responses to oxidative stress and redox-sensitive transcription factors. Her lab made the exciting discovery that the activity of the E. coli transcription factor OxyR is regulated by reversible disulfide bond formation, establishing a paradigm for redox-sensing proteins. As a result of the serendipitous detection of the peroxide-induced OxyS RNA, one of the first small, regulatory RNAs to be discovered, work in her lab shifted to the genome-wide identification of small RNAs. The pioneering characterization of many of these small RNAs revealed that they are integral to most regulatory circuits in bacteria. Recently, work in the Storz lab has extended to the detection and characterization of proteins of less than 50 amino acids, another class of molecules that is overlooked by traditional methods of investigation.

Keynote Speaker

Elçin Ünal, Ph.D., University of California, Berkeley

“LUTI mRNAs: a fresh perspective on gene regulation”

Dr. Elçin Ünal is Assistant Professor of Genetics, Genomics and Development at the University of California, Berkeley. She obtained her Ph.D. from Johns Hopkins University. Dr. Ünal was a Jane Coffin Childs Memorial Fund postdoctoral fellow with Angelika Amon at the Massachusetts Institute of Technology, where she began her studies on the link between gametogenesis and cellular rejuvenation. She was named a Pew Scholar in 2014, a Damon Runyan Rachleff Innovator in 2015 and a NIH Director’s New Innovator in 2016, and was awarded the R.R. Bensley Award in Cell Biology in 2018. Her lab studies the principles and regulation of meiotic differentiation. Specifically, Dr. Ünal and her team aim to understand (1) how the dynamic gene expression program of meiosis controls specialized events such as stepwise chromosome segregation and (2) how gamete formation promotes cellular rejuvenation and how cellular quality control pathways mediate organelle segregation and protein homeostasis during meiotic differentiation.
Keynote Speaker

Eric Kool, Ph.D., Stanford University
“Small molecule reactive probes of RNA”

Dr. Eric Kool’s research interests lie in the overlap between organic chemistry, biophysics, and chemical biology of nucleic acids. His work is aimed at the design of new functionally useful molecular tools, and applying them to gain basic understanding of biological and biomedical interactions and mechanisms. His laboratory has focused recently on mechanisms of DNA repair, and on development of tools for studying RNA biology. Dr. Kool received his Ph.D. in organic chemistry at Columbia University, and was trained in chemical biology as a postdoctoral fellow at Caltech. He has published over 280 papers during his career. He is an inventor on 30 patents granted or pending, and his inventions have been used as founding technologies for three biotechnology companies. He has trained more than 120 graduate students and postdoctoral researchers in his laboratory; over thirty of them have taken academic positions worldwide. A popular teacher at Stanford, he has twice been awarded the Humanities & Sciences Dean’s Award for Distinguished Teaching.

Keynote Speaker

Matt Disney, Ph.D., The Scripps Research Institute
“Translating RNA sequence into lead small molecule medicines”

Dr. Matthew Disney is a professor in the Department of Chemistry at The Scripps Research Institute. He obtained his Ph.D. from the University Of Rochester in 2003. He was named the BioFlorida Entrepreneur of the Year in 2018. The Disney Group is focused on developing rational and predictable approaches to design highly selective therapeutics from the genome sequence. One of the major articulations of the utility of genome sequencing efforts has been in advancing patient-specific therapies. Dr. Disney’s lab has developed a general approach to provide targeted therapeutics and precise medicines that target RNAs that cause disease broadly and include rare neuromuscular (muscular dystrophy), neurodegenerative (Alzheimer’s, ALS), infectious diseases as well as difficult-to-treat cancers (breast, pancreatic, prostate, and others), and infectious diseases.
**Keynote Speaker**

**Eliezer Calo, Ph.D., Massachusetts Institute of Technology**
“DNA-PK leverages non-coding RNA binding to regulate nucleolar functions”

Dr. Eliezer Calo is the Irwin W. and Helen Sizer Career Development Assistant Professor at Massachusetts Institute of Technology. He obtained his Ph.D. in 2011 from Massachusetts Institute of Technology. His lab focuses on the molecular entities controlling and coordinating RNA metabolism — that is, the compendium of processes that involve RNA, including protein synthesis, processing, modifications, export, translation and degradation. Dr. Calo works to understand how different aspects of RNA metabolism are controlled to generate structure and function during development, as well as how mutations in components of the RNA metabolic program lead to congenital disorders and cancer with a goal of providing unique insights into the structure and function of our cell-type diversity and potential opportunities for therapeutic interventions of a broader spectrum of complex human disorders.

**Keynote Speaker**

**Charles Thornton, M.D., University of Rochester Medical Center**
“RNA as perpetrator and victim in Myotonic Dystrophy”

Dr. Charles Thornton received his M.D. from the University of Iowa College of Medicine. He is the Saunders Distinguished Professor of Neuromuscular Research at the University of Rochester. He was the second fellow in the experimental therapeutics fellowship at the University of Rochester. Since graduating as a fellow, he has remained in the program as key mentoring faculty providing expertise in translational research and clinical trial design. He directs the Wellstone Muscular Dystrophy Cooperative Research Center in Rochester, now in its 16th year. Dr. Thornton’s research specializes in neurogenetic disorders, including myotonic dystrophy and therapeutics, including antisense approaches. Dr. Thornton has been the recipient of awards including the Carrell-Krusen Award, the Hans Steinert Award for Myotonic Dystrophy Research, and the Norman Saunders Jacob’s Ladder International Research Prize.
Keynote Speaker

Cara Pager, Ph.D., Department of Biological Science, University at Albany
“RNA modifications fine-tune viral gene expression”

Dr. Cara Pager is an Assistant Professor in the Department of Biological Sciences and The RNA Institute at the University at Albany-SUNY. She obtained her Ph.D. from the University of Kentucky in Lexington and joined Dr. Peter Sarnow’s laboratory at Stanford University as a Damon Runyon postdoctoral fellow. The overarching theme of her lab is to elucidate the interactions and mechanisms by which single-stranded positive-sense RNA viruses subvert host RNA metabolism pathways. Dr. Pager’s research has shown that RNA-binding proteins localized in processing bodies and stress granules function to promote and limit Zika virus gene expression. More recently her lab identified a plethora of epitranscriptomic marks on cellular RNAs, and the genomes of single-stranded positive-sense RNA viruses. Her recent published work showed that Zika virus, Dengue virus, HCV, poliovirus and HIV-1 modulate not just the methylome but the whole epitranscriptome. Her lab is currently focused on deciphering the regulatory importance of RNA modifications to cellular and viral RNAs following viral infection.

Keynote Speaker

Wendy Gilbert, Ph.D., Yale Center for RNA Science and Medicine
“Thousand-fold regulation of translation initiation by transcript leader features”

Dr. Wendy Gilbert is Associate Professor of Molecular Biophysics and Biochemistry at Yale University. She obtained her Ph.D. from the University of California, San Francisco, and then joined the laboratory of Dr. Jennifer Doudna at the University of California, Berkeley, as a Damon Runyan Cancer Foundation postdoctoral fellow. Dr. Gilbert was the recipient of The RNA Society Early Career Award in 2017. Her lab focuses on studying the features of mRNAs and ribosomes that control the level of protein production. Dr. Gilbert’s research program is unified by a global interest in RNA-dependent regulatory mechanisms and currently includes investigations of translation efficiency determinants, alternative 5’ UTRs, ribosomes, snoRNAs, and regulated RNA modifications.
Friday March 22, 2019
Performing Arts Center
Main Theater

Selected Speaker Abstracts
Selected Speaker                Session 1                9:55—10:10 AM

Nus Factors Prevent Premature Transcription Termination of Bacterial CRISPR Arrays

Anne M. Stringer¹, Gabriele Baniulyte², Erica Lasek-Nesselquist¹, and Joseph T. Wade¹²

¹Wadsworth Center, New York State Department of Health, Albany, NY
²Department of Biomedical Sciences, School of Public Health, University at Albany, Albany, NY

A hallmark of CRISPR-Cas immunity systems is the CRISPR array, a genomic locus consisting of short, repeated sequences (“repeats”) interspersed with short, variable sequences (“spacers”). CRISPR arrays are transcribed and processed into individual CRISPR RNAs (crRNAs) that each include a single spacer, and direct Cas proteins to complementary sequence in invading nucleic acid. Thus, the length of a CRISPR array correlates with the number of invaders that an organism is immune to. There is enormous variability in CRISPR array length between species, suggesting species-specific limits on the number of spacers that can be accommodated in a single CRISPR array. We hypothesized that the bacterial transcription termination factor, Rho, is one such limit. Rho is a widely conserved protein that terminates transcription of non-coding RNA. However, many bacterial CRISPR array transcripts are unusually long for untranslated RNA, suggesting the existence of mechanisms to prevent premature transcription termination by Rho. We show that CRISPR arrays of Salmonella Typhimurium are protected from Rho by the Nus factor anti-termination complex, and we provide evidence that this is an evolutionarily ancient mechanism to facilitate complete transcription of bacterial CRISPR arrays. Moreover, we show that Nus factor anti-termination is only partially protective against the activity of Rho, limiting the effective length of CRISPR arrays in bacteria that express Rho.

NOTES:
A high-resolution in vitro single-molecule assay for eukaryotic cap-dependent initiation

Hongyun Wang¹, Anthony Gaba¹, Lexi Sun², Xiaohui Qu¹

Molecular Biology Program, Memorial Sloan Kettering Cancer Center¹, Department of Physiology, Biophysics and Systems Biology, Weill Cornell Graduate School of Medical Sciences, Cornell University²
qux@mskcc.org

Protein synthesis rates differ by up to 1000 folds between eukaryotic mRNAs. Only less than half of such variability can be attributed to the difference in mRNA levels, highlighting the importance of translational control on regulating gene expression levels. Cap-dependent initiation is the predominant initiation pathway in eukaryotes and is the main target of translational control mechanisms. There is a lack of high-resolution techniques for kinetic characterization of the cap-dependent initiation pathway. We developed an in vitro single-molecule assay that addressed this lack. Our assay uses reporter mRNAs that code for N-terminal tagged proteins. The initiation kinetics is measured from single-molecule imaging of the binding of fluorescently labeled antibodies to the N-terminal tagged nascent peptides when they emerge from ribosome exit tunnel during active in vitro translation. We used this assay to study Arginine Attenuator Peptide (AAP) dependent translational control. For an AAP-regulated mRNA, its uORF (upstream open reading frame) codes for AAP, a short peptide that can stall ribosome in the exit tunnel at high Arginine concentration and consequently downregulate the main ORF translation. Arginine-regulation of the main ORF translation was well preserved in our assay. The single-molecule observation of the initiation kinetics for both the uORF and main ORF revealed very interesting interplay between the multiple ribosomes traveling on the same mRNA. Instead of a single AAP acting as a switch to turn off the main ORF initiation at high Arginine concentration, we found that each AAP only causes a short stalling of a single ribosome on mRNA. Intriguingly, due to the interplay between ribosomes, a mild stalling event is amplified over multiple ribosomes, which collectively cause a major halt of the main ORF initiation.

NOTES:
ClampFISH detects individual nucleic acid molecules using click chemistry-based amplification

S. Rouhanifard\(^1\), I. Mellis\(^2\), M. Dunagin\(^2\), S. Bayatpour\(^2\), C. Jiang\(^2\), I. Dardani\(^2\), O. Symmons\(^2\), B. Emert\(^2\), E. Torre\(^2\), A. Cote\(^2\), A. Sullivan\(^3\), J. Stamatyannopoulos\(^3\), A. Raj\(^2\)

\(^1\) Department of Bioengineering, Northeastern University, Boston MA, \(^2\) Department of Bioengineering, University of Pennsylvania, Philadelphia PA, \(^3\) Altius Institute for Biomedical Sciences, Seattle, WA

s.rouhanifard@northeastern.edu

Non-enzymatic, high-gain signal amplification methods with single-cell, single-molecule resolution are in great need. Our goal was to create a non-enzymatic, exponential amplification scheme with high sensitivity (detection efficiency), very high gain (signal amplification), and specificity (low background). We present click-amplifying FISH (clampFISH) for the fluorescent detection of nucleic acids that combines the specificity of oligonucleotides with bioorthogonal click chemistry in order to achieve high specificity and extremely high-gain (>400x) signal amplification. We show that clampFISH signal enables detection of multiple RNA species with low magnification microscopy and separation of cells by RNA levels via flow cytometry. Additionally, we show that the modular design of clampFISH probes enables multiplexing, that the locking mechanism prevents probe detachment in expansion microscopy, and that clampFISH works in tissue samples. Finally, we show that clampFISH enables the detection of DNA and RNA together in single cells. Of note, clampFISH probes behave as a proximity ligation wherein the click reaction will occur if and only if the two arms are hybridized adjacent to each other, suggesting future utility for probing RNA subsets such as splicing junctions, short alternatively spliced variants, or edited RNAs.

NOTES:
The mir-465 family is upregulated with age and attenuates growth hormone signaling

Amy Elias, Andrea Cespedes Zablah, Ian Sabula, and Jill A. Kreiling.

Department of Molecular Biology, Cell Biology and Biochemistry, Center for the Biology of Aging, Brown University, Providence, Rhode Island 02903, USA.

Aging is associated with a relaxation of heterochromatin that can lead to expression of sequences that are normally repressed. Several of these heterochromatic regions contain miRNA genes, and dysregulation of these sequences can result in altered expression levels of their mRNA targets. To identify age-associated changes in the miRNA transcriptome, we performed small RNA sequencing (<200 nt) on young (5 months), old (24 months) and very old (36 months) mouse liver. Of the 40 miRNAs identified that showed changes in expression levels with age, 27 were upregulated. Among these were a cluster of 15 miRNAs that were upregulated between 50 and 1000 fold at both 24 months and 36 months of age. This cluster is located in a 60 kb region of the X chromosome that is devoid of other coding sequences. This region is also part of a lamin-associated domain, indicating it is part of the constitutive heterochromatin. Expression of these miRNAs has only been found in the testes of young mice and their functions are not well established. Bioinformatic analyses of the potential targets of the miRNAs suggested they may regulate several pathways known to be altered in aging, including the PI3K-Akt pathway. Of the 64 putative targets in the PI3K-Akt pathway, RNA-seq revealed that 23 were downregulated in the liver from 24 and 36 month mice. Five miRNAs in this cluster are members of the mir-465 family: mmu-mir-465a, mmu-mir-465b (2 copies), and mmu-mir-465c (2 copies). These miRNAs share the same seed sequence and only differ by 1-2 nucleotides. There are 10 potential targets of the mir-465 family in the PI3K-Akt pathway that showed reduced expression with age in liver. One of these targets, the growth hormone receptor (GHR), contains a single predicted target site for the mir-465 family in its 3’ UTR. This site is highly conserved among vertebrates, signifying a strong evolutionary selection for the target sequence. We found that all 3 mir-465 miRNAs target the GHR mRNA equally. Transfection of the liver cell line AML12 with mir-465 family members led to a 40-50% reduction of the GHR at both the mRNA and the protein levels. There was a corresponding reduction in growth hormone signaling indicated by an attenuation of JAK2 and STAT5 phosphorylation upon growth hormone (GH) stimulation. There was also a dampening in the upregulation of the GH responsive genes, including the insulin-like growth factor 1 (IGF-1) and IGF-1 binding protein 3. It is known that GH signaling decreases with age and there is a corresponding reduction in circulating IGF-1. These data suggest that an increase in expression of the mir-465 family may contribute to the reduction in the GH signaling with age.

NOTES:
Bio-orthogonal chemistry based method for labeling of cellular RNA

Maksim Royzen, Dr. Kui Wi, Dr. Irfan Khan, Muhan He

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A bio-orthogonal chemistry-based approach for fluorescent labelling of cellular RNA will be described. It involves an adenosine analog modified with trans-cyclooctene and masked 5’-phosphate group using aryl phosphoramidate. The incorporation of the adenosine analog into RNA has been confirmed using a highly sensitive UHPLC-MS/MS method. Fluorescent labelling of RNA has been achieved in live HeLa and HEK-293 cells via an inverse electron demand Diels-Alder reaction with a tetrazine conjugated to an Oregon Green fluorophore. The obtained results demonstrate a new strategy towards development of future fluorescent probes to investigate the biochemistry of nucleic acids.

NOTES:
A Cryo-EM-derived Structural Ensemble of Zn-deficient Ribosomes Implicated in Mycobacterial Hibernation

Banavali, Nilesh K.1,2,* , Sharma, Manjuli R.1,*, Koripella, Ravi K.1, Keshavan, Pooja1, Yunlong, Li3, Ojha, Anil K.2,3, Agrawal, Rajendra K.1,2

* These authors contributed equally to this work

Zinc starvation in Mycobacteria results in synthesis of remodeled ribosomes with five ribosomal proteins replaced by paralogs lacking the Zinc-binding CXXC motif, and the ribosome is referred to as C-ribosomes. Biochemical evidence suggests that synthesis of C-ribosomes occurs during mycobacterial infection and increases the ribosome stability and antibiotic resistance [1]. We have solved a cryo-EM structure of the Zinc-depleted Mycobacterium smegmatis ribosome bound to the hibernation-promoting mycobacterial-specific protein Y (MPY) at 2.87Å resolution. This structure clarifies multiple mycobacterial ribosome features that remained unresolved in previous structures with resolutions lower than 3 Å [1-4], such as the details of the link provided by the L31 ribosomal protein between the two ribosomal subunits. Structural classification also reveals distinct conformational states involving larger-scale rotation of the two ribosomal subunits, presence or absence of ribosome-binding ligands (MPY, tRNA), and identification of stable binding poses of the typically-elusive S1 ribosomal protein. This ensemble also shows that a hibernating mycobacterial ribosome has both compositional and structural heterogeneity and provides the details necessary for a structure-based route to address its antibiotic resistance.


The work is supported by NIH grants GM061576 (to R.K.A.) and AI107595 (to A.K.O.)

NOTES:
Thursday March 21, 2019
Performing Arts Center
Main Theater

Trainee Presentation Session 1
3:10-4:10 PM
Imaging metabolite dynamics in live cells using Corn, a fluorogenic homodimer RNA reporter

Kim, Hyaeyeong¹, Jaffrey, Samie R. Ph.D.¹

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RNA-based genetically encodable sensors represent a new technology for imaging small-molecule metabolites in live cells. RNA-based sensors comprise a metabolite-binding aptamer fused to a fluorogenic aptamer, i.e., an aptamer that binds and switches on the fluorescence of a small-molecule fluorophore. Metabolite binding to its cognate aptamer triggers folding of a fluorogenic aptamer and activating fluorescence of the fluorophore by binding. We previously engineered RNA-based metabolite sensors using two related fluorogenic aptamers, Spinach and Broccoli. These metabolite sensors demonstrate the versatility of RNA-based sensors for imaging essentially any metabolite. However, a major challenge with the Spinach- and Broccoli-based sensors is their low photostability. If the fluorescence signals are changing over time, it is not clear if those changes in fluorescent signals reflect a loss of metabolite levels or a change in fluorescence due to photobleaching.

Recently we developed Corn, a fluorogenic RNA aptamer with remarkable photostability. Corn binds and induces fluorescence of 3,5-difluoro-4-hydroxybenzylidene-imidazolinone-2-oxime (DFHO), which resembles the fluorophore found in red fluorescent protein (RFP). The marked photostability of Corn–DFHO complexes is a result of the specific interaction of DFHO with the Corn RNA. Corn forms a homodimer in which the dimer interface encapsulates DFHO. Although photostable metabolite sensors would notably improve metabolite imaging and quantification in living cells, it is not clear how a metabolite-binding aptamer can be fused to Corn such that its fluorescence is regulated by metabolite levels.

Here we describe a new approach to engineer Corn-based metabolite sensors. Using this newly developed Corn-based sensor, we imaged S-adenosyl methionine (SAM) in live mammalian cells and monitored SAM dynamics in real-time. Notably, the dimerization of the Corn-based SAM sensor is induced upon the addition of SAM. Furthermore, SAM-induced dimerization of the sensor is reversible enabling imaging of SAM dynamics in real-time in live cells. Overall, this study demonstrates a general strategy for generating photostable metabolite sensors that function by metabolite-induced Corn dimerization and fluorescence.

NOTES:
Osteosarcoma (OS) is the most prevalent primary bone tumor that affects children and young adults. Despite several years of research, survival outcome of OS patients has stayed unchanged in last three decades. OS is treated with multi-modal chemotherapy, which is highly toxic and ineffective for chemoresistant tumors. Unlike most pediatric tumors, OS tumors show high genomic instability and lack of recurrent targetable mutations, precluding development of targeted therapy solely based on genomics. There is no FDA approved drug that can serve as an alternative to chemotherapy, warranting an urgent need to find efficacious therapeutics for OS. Here, we report that RNA methylation may hold opportunity to understand osteosarcoma etiology and develop effective therapeutic strategies. Our research shows that RNA demethylase AlkB Homolog 5 (ALKBH5) may serve as a novel therapeutic target for treating OS. N6-methyladenosine (m^6A) is the most common internal mRNA modification, which is modulated by the multi-component RNA methyltransferase complex, RNA demethylase (ALKBH5) and m6A readers. We found that ALKBH5 is amplified in sarcomas and its expression is highly elevated in osteosarcoma patient derived xenografts. We demonstrate that silencing of ALKBH5 inhibits the OS growth and migration without affecting the viability of normal human fetal osteoblast cells. Using catalytically mutant ALKBH5 overexpression plasmids, we found that demethylase activity of ALKBH5 is critical for its role in supporting OS cells migration. Supporting our in vitro data knockdown of ALKBH5 led to reduced tumor xenograft growth in mice. Interestingly, ALKBH5 depletion impairs the cell cycle progression and induces apoptosis in OS cells. Interestingly, reduction in ALKBH5 levels suppressed the DNA damage repair capacity of osteosarcoma cells rendering them sensitive to DNA damaging agent like Doxorubicin. In particular, we show that ALKBH5 depletion leads to reduced homologous recombination (HR)-and Non-Homologous End Joining (NHEJ) mediated DNA repair capacity of U2OS cells. Supporting this, we observed significantly reduced levels of several genes that are known to play critical roles in cell cycle progression, DNA damage repair as well as replication. Taken together, our study unveils a previously undefined role for m^6A methylation in maintaining osteosarcoma progression and Doxorubicin response.

NOTES:
Nascent RNA Sequencing Reveals Neuronal Activity-Dependent Transcription Start Sites

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In the brain, tightly controlled transcription is critical for proper neurodevelopment, learning, and memory. Since over half of mammalian genes are predicted to have multiple transcription start sites (TSS), the question arises: how do neurons selectively utilize alternative TSS? Studies have described steady-state, cell type-specific TSS usage in brain tissue, but recent evidence from plant biology hints that alternative TSS may also be regulated in a dynamic, stimulus-dependent manner. We therefore wondered if neuronal activity, a key signal in the brain, directs dynamic use of alternative TSS. To test this, we utilized a form of nascent RNA-sequencing, BrU-seq, which allows sensitive detection of short-term changes in transcription. We purified nascent RNA from mouse primary forebrain cultures treated with drugs to reduce or increase neuronal firing rate, and prepared next generation sequencing libraries using direct ligation of adapters to RNA, which enriches for read density at TSS. Our custom analysis pipeline revealed over 30 genes with activity-dependent usage of alternative TSS. Usage of these alternative TSS is predicted to alter the encoded protein primary structures, and in some cases to alter the protein’s subcellular localization. Using a CRISPR/Cas9-mediated protein-tagging technique (SLENDR), we tested if altered neuronal activity indeed results in a switch in protein localization of alternative TSS gene products. Electrophysiological studies of these same genes will determine a role for alternative TSS usage in neuronal plasticity. Lastly, we examined if these changes in TSS usage occurred in neurons in vivo, using next generation sequencing of ribosome-bound RNA after a paradigm of neuronal activation. Taken together, this work renders TSS selection as a key layer of gene regulation during the brain’s response to its environment. Future work aspires to illuminate mechanisms of TSS-specific transcriptional regulation.

NOTES:
Impact of MicroRNAs on Renal Disease Induced Calcification

Ying, Tang¹ Youhua, Zhu¹ ,Melissa B, Rogers¹
Rutgers - New Jersey Medical School, Microbiology, Biochemistry, & Molecular Genetics, Newark, NJ¹
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Cardiovascular calcification is a major pathological feature of atherosclerosis and other cardiovascular diseases. The American Heart Association projects that 40% of Americans will have some form of heart and/or vascular disease within the next 2 decades. Understanding the underlying mechanisms driving calcification is required to develop needed therapeutic approaches. Abnormally elevated levels of the pro-osteogenic bone morphogenetic protein 2 (BMP2) and BMP signaling are implicated in all forms of pathological cardiovascular calcification. MicroRNAs (miRNAs) that post-transcriptionally modulate the synthesis of BMP2 and downstream mediators have clinical potential as biomarkers and therapeutic agents in calcific disease. Mice deficient in Klotho develop ectopic calcification of soft tissues such as aorta, aortic valves and kidneys. We compared the profiles of miRNAs in the aorta from healthy mice relative to Klotho deficient mice with severe vascular calcification and tested the effect of selected miRNAs on the synthesis of BMP2 and downstream osteogenic events leading to calcification.

MicroRNA expression analysis showed that, compared to control mice, 135 miRNAs significantly increased and 77 miRNAs significantly decreased (p<0.05) in abundance in Klotho mutant male aortas. In Klotho mutant females, 62 miRNAs significantly increased and 88 miRNAs significantly decreased (p<0.05). Because miRNAs that target more than one BMP signaling mediator may act coordinately, we prioritized miRNAs predicted to target several members of this path. Bioinformatic analyse indicate that miR-145a-5p targets Bmp2, Smad1 and Smad5; miR-145a-3p is predicted to target Bmp2. RT-PCR assays confirmed the microarray results indicating that miR-145a-5p and miR-145a-3p levels were significantly decreased in Klotho mutant mice. A smooth muscle cell-targeted lentiviral vector bearing mir-145 was injected into the tail veins of Klotho mutant mice. The empty vector was used as negative control. RT-PCR results showed that aortic levels of miR-145a-5p and miR-145a-3p were increased by 1.3 to 2-fold in mice injected with the miR-145 virus, but not the empty vector. Having successfully optimized lentivirus delivery, the impact of increased miR-145 levels on Bmp2 and Smad expression and on calcification was evaluated. RNAs encoding the ligand BMP2 and the BMP signaling mediator SMAD5 were reduced in the aorta of mice injected with the miR-145 virus relative to the empty vector and SMAD1 expression was reduced only in females. These results demonstrate that restoring the normal healthy levels of specific miRNAs will repress the synthesis of BMP2 and other pro-osteogenic proteins. Finally, forced expression of miR-145 significantly decreased aortic calcium levels in the Klotho mutants. This important result suggests that restoring miR-145 levels in vivo can normalize the elevated levels of BMP signaling and the resulting vascular calcification.

This study demonstrated that miR-145 is a novel in vivo therapeutic target to limit pathological calcification by reducing BMP signaling. Our profiling efforts identified several other promising miRNAs now being tested in Klotho mice. This work shows great potential for identifying novel therapeutic approaches for treating calcification pathologies.

NOTES:
Thursday March 21, 2019
Performing Arts Center
Main Theater

Trainee Presentation Session II
4:30 – 5:00 PM
Identification of large noncoding RNA-protein interactions and their effects on stress response in bacteria

Kimberly A. Harris1,2,*, Zhiyuan Zhou2, Danielle L. Widner3, Michelle L. Peters2, Sarah G. Wilkins2, Nicole B. Odzer2, and Ronald R. Breaker1,2,3

1Howard Hughes Medical Institute, 2Department of Molecular, Cellular and Developmental Biology, 3Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, USA

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Noncoding RNAs (ncRNAs) are responsible for a remarkable variety of biological functions. Large ncRNA classes such as rRNAs, RNase P, and tmRNAs accomplish biochemical functions that rival the activities of proteins. However, large ncRNA classes are rare in bacteria. In the last decade, ~20 additional large, structured ncRNAs classes have been uncovered in bacteria that are unique, highly conserved, and likely to have interesting functions. Of these, the OLE (Ornate, Large, Extremophilic) RNA class is among the most complex and well-conserved ncRNAs discovered to date. This class includes 795 distinct representatives, each ~600 nucleotides long, from a wide range of species, including several human pathogens. These RNAs are abundantly expressed and lack homology to known RNA classes, suggesting they have an important and novel function. One possibility is that OLE RNAs have a role related to bacterial cell membrane stress. OLE RNAs localize to membranes through association with the transmembrane OLE-associated protein OapA. In Bacillus halodurans, Δole, ΔoapA, and Δole-oapA strains are less tolerant of cold temperatures, ethanol, and, surprisingly, Mg^{2+}. We have identified a dominant OapA mutant that reduces growth beyond the knockouts under cold, ethanol, and Mg^{2+} stresses. This defect can be rescued by mutations that render OLE RNA nonfunctional, suggesting that the dominant negative effect requires an intact RNA and that the ribonucleoprotein complex (RNP) interacts with other molecules. A genetic screen revealed several suppressor mutations in a gene for a protein of unknown function, named OapB. We found that OapB specifically binds OLE RNA in vitro with high affinity and specificity, whereas a mutation identified in the screen negates binding. These results indicate that the OapB-OLE RNA interaction is important for the RNP function. Furthermore, we investigated one of the first observations of Mg^{2+} toxicity in bacterial cells. By isolating suppressor mutants from cells exposed to Mg^{2+}, a potential strategy cells use to overcome Mg^{2+} stress was identified.

NOTES:
mRNA stem-loops can induce ribosome pausing by hindering A-site tRNA binding

Bao, Chen\textsuperscript{1}, Ling, Clarence\textsuperscript{1}, Ermolenko, Dmitri\textsuperscript{1}

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During translation elongation the ribosome moves along mRNA in a codon-by-codon manner. Although the elongating ribosome is an efficient helicase, certain RNA stem-loop structures are known to impede ribosome movement along mRNA and stimulate programmed ribosome frameshifting in both bacteria and eukaryotes. Molecular mechanisms by which these mRNA stem-loops, also known as Frameshifting Stimulating Sequences (FSSs), perturb translation elongation are poorly understood. To delineate the effect of the FSS RNA hairpin on ribosome pausing from frameshifting, which may partially alleviate ribosome pausing, we study FSSs from \textit{E.coli dnaX} mRNA and the \textit{gag-pol} transcript of Human Immunodeficiency Virus (HIV) in the context of “non-slippery” codons that were previously shown to dramatically diminish frameshifting efficiency. Using single-molecule Förster resonance energy transfer (smFRET), we find that dnaX and HIV FSSs efficiently induce stalling of the 70S ribosome and inhibit ribosome intersubunit rotation that normally accompanies each translation elongation cycle. mRNA-induced ribosome pausing occurs when the ribosome encounters the FSS, i.e. when the FSS is positioned at the mRNA entry channel of the small subunit. Biochemical assays show that upon encountering the ribosome, dnaX and HIV FSSs interfere with A-site tRNA binding. The inhibition of the A-site tRNA binding by mRNA stem-loops might be the common mechanism of the regulation of translation elongation shared by FSSs and other mRNA stem-loops that trigger ribosome pausing.

NOTES:
Thursday March 21st
and Friday March 22, 2019
Lecture Center East Concourse

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A Single-molecule Investigation on Interfacial Base-stacking Interaction Using a Centrifuge Force Microscope

Jibin Abraham Punnoose, Lifeng Zhou, Andrew Hayden, Arun Richard Chandrasekaran, Thomas Banco, Lillie Carnell, Ken Halvorsen*

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Single-molecule force experiments can be critical in providing a mechanistic understanding of biomolecular interactions. The recently developed Centrifuge Force Microscope (CFM) enables massively parallel single-molecule force manipulation with a low cost and easy to use instrument. Experiments are performed by subjecting surface tethered microspheres to centrifugal force while observing their motion with a microscope objective coupled to a CMOS camera. In this project, we have developed a plug-and-play CFM module that is self-contained in a commercial centrifuge bucket, and is able to live-stream images to an external computer during centrifugation. The whole system including data acquisition is controlled wirelessly through a LabVIEW interface. Using this system, we have been investigating the influence of the interfacial DNA base stacks on the stability of DNA structures held together by sticky-end hybridization. Through systematic elimination of interfacial base-stacks on one or either strand we were able to analyze the contribution of DNA base-stacking. DNA duplexes held together only by base-pairing showed significantly faster force dependent dissociation rates compared to those held together by the same number of base-pairs with interfacial base-stacks. The high-throughput CFM can collect hundreds of data points in a single minutes-long experiment, enabling us to probe many different duplex variants and conditions. Additionally, the simple operation of our instrument facilitates use by undergraduate researchers, introducing a new generation to biophysics.

NOTES:
Long Noncoding RNA AK001796 is associated with epithelial-to-mesenchymal transition in breast cancer and is essential for its survival

Sumayya Al-Chalabi1, Maneesh Kumar1, Ye Hsuan Tsai2, Joel Parker2, Marcel E. Dinger3, John S. Mattick3, Charles M. Perou4, Jeffrey M. Rosen5, Sendurai A. Mani6, and Jason I. Herschkowitz

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# These authors contributed equally to this manuscript

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Long non-coding RNAs, which are defined as transcripts that are longer than 200 nucleotides with no protein coding capacity, emerged as important players in cancer development and progression in the last decade. In this study, we examined the expression profiles of more than 17,000 lncRNAs in a large set of patients’ derived breast cancers and identified the lncRNA AK001796 to be enriched in aggressive breast cancers and to be associated with epithelial to mesenchymal transition (EMT), a molecular reprogramming process that play a vital role in cancer metastasis.

Loss-of-function studies using modified antisense oligonucleotides (ASOs) revealed the importance of this lncRNA for cell survival in EMT-positive breast cancer cells. Using cellular fractionation, we found that AK001796 is mainly localized to the nucleus. Most of lncRNAs regulate the expression of nearby genes; therefore, we attempted to find its association with the genomic downstream gene, BCL2L11, an apoptosis-associated protein. Interestingly, silencing of AK001796 resulted in upregulation of BCL2L11 in MDA-MD-231 cell line; while overexpression of AK001796 in the epithelial cells MCF7 results in upregulation of BCL2L11.

In addition to that, knockdown of this lncRNA in triple-negative breast cancer cell lines showed concomitant increase in the epithelial phenotype-associated gene E-cadherin.

Further, molecular characterization of this lncRNA revealed it to be regulated by the EMT-associated transcription factor BHLHE40.

For the purpose of understanding the mechanism of action of AK001796, we tried to identify the interacting protein partner. We found that AK001796 interacts with Poly (ADP-ribose) polymerase (PARP1) and affects its function. Furthermore, we used the chromatin isolation by RNA purification technique to identify the genomic loci that are targeted by this lncRNA. We found that AK001796 is enriched on markers for EMT, apoptosis, and some other cancer associated genes. These findings suggest AK001796 is a potential therapeutic target for metastatic breast cancer.

NOTES:
Regeneration is the process of renewal, restoration or growth resulting from injury or damage. Regenerative ability varies between organisms, with some invertebrates retaining the ability to regenerate their whole body from a small group of cells, while mammalian regeneration is much more limited. Within mammals, it is unknown why some organs, such as the liver, have significant regenerative capacity, while other organs, such as the submandibular salivary gland have limited regenerative ability. To better understand the signaling pathways that may limit salivary gland regeneration, we developed a partial salivary gland resection model, where approximately 40% of the left salivary gland is resected, leaving the right gland as a contralateral control. The gland was allowed to recover for 3 or 14 days. To get a broad understanding of changes in the transcriptome occurring at both 3 and 14 days post-resection, we performed RNA-sequencing on partially resected salivary gland and glands that had undergone mock surgery. Transcriptome analysis revealed an increase in cell cycle genes at 3 days that were largely reduced by 14 days, suggesting that the cells entered the cell cycle but that there was not a sustained proliferative response. Additionally, there was an increase in extracellular matrix transcripts that may represent a fibrotic response that could create a non-permissive environment for gland regeneration. In future studies, we will identify the cells and factors that contribute to the fibrotic response and identify pathways that can be manipulated to remediate fibrosis to facilitate regenerative mechanisms.
In an effort to observe and characterize the formation of parallel polyadenylic helices, a series of experiments was conducted utilizing a detection tool known as a DNA nanoswitch. Designed to permit parallel duplex formation, our modified use of the nanoswitch provides a direct visualization of interaction when determining if two different polyadenylic strands can duplex. We compared detection of parallel duplexes formed between RNA polyadenylic strands paired with strands of modified polyadenylic RNA that contain locked sugar moieties (i.e. locked nucleic acids or LNAs). We reveal important details regarding the capability of parallel duplexes to form or not form when LNAs are involved.
Characterizing RNA Aptamers as tools to dissect the distinct molecular functions of HSF1

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The Heat Shock Response is a highly conserved protective mechanism that is regulated at the transcriptional level by transcription factors called Heat Shock Factors (HSFs). These factors, when activated by high temperatures or stress, strongly induce the expression of Heat Shock genes (HS genes), that encode Heat Shock Proteins (HSPs). Of the family of HSFs in mammals, HSF1 is the functional homolog of the single HSF in yeast and the fruit fly, and, is required for the rapid activation of HS genes. HSF1 consists of at least three functional domains; the DNA binding domain (DBD), Trimerization domain (TD) and the Trans-Activation domain (TAD). Each of these domains function to coordinate HSF1’s ability to bind to its target DNA elements and trans-activate HS genes under HS conditions. However, the mechanistic roles of each domain are not entirely understood. To elucidate these functions in living cells, I am using the novel approach of blocking domains with RNA aptamers. RNA aptamers are short, single stranded RNAs that bind specifically and with high affinity to their selected target, whether it is a protein domain, transcription factor or small molecule.

We have thousands of selected candidate aptamers for HSF1, however, we needed to identify those that have domain specific binding in order to test a domain specific effect. Initially, to identify the domains bound by the aptamers, each HSF1 domain was purified to be used in Electrophoretic Mobility Shift Assays (EMSA). It appears that the initial selection yielded many high affinity selected aptamers, however this method of characterization does not take into account whether the aptamers bind across domains, or require a multimerized HSF1. Instead, I proceeded to use a higher resolution, biochemical, UV crosslinking approach to characterize the specific RNA-Protein interactions that occur between the aptamers and HSF1. I tested 21 aptamers, with a range of multiplicities and enrichments, and observed that they all bind to the DBD-TD portion of HSF1. To identify further whether these aptamers function as molecular inhibitors of the DBD, I tested their ability of compete with a Heat Shock Element (HSE) for HSF1 binding and found at least one candidate aptamer that appears to inhibit the functions of the DBD.

I have begun to test a few selected aptamers in mammalian cells to identify the extent to which they inhibit HSF1 induced transcription in vivo. We will measure the primary effects of aptamer expression on genome-wide transcription using Precision Run-On sequencing which allows for base-pair resolution mapping of actively transcribing Pol II.

NOTES:
Regulation of *yjhX-yjhQ* Toxin-Antitoxin system in *Escherichia coli*

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Toxin-antitoxin (TA) systems are widespread in bacteria and are utilized to target a wide variety cellular processes. While the expression of the toxin leads to cell growth arrest under unfavorable environmental conditions, the antitoxin neutralizes the corresponding toxin under normal conditions. Recently identified *yjhX-yjhQ* TA system, which is conserved in *E. coli* and related bacteria, contains an unusually long 5’ untranslated region (UTR). In this work, we show that *yjhX* 5’ UTR encodes a small open reading frame (sORF) which regulates the expression of the downstream *yjhX-yjhQ* operon by sensing nutrient limiting conditions and the presence ribosome targeting antibiotics. Our model suggests that under normal growth conditions 5’ UTR adopts an mRNA structure that renders ribosomal binding site (RBS) for the *yjhX* gene inaccessible, thus directly repressing translation. Transcription of *yjhX* is then further suppressed by Rho-dependent transcription termination. In the presence of antibiotics and limited nutrients, the ribosome stalls in the upstream sORF and causes the downstream 5’ UTR region to change conformation that permits the expression of the TA system. Finetuning of antitoxin and the corresponding toxin expression via ribosome fitness sensing mechanism could allow the cell to switch between active proliferation and cell growth arrest in response to changing environmental conditions.

NOTES:
Maternal mRNAs produced during oogenesis are essential for the next generation; any mistakes in this process are detrimental to the offspring. It is not known if mRNAs are selected for maternal deposition or if a mechanism is in place to curate the maternally deposited mRNAs. We have identified the RNA helicase Twister (Tst), catalyst of the conserved Super Killer (Ski) complex, as a guardian of maternal RNA inheritance in *Drosophila*. Tst, the homolog of human SKI2VL, facilitates mRNA decay by funneling target mRNAs from surveillance pathways to degradation machinery. Our data show that *tst* mutants are viable yet female sterile; expression of *tst* in the germ line alone rescues sterility defects. Loss of *tst* in the germ line triggers an accumulation of mRNAs that are otherwise only expressed in undifferentiated cells and germline stem cells (GSCs). Unexpectedly, a large fraction of these Tst-regulated ovarian-GSC transcripts are also endogenously expressed during spermatogenesis. In human cancers, expression of spermatogenesis proteins, called cancer-testis (CT) antigens, correlate with tumor metastasis. We find that some of the Tst-targets that are expressed during spermatogenesis are required in the female GSCs for proper stem cell self-renewal. Conversely, over expression of a Tst-target mRNA in differentiated stages of the female germ line impedes late oogenesis. RNA-Seq analyses show that Tst-regulated mRNAs are surveilled by Pelota (Dom34), an effector of the No-Go Decay (NGD) pathway. We find that the Ski complex detects spermatogenesis-expressed as well as oogenesis-regulated mRNAs undergoing translation and elicits degradation through NGD during differentiation to an oocyte. Intriguingly, we find that the expression of *tst* is attenuated in the GSCs and in testis, allowing for the expression of its targets there. Our data suggest that transcript-specific degradation via mRNA surveillance is a novel mechanism that contributes to GSC progression, which we hypothesize is linked to maternal mRNA transmission to the next generation. We propose that Ski complex-mediated culling of maternally produced mRNAs during oogenesis alleviates the presence of transcripts that sequester ribosomes and trigger NGD from being passed on to the next generation.
**Poster #8**

**Kdm5 controls germline stem cell differentiation by modulating ribosome biogenesis**

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**Abstract:** Stem cells display higher rates of ribosome biogenesis than their differentiated daughter cells. Decreasing ribosome biogenesis in stem cells promotes their differentiation suggesting levels of ribosomes can control stem cell fate. However, the mechanism by which ribosome biogenesis is regulated during stem cell differentiation is not fully understood. To address this question, we used *Drosophila* female germline stem cells (GSCs) as a model system due the availability of mutants, markers and short generation time. We find Drosophila female GSCs exhibit high level of ribosome biogenesis that decrease during differentiation. We identified Kdm5, a histone demethylase, as critical regulator of ribosome biogenesis and GSC differentiation. Kdm5 demethylates Histone 3 lysine 4 trimethylated (H3K4me3) containing nucleosomes to cause transcriptional silencing. We find that loss of Kdm5 or its catalytic activity in the germ line leads to an accumulation of stem cell daughters that cannot efficiently differentiate. RNA-seq analysis comparing Kdm5 mutants to control revealed that there are 1436 upregulated targets. Gene Ontology (GO) analysis of the upregulated targets unveiled an overwhelming number of genes associated with components of ribosome biogenesis that decrease during differentiation. As ribosome biogenesis is regulated by the Target of rapamycin (TOR) pathway, we asked if Kdm5 regulates ribosome biogenesis directly or indirectly by regulating the TOR pathway. We found that while only one protein of the TOR complex called Regulatory Associated Protein of TOR (Raptor) was upregulated the rest were not upregulated but alternatively spliced. Differential exon usage analysis of Tor pathway members such as TOR and Ribosomal s6 Kinase, revealed exons coding for regulatory domains were selectively excluded creating active versions of these proteins. Global bioinformatics analyses revealed that this alternative splicing was global with exons flanked by short introns being often excluded. Overexpression of active form of Tor pathway components also cause defects in GSC differentiation suggesting that Kdm5 promotes GSC differentiation by controlling ribosome levels. We hypothesize that during GSC differentiation, Kdm5 reprograms gene expression profile by modulating both levels and splicing of critical genes that control ribosome biogenesis promoting GSC differentiation.

**NOTES:**
Messenger RNA (mRNA) represents quite a small portion of all RNA molecules in the living organism. Besides mRNA there are many small non-coding RNAs whose biological significance has not been understood completely yet. Here we introduce the recognition of double-stranded RNA (dsRNA) using peptide nucleic acid (PNA) forming the triple helix in the major groove of RNA. PNA is an oligonucleotide analogue that has a neutral backbone with artificial synthetic nucleobases attached to it. We use nucleobases that bind with high selectivity to A, G, C, U nucleobases of dsRNA via Hoogsteen hydrogen bonding. The PNA-dsRNA triplex is stabilized by cationic nucleobase analogues, which interact with negatively charged backbone of RNA and by the improved pi-stacking of nucleobases. Recognition of guanosine (G) with cytosine (C) is problematic because of its low pKa (it has to be protonated in order to recognize G. Artificial nucleobase 2-aminopyridine (M) has a higher pKa than C and can be protonated at physiological conditions. Positive charge helps to stabilize triplex since in interacts with negatively charged backbone of dsRNA. Our goal is to recognize both nucleobases in the base pair (Figure 1) with additional heterocycle. The final goal is to design heterocycles that are linked together in order to increase binding between PNA and dsRNA and also pi-stacking.

This work was supported by the US National Institute of Health

Hepatitis C virus gene expression is modulated by fibrillarin abundance.

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RNA can be decorated with a plethora of chemical modifications that have recently been found to have critical functions within the cell. Some viruses encode their own RNA writer enzymes. For example, the methyltransferase activity of the Dengue virus (DENV) and Zika virus NS5 protein install a guanosine 5’ cap structure on the viral RNA and then further methylate the N7-position on guanosine and the 2’-O position of the adenosine sugar. Interestingly, DENV NS5 was also shown to modify the 2’-O position of internal adenosines in the viral and cellular RNAs, which impacted DENV translation and replication. Using mass spectrometry, we recently investigated the effect of hepatitis C virus (HCV) infection on the global landscapes of post-transcriptional RNA modifications (PTMs). In these studies, we observed the appearance of two dimethylcytosine variants in cellular RNA and on HCV RNA. Interestingly, we also observed 2’-O-methylation on the HCV genome. These data were surprising particularly because, unlike DENV NS5, the HCV NS5B RNA-dependent RNA polymerase lacks methyltransferase activity. These data suggested a cellular 2’-O-methyltransferase might be responsible for installing these marks on the viral RNA.

Fibrillarin functions with snoRNAs to 2’-O-methylate rRNA. A recent siRNA screen identified fibrillarin as a key modulator of Henipah virus infection. In this study therefore, we investigated the effect of fibrillarin on HCV gene expression. Specifically, we used target-specific siRNAs to deplete fibrillarin levels in Huh7 cells, infected with JFH-1 infectious HCV clone, and examined HCV gene expression three days post-infection. While depletion of fibrillarin, modestly affected cell viability, this effect contrasted the dramatic decrease in HCV core protein and viral RNA abundance. In current studies we are investigating the effect of fibrillarin depletion on HCV translation and replication, as well as examining the consequence of fibrillarin knockdown on the distribution of 2’-O-methyl groups on different cellular RNAs.

NOTES:
Utilization of a DNA Nanoswitch to Detect Parallel Polyadenylic Duplex Formation

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Polyadenylic molecules (poly A) are single stranded nucleic acids containing a series of repeating adenine bases. Polyriboadenylic (poly (rA)) strands form parallel duplexed helices in acidic conditions. Our group has generated and utilized a technique to determine if polyadenylic sequences can pair when each sequence differs in composition. We have adapted a DNA nanoswitch developed by others to detect duplex formation where we can control the composition of each strand that is being tested for interaction. This method is superior to traditional UV melting studies, given the low specificity for detection of duplex species in a complex mixture of polyadenylic strands with different compositions. Our results show that nanoswitches can be used to detect poly(rA) duplex formation as a function of pH and we have identified interesting binding between polyadenylic strands that have different compositions. Our methodology not only provides fundamental information regarding parallel polyadenylic duplex formation, but also can help in the design of future and potentially pH-sensing biological nanotechnology.

NOTES:
Poster #12

Investigating MBNL contributions to RNA SHAPE

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It is widely appreciated that post-transcriptional RNA processing is driven by combinatorial interactions with multiple RNA-binding proteins (RBPs). While individual binding motifs have been derived for many RBPs, there are often many more instances of a particular consensus sequence than appear to be utilized. The MBNL protein family is an evolutionarily-conserved set of RBPs which are best characterized as splicing regulators, but also function in alternative polyadenylation and mRNA localization. Loss of MBNL function is detrimental to the cell as sequestration of MBNL proteins by expanded CUG or CCUG repeat-containing RNAs results in multiple downstream effects on RNA processing and contributes to pathogenesis in myotonic dystrophy.

Determining what discriminates an active motif from an inactive one likely requires an understanding of the local topology of the RNA around the binding site. This is possible using the rapidly-evolving field of RNA SHAPE analysis. SHAPE technologies are very facile for abundant, short and structured targets such as IncRNAs but are still evolving for use in mRNA and pre-mRNAs targets as well as whole transcriptomes. Our current focus is on adapting the SHAPE and mutational profiling approach (SHAPE-MaP) for monitoring endogenous RNAs of low abundance such as pre-mRNAs. Among its many targets, MBNL autoregulates the inclusion of an alternative exon in its own pre-mRNA. This regulation is controlled by an upstream region that includes several putative MBNL1 sites, however only one serves as the dominant site. Using a cell line with inducible MBNL1 expression, we are interested in examining changes in local structure within the exon 5 regulatory region in the MBNL pre-mRNA in response to protein levels.

NOTES:
Despite the lack of proofreading mechanisms, the rate of error-prone replication of flaviviruses is thought to be tightly regulated for optimal fitness within and between hosts. In West Nile virus (WNV) specifically, high fidelity mutations V793I and G806R within the RNA-dependent RNA polymerase (RdRp) and low fidelity mutation T248I within the methyltransferase (Mtase) were previously shown to attenuate infectivity and replicative fitness in Culex mosquitoes and Culex tarsalis (CxT) cells, respectively, but did not alter in vitro growth kinetics in vertebrate cells. In addition to fidelity differences, mutational bias was previously noted. We hypothesized that alterations to the pace and frequency of mutations would alter the capacity for evolution and adaptation in a host-specific manner. To test this hypothesis, mutant high fidelity (HiFi; V793I/G806R) and low fidelity (LoFi; T248I) strains were serially passaged 8 times in either avian cell line PDE or mosquito cell line CxT or passaged alternatively in each. Passaged fidelity variants were deep-sequenced to ensure the presence of the relevant fidelity mutations, and then grown in PDE and CxT cell lines to characterize changes to replicative fitness resulting from passage. Deep sequencing results showed multiple synonymous and non-synonymous consensus and non-consensus mutations unique to both cell type and viral strain, suggesting evolutionary trajectories were both host and strain-dependent. In addition, evolution was constrained by alternating hosts. Analysis of growth kinetics of passaged and unpassaged fidelity variants confirmed attenuation of LoFi variants in avian cells, and both HiFi and LoFi variants in mosquito cells. Although attenuated fidelity variants did not achieve fitness levels equivalent to WT WNV these results demonstrate that they are capable of adaptation to specific and non-specific cell lines via differential mutations.
Transcriptional regulatory regions are critical elements driving transcript abundance within an organism. DNA sequences, known as enhancers, act as scaffolds for transcription factors which control the various steps required for RNA synthesis. Enhancers orchestrate the temporal and spatial expression of their linked genes, however, the rules governing factor identity, abundance, and position at the enhancer remain elusive. Further, the number of predicted enhancers far exceeds the number of genes creating additional layers of regulation that must be deciphered. In order to better understand the regulatory potential of enhancers bound by the transcription factor p53, we designed and executed a massively parallel reporter assay (MPRA). This approach allowed us to measure the transcriptional activation potential of thousands of DNA regulatory elements in parallel. We utilized molecular DNA barcodes that are retained in the RNA product in order to measure the activity of our candidate enhancers. Using this approach coupled with systematic mutagenesis and high throughput DNA sequencing, we dissected how DNA regulatory sequences within p53-bound enhancers affect RNA synthesis. Our data indicate that p53-bound enhancers require other DNA binding co-factors, but specific factors are difficult to predict by sequence or conservation alone. The use of an additional unique molecular identifier (UMI) within the RNA and DNA products reduced sequencing costs and increased the efficiency of sequencing library preparation. Optimization of MPRA and integrated molecular barcoding approaches will allow more cost-effective and efficient analysis of DNA and RNA regulatory pathways.

NOTES:
Developing an accurate all-atom fixed-charge force field for RNA with implicitly polarized charges

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Molecular dynamics (MD) simulations are powerful tools for modeling the structural ensembles of nucleic acids that mediate their biological functions. MD simulations generate hypotheses about molecular structure that can then be tested experimentally. The utility of computer simulations for biomolecules relies on the accuracy of the energy model, a parameterized function called a force field. Currently available fixed-charge force fields for RNAs can accurately describe A-form helices composed of canonical Watson-Crick base pairs. However, there is a consensus among the RNA simulation community that these force fields fail to model other common features of RNA tertiary structure, such as noncanonical base interactions in loop regions and the relative orientations of helices interrupted by bulge loops or asymmetric internal loops. The failures of current RNA force fields limit the applicability of MD simulations to provide useful information about the tertiary structures of RNAs. Thus, development of an accurate fixed-charge force field that is transferable to structurally diverse RNAs remains an open challenge.

We report a new parameter set for the Amber RNA force field using the ff99 functional form and bonded terms. The nonbonded terms describing the electrostatic and van der Waals interactions are fit to quantum mechanical interaction energies, obtained using symmetry-adapted perturbation theory (SAPT), for a diverse set of nucleoside dimers and base-phosphate dimers. Following the implicitly polarized charges (IPolQ) strategy used for the ff15ipq force field, we account for the influence of solvent in the SAPT calculations by including a field of point charges representing the time-averaged solvent density obtained from classical MD simulations of water and ions around a restrained RNA.

NOTES:
Differentiated vascular smooth muscle cells (VSMCs) are crucial to maintain vascular tone for normal blood pressure. However, the differentiated/contractile phenotype of VSMCs is not terminal but switched to a dedifferentiated/synthetic mode upon pathophysiological cues such as vascular injury, contributing to the progression of vascular diseases. VSMC differentiation is primarily regulated by serum response factor (SRF), and its cofactor, myocardin (MYOCD), which transactivate contractile VSMC marker genes through binding conserved CArG elements in regulatory regions of the VSMC markers. While the transcriptome of coding genes to define the VSMC contractile phenotype has been intensively investigated and therefore well-established, we know little about SMC-specific noncoding genes, particularly the dominant class of noncoding transcripts, long noncoding RNAs (lncRNAs). Here, by lncRNA array analysis of cultured mouse aortic SMCs with either MYOCD overexpression or SRF deletion, we identified a novel Myocardin-induced muscle-specific lncRNA (Mymsl), which is markedly regulated by both MYOCD and SRF in mice. Mymsl was found to have no coding potential by PhyloCSF computational prediction and in vitro transcription/translation assay. RT-PCR and RNA in situ hybridization showed its abundance in SMCs and cardiomyocytes. To determine whether Mymsl is a direct transcriptional target of MYOCD-SRF, we screened its proximal promoter region and found a consensus CArG box. Luciferase reporter containing the CArG was robustly activated by both MYOCD and SRF, and mutation of the CArG box abolished this activation. To evaluate in vivo functionality of this CArG, we generated a mouse line carrying the 3-nucleotide mutation on the CArG by CRISPR-CAS9. The CArG mutant mice completely lost Mymsl expression in SMCs but not in cardiomyocytes, indicating that Mymsl transcription in SMCs is exclusively dependent on MYOCD-SRF-CArG regulatory axis. Chromatin immunoprecipitation further showed that SRF binding to the CArG region in SMC tissues was impaired in CArG mutant mice. To begin to elucidate the functional roles of Mymsl in VSMC phenotypic transition, we first assessed its mRNA expression in several VSMC phenotypic modulation and vascular pathology models. We found that injured carotid arteries and atherosclerotic arteries display much less Mymsl expression compared with their individual control arteries. Unexpectedly, SMC-specific loss of Mymsl in the CArG mutant mice inhibited gene expression of proinflammatory cytokines in injured carotid arteries compared with wild type mice. This indicates that Mymsl is a critical regulator in vascular inflammation triggered by injury. Taken together, our data demonstrate Mymsl transcription in VSMCs is solely dependent on MYOCD/SRF through a single consensus CArG box within its promoter region. Our data also indicate that Mymsl is a novel lncRNA associated with the VSMC contractile phenotype but positively regulates inflammation, which may play important roles in vascular disease.

NOTES:
Visualization of zinc regulated ribosome remodeling and hibernation in *Mycobacterium smegmatis*

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In many bacterial species, zinc regulates ribosome assembly. During zinc starvation, all mycobacterial species reprogram their ribosomes by replacing ribosomal proteins containing the zinc-binding CXXC motif, called C+, with their paralogues without the motif, called C-. Slow growing species, such as *Mycobacterium tuberculosis*, have four C+/C- paralogues, S18, S14, L28, and L33 while the fast growing, such as *Mycobacterium smegmatis*, encode a fifth paralogue, L31. The C+ proteins are constitutively expressed and are post-translationally downregulated during zinc starvation. The C- proteins are transcriptionally regulated and are located within an operon controlled by ZurB. ZurB binds to zinc and represses expression of the C- operon. When zinc is depleted, it de-represses the C- operon, leading to their robust transcription and subsequent replacement of the C+ proteins in the ribosome. Moreover, the C-ribosome appears to be the target of two proteins – MPY (Mycobacteria Protein Y) and MRF (MPY Recruitment Factor) – that stabilize the 70S ribosome in a inactive but drug resistant state. Furthermore, MRF expression is post-transcriptionally controlled. The purpose of this study was to test if ribosome remodeling and ribosome hibernation occur at different stages of mycobacterial growth. To monitor the ribosome remodeling, the operon encoding C-ribosomal protein was transcriptionally fused to Dendra2. To monitor ribosome hibernation, the C-terminus of MRF was fused in-frame with Dendra2. Both the reporter strains were grown in zinc-limiting medium. We observed that ribosome remodeling occurs at a higher zinc concentration than that necessary for ribosome hibernation. Thus, the findings suggest that assembly of C-ribosomes occurs during active growth of mycobacteria, and that these ribosomes likely assume the primary role of protein synthesis for a significant period before winding down into a hibernating state.

NOTES:
Developing countermeasures against the biothreat agent ricin toxin is extremely challenging, because the toxin is readily internalized into mammalian cells via endocytosis. Once inside a cell, ricin traffics retrograde to the endoplasmic reticulum, where the enzymatic subunit (RTA), an RNA N-glycosidase specific for a conserved residue within the sarcin-ricin loop of the 28S rRNA, is liberated from the binding subunit (RTB) and is translocated into the cytosol. In the cytosol, the acidic P-stalk proteins of the 60S ribosome bind RTA and guide it to the SRL, whereupon it depurinates a single adenine residue that disables the ribosome and arrests protein synthesis. In this poster, we report efforts to develop intracellular antibodies (intrabodies) capable of neutralizing ricin within the cytosol of mammalian cells. We have recently identified a collection of 10 alpaca-derived, heavy chain-only antibody VH domains (VHs) against RTA’s two ribosome-interacting surfaces: the active site (AS) and ribosome binding site (RBS). In a cell-free translation assay (IVT), the 10 VHs displayed a wide range of neutralizing activities against RTA that strongly correlated with antibody binding affinity, as determined by surface plasmon resonance (SPR). To assess the VH neutralizing activities within cells, we generated mammalian expression vectors encoding each of the seven AS VHs and one RBS VH (V9E1) and transfected them into Vero cells prior to ricin challenge. Functional expression of the VHs was demonstrated via ELISA with cell lysate. Vero cells transfected with the AS VHs were 50-fold more resistant to ricin than control cells transfected with a botulinum neurotoxin-specific VH. These results recapitulate IVT and SPR data. V9E1 expression results in cellular protection to the same extent as the most potent AS VHs. This is consistent with V9E1 exerting its neutralizing effects by physically occluding RTA access to the ribosome. We postulate that high affinity intracellular VHs targeting the ribosome interfaces on RTA may not only provide a powerful therapeutic option for ricin intoxication, but also an invaluable tool for better understanding the elusive mechanisms of RTA or other RIPS, such as Shiga Toxin, after exit from the ER.

NOTES:
BHLHE40-AS1 is a noncoding RNA that regulates IL6 signaling to support breast cancer progression

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Increased emphasis on breast cancer screening has led to a dramatic increase in diagnosis of ductal carcinoma in situ (DCIS). DCIS lesions are nonobligate precursors of invasive ductal carcinoma (IDC) and thus, the current standard-of-care is aggressive therapy to prevent invasive and metastatic disease. However, only ~30% of DCIS cases are predicted to progress leading to a current state of overtreatment and overdiagnosis. Thus, there is a critical need to identify functional determinants of progression of DCIS to IDC to allow discrimination between indolent and aggressive breast cancers and refine patient treatment strategies. We propose that long noncoding RNAs (lncRNAs) functionally drive breast cancer progression and their expression can discriminate between innocuous and potentially invasive DCIS.

Using biopsies from women with tandem DCIS and IDC lesions, we identified 35 lncRNAs whose expression can distinguish between DCIS and IDC. From this candidate list, the lncRNA BHLHE40-AS1 is found enriched in multiple breast cancer progression models, in HER2+ cell lines, and HER2+ patient tumors. Functionally, BHLHE40-AS1 is found to support tumor cell cycle and motility and may serve as a clinically relevant biomarker and therapeutic target in invasive breast cancer.

To mechanistically elaborate the function of BHLHE40-AS1, we overexpressed the lncRNA in normal MCF10A cells. Overexpression resulted in increased motility and invasion. Microarray analysis revealed an increased expression of IL6 and STAT3 and predicted the STAT3 pathway to be activated. Indeed we find that overexpression and depletion of BHLHE40-AS1 regulates the STAT3 pathway, potentially through an interaction with the RNA binding protein ILF3. Together these data suggest BHLHE40-AS1 supports early breast cancer progression as a key mediator of IL6 signaling.

NOTES:
Low temperature adaptation of a group II intron

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Group II introns disseminate across all domains of life by interrupting genes. The Lactococcus lactis LtrB (LI.LtrB) group II intron resides in a conjugative plasmid pRS01 within its host relaxase gene that is responsible for the plasmid's conjugal transmission. Our previous work showed that the LI.LtrB intron inhibits expression of the host relaxase and thus the horizontal transfer of pRS01 by mRNA targeting. However, whether and how this regulatory pathway responds to environmental cues have not been determined. Here we show that the splicing of the LI.LtrB intron in the native lactococcal host is inhibited at temperatures that are lower than the physiological one (10/20°C vs. 30°C). In-cell structure profiling reveals that key tertiary interactions essential for maintaining the scaffold structure of the intron RNA are disrupted at those lower temperatures. In addition, in vitro assays indicate that such splicing inhibition by cold takes place in a protein-independent fashion. However, despite suppression of splicing, levels of the host relaxase gene-encoded mRNA (ligated exons) increase, likely due to significant reduction in the mRNA-targeting activity of the intron, leading to increased levels of translatable mRNA. Taken together, this study suggests that the bacterial group II intron can sense and adapt to low temperatures and regulate the function of the host gene.

NOTES:
Kinetic Analysis of a DNA-Cleaving DNA Enzyme

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A class of proteins, referred to as enzymes, act as the main catalysts for various essential biological reactions. However, it has also been discovered that macromolecules other than proteins, like certain DNA and RNA, possess the same functional capability as enzymes and facilitate specific nonspontaneous reactions. One particular synthetic catalyst, referred to as the ssDNA-cleaving DNA enzyme, binds to and cleaves specific a ssDNA substrate when found in the presence of zinc cations. Understanding the mechanism behind the DNA enzyme will enhance our understanding of certain biological processes, as well as provide more insight into the instability associated with cellular gene expression.

To test the functionality of the DNA cleaving DNA enzyme, a non-radioactive kinetics assay was used to determine the rate of the reaction in terms of percent cleavage per unit of time. While conducting the experiment, several factors were altered to test which conditions allowed for the most effective DNA cleavage. To do this, varying concentrations of zinc (II) chloride were used in combination with the DNA enzyme to achieve the highest value of percent cleavage per unit of time.

At high DNA concentration, 10 mM ZnCl₂, yield the most cleavage the substrate DNA. This is in contradiction to the published results with radioactive trace-amount kinetics where an optimal ZnCl₂ concentration was at around 0.5 mM (10 mM ZnCl₂ showed total cleavage inhibition). We are currently investigating if the zinc cations favor binding to the negatively charged phosphate backbone with more affinity than to that of the active site, which lowers the activity of the enzyme. Furthermore, we are in the process of converting the non-radioactive kinetic gels to fluorescence spectroscopy to measure kinetics more precisely.
Rbfox1 splicing regulation across a broad concentration range

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Alternative RNA splicing is a fundamentally important process that cells use to generate multiple proteins through differential splicing of a single pre-mRNA transcript. The process that regulates the alternative ‘splicing code’ is far from completely understood. RNA binding proteins (RBPs) play a crucial role in the regulation of alternative splicing through various mechanisms such as position-dependent effects and recruitment of other splicing factors. Rbfox1 codes for Rbfox1 RBP that has been shown to have a role in splicing and development in the brain and skeletal muscle. Rbfox1 binds to the (U)GCAUG motif and interacts with a complex of other proteins to regulate splicing. How Rbfox1 mechanistically regulates splicing has still yet to be fully understood. Here we show using an inducible-Rbfox1 cell line that splicing is regulated in a dose-dependent manner. Using this system, we will identify which pre-mRNA transcripts are regulated at low Rbfox1 concentrations versus high concentrations. Furthermore, we will identify splicing events that respond over shallow or broad concentrations ranges and correlate these measurements with RNA elements in the pre-mRNA and other splicing factors with the long-term goal of better understanding Rbfox1’s role in the splicing code.
Chemical modifications that regulate protein expression at the translational level are vital components of the cellular response to oxidative stress and defend against the damaging effects of reactive oxidative species (ROS). tRNAs are significant targets for methyl-based modifications, which are catalyzed by tRNA methyltransferases, or TRMs. tRNA modifications within the anticodon stem-loop are essential determinants of messenger RNA codon selection and influence the translation of certain open reading frames (ORFs) with a codon-bias. That is, ORFs that preferentially use one synonymous codon over another. Previous work shows that TRM7 deficiency in *S. cerevisiae* leads to oxidative stress sensitivity. One of the targets of TRM7-dependent methylation is the 2'-O-ribose position of tRNA-Phe(GAA). Due to this methylation, we expect that the translation of ORFs high in UUC are favored because of stronger wobble-base pairing when compared to the synonymous codon for Phe, UUU. We further expect that genes with a UUC codon bias are involved in the oxidative stress response.

*S. cerevisiae* was used here as a model organism to test the hypothesis that UUC-biased genes are involved in pathways that respond to oxidative stress. We first used the Codon Usage Tool to identify ORFs that have significant z-scores for the number of UUCs in their transcripts. Then, we obtained *S. cerevisiae* deletion mutants of these genes and performed acute exposures to hydrogen peroxide and chronic exposures to hydrogen peroxide, acetic acid, and rotenone to assess the sensitivity of these mutants to these oxidative stress-inducing compounds. We found that the deletion mutant strains, *CRT10-Δ, HIR3-Δ,* and *GNP1-Δ,* had significantly lower colony forming potential and survival post-exposure compared to the wild type (BY4741) strain. These results support the idea that genes enriched with UUC transcripts are involved in the oxidative stress response due to codon biased translation linked to the methylation of tRNAPhe(GAA) by TRM7.

**NOTES:**
The Protective Role of the Epitranscriptome Against Acetaminophen Toxicity

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Epitranscriptomic marks have been shown to play important roles in sensing environmental and physiological conditions as well as corresponding RNA modifications which regulate the translation of specific proteins to coordinate gene expression. Little is known about the role of epitranscriptomic systems during the response to common pharmaceuticals, and my project addresses this gap in information. The use of acetaminophen causes nearly 50% of all U.S. acute liver failure. Chronic use or toxic doses of acetaminophen promote increased concentration of the reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), which can cause depletion in glutathione levels and promote increased intracellular reactive oxygen species (ROS), DNA damage and lipid peroxidation in both kidney and liver tissue. The epitranscriptomic writer Alkylation Repair Homolog 8 (Alkbh8) plays critical roles in regulating the levels of ROS detoxifying selenoproteins that include glutathione peroxidases (Gpx) and thioredoxin reductases (TrxR). Alkbh8 modifies tRNA selenocysteine (tRNA^Sec) to promote the formation of 5-methoxycarbonylmethyluridine (mcm^5U) or the ribose-methylated derivative, 5-methoxycarbonylmethyl-2'-O-methyluridine (mcm^5Um) with both modifications playing critical roles in the translation of the selenoproteins Gpx and TrxR enzymes. Gpxs and TrxRs have been shown to have protective roles in hepatic tissues when exposed to toxic doses of acetaminophen. My primary hypothesis is that Alkbh8 and other epitranscriptomic writers provide protection against pharmaceutical stressors, by catalyzing tRNA modifications which regulate the translation of stress response genes and protein levels, against resulting cellular insults. In vivo exposures utilizing acetaminophen will be used to test my hypothesis. We have demonstrated that there is increased levels of DNA damage and inflammation gene markers in kidney and liver tissue from Alkbh8 deficient mice with chronic acetaminophen exposure indicating the protective role of epitranscriptomic marks. Specifically, in liver tissue from Alkbh8 deficient mice, oxidative stress gene markers are also elevated. Further studies will involve evaluating macro, micro, and nano marks of cellular stress phenotypes such as oxidative stress, DNA damage, as well as epitranscriptomic response to acetaminophen. My study will be the first to define the role of epitranscriptomic writers and their response to acetaminophen.

NOTES:
Splicing is the process where pre-mRNA is transformed into mRNA by removing introns and joining exons together. The pattern of which introns are removed, and which exons are joined together affects the characteristics of the translated protein. We have developed a gene therapy vector that delivers antisense RNA sequences that bind splicing elements blocking pre-mRNA splicing. Our therapy induces alternative patterns of splicing, reducing the expression of oncogenic proteins. We are using this strategy to target oncogenic epidermal growth factor receptor (EGFR) transcripts which are overexpressed in 60% of glioblastoma multiforme tumors. To optimize efficacy of our current antisense RNAs, we are examining the EGFR RNA structurome to identify regions of transcripts which are not bound by protein or involved in RNA secondary, tertiary, or quaternary structure.

The pre-mRNA structurome affects the splicing pattern. In order to therapeutically modify the pre-mRNA transcript, we are using tools to uncover the RNA structurome of oncogenic transcripts. We have begun experiments to analyze the EGFR pre-mRNA structure using selective 2′ hydroxyl acylation and primer extension followed by mutational profiling (SHAPE-MaP). The SHAPE reagent (1M7) reacts with the 2′ hydroxyl of RNA molecules when the RNA molecule is in a conformationally flexible position creating a 2′ O-adduct. The modified RNA is reverse transcribed, incorporating mismatches at the acylated positions; a comparison of unmodified to modified RNA will allow us to determine RNA nucleotides that are involved in secondary structure, part of RNA-binding-protein complexes, or single stranded. Single stranded RNAs and RNAs with minimal structure are a preferential target of our therapy. We hypothesize that the secondary structure of exon15-intron-15-exon16 of the pre-mRNA transcript will determine the most effective way to therapeutically alter the splicing of EGFR. Also, the secondary structure of the pre-mRNA will give further insight into understanding the mechanism of alternative transcripts induced by nature.

SKMG-3 cells were grown in DMEM with 10% FBS. Cells were subjected to 1M7, 5-NIA or DMSO (control) in cellular and cell-free conditions. RNA was isolated using Trizol and phenol:chloroform:isoamyl alcohol respectively. The RNA was subjected to DNA degradation followed by reverse transcribed with Superscript IV under SHAPE conditions using a gene specific cocktail primer. Reverse transcription under SHAPE conditions includes the use of manganese chloride as the divalent ion for the RNA-dependent DNA polymerase subunit and is significantly less effective. It was determined that the most effective way to isolate pre-mRNA was to reverse transcribe with a gene specific cocktail primer mix. Primers were designed to target the EGFR transcript ranging from intron 15 to the 3′ UTR. The cocktail consisted of 10 primers at 2 pmol/primer. cDNA was converted to double stranded DNA and prepared for Oxford Nanopore sequencing.

NOTES:
Transfer RNA fragments (tRFs) are a class of small RNA molecules derived from mature or precursor tRNAs. Although characterized very recently, tRFs have been gradually attracting more attention. They are found across a wide range of organisms and tissues in cytoplasmic compartments or loaded to RISC complexes, often in numbers comparable to microRNAs.

We analyzed sequences of chimeras formed in vivo between Argonaute-loaded tRFs and their targets, corresponding to various gene types, in addition to protein-coding transcripts. In the latter, 3' UTRs were the likely primary target regions, although we observed interactions of tRFs with coding sequences and 5' UTRs. We also report a novel phenomenon – a large number of putative interactions between tRFs and introns, compatible with the role of Argonaute in the nucleus.

We clustered tRF binding patterns and identified enriched motifs that may be responsible for tRF-target interactions. Such interaction sites appear to be primarily located on the 5' end of a tRF, often involving additional binding of the 3' nucleotides of guide tRFs, similar to microRNAs. Strikingly, our results match interaction sites detected in a recent experimental screen, confirming the validity of our approach to predict the sites and mechanisms of tRF/target interactions computationally.

NOTES:
Selection of an Aptamer to bind 2-HydroxyGlutarate through SELEX

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Glioma and Acute Myeloid Leukemia are both cancers that have been linked to the formation of 2-HydroxyGlutarate (2-HG) during the Krebs Cycle. A point mutation (R132H) of IDH1 enzyme causes a gain of function that converts α-Ketoglutarate (α-KG), the correct metabolite, into 2-HG, an inhibitor. The carbonyl group of the molecule is replaced with a hydroxyl, during the gain of function performed by the enzyme. Excess levels of 2-HG have been found to have tumors relating to the above cancers.

The goal of this research is to isolate an aptamer, or a single strand of RNA, that can bind to the 2-HG molecule with high specificity and accuracy. Through cycles of SELEX, Systematic Evolution of Ligands by Exponential Enrichment, a large pool of randomized RNA sequences is narrowed down until eventually the addition of 2-HG to the RNA pool shows a high percentage of cleavage, while also showing little to no cleavage with the addition of random molecules, like magnesium. Once the aptamer selected, it can then be cloned by use of a plasmid and incorporated into a riboswitch, which will act as the mechanism to turn on and off translation of a desired gene that is placed in the plasmid, to create a biosensor or cancer therapy.

NOTES:
Congenital myotonic dystrophy patients exhibit unique splicing dysregulation compared to adult-onset myotonic dystrophy

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Myotonic dystrophy type-1 (DM1) is the most common form of adult-onset muscular dystrophy and impacts approximately 1 in 2300 individuals worldwide. In 10-20% of individuals with DM1, the onset of symptoms begins at birth in a form of the disorder termed congenital myotonic dystrophy (CDM). Both diseases are caused by an expansion of a CTG trinucleotide DNA repeat in the DMPK gene, although cases of CDM are often associated with a larger repeat number. In contrast to adult-onset DM1, which is characterized by symptoms of progressive distal muscle weakness, cardiac conduction defects, and myotonia, CDM patients present at birth with respiratory failure, feeding difficulties, and hypotonia. Despite a common genetic basis for disease, the symptomatic profile of CDM patients is distinct. In fact, common adult DM1 symptoms, such as myotonia, are often absent in CDM until adolescence.

With such variation of symptomatic presentation between these patient populations, questions arise regarding unique and shared molecular mechanisms between DM1 and CDM. Sequestration of muscleblind-like (MBNL) proteins by the “toxic” RNA produced from the repeat expansion and subsequent global dysregulation of RNA metabolism, most notably alternative splicing (AS), is considered the major pathogenic molecular mechanism responsible for disease phenotypes. However, most of these studies to date have been performed in the context of adult-onset DM1. To characterize the spliceopathy of CDM, RNAseq was performed on a large cohort of muscle biopsies from CDM/DM1 patients and adult/pediatric controls.

As expected, many AS changes were conserved between CDM and DM1 patients. Interestingly, a group of AS events specifically mis-regulated in CDM were also identified, many related to proper muscle development. Preliminary motif enrichment analysis of these CDM specific mis-splicing events and identification of gene expression changes suggest that two other RNA binding proteins, RBM4 and RBM5, may contribute to the splicing dysregulation of these RNAs. RBM4 and RBM5 are both regulators of multiple facets of RNA metabolism including AS and have been implicated in developmental processes such as muscle differentiation. Overall, analysis of this comprehensive collection of DM samples illuminates new modes of AS dysregulation that may factor into the distinct disease presentation of CDM.

NOTES:
Synergistic Delivery of Au(III) Compounds and siRNA for Improved Cytotoxicity

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Cisplatin is one of the most widely used DNA-damaging anti-cancer drug. However, many metallodrugs suffer from low selectivity and harmful side effects. The present study focuses on two potent Au(III) compounds, AP209 and AP228. These compounds, designed as an alternative to platinum-based metallodrugs, show cytotoxicity in various cancer cell lines, although they suffer from limited solubility in aqueous environments. Encapsulating the Au(III) compounds into the hydrophobic core of a recently developed nucleic acid nanoparticle (NAN) construct showed increased cellular uptake, analyzed by confocal microscopy and inductively coupled plasma mass spectrometry (ICP-MS). Cell viability was also significantly decreased with lower concentrations of encapsulated drug vs. free drug. After confirming the enhanced uptake and toxicity of the encapsulated compounds, it became of interest whether combination therapy with synergistic co-delivery of a therapeutic oligonucleotide would further increase the cytotoxic effects of the drugs. siRNA targeting Bcl-2, an apoptosis regulating gene, was ligated to the surface of the NAN. Treatment of HeLa cells with the siRNA-NAN showed up to a 60% knockdown of Bcl-2 mRNA. Co-delivery of the encapsulated metallodrug with the Bcl-2 siRNA showed increased toxicity over the drug or siRNA alone. These results show that the nucleic acid-functionalized nanoparticle-based combination therapy with a small molecule drug and a therapeutic nucleic acid can both improve delivery and sustained toxicity, while maintaining lower overall drug concentrations.

NOTES:
Selouridine synthetase (SelU) recognition of specific anti-codon stem loops (ASL) and geranyl-pyrophosphate analogues.

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Natural modification maximizes the structural and functional diversities of the existing nucleic acid building blocks. Geranyl group is the most hydrophobic modification that has been recently identified on tRNA system in several bacteria. Geranylated-tRNA_{Lys}^{UU(ges2U)} affected the protein translation level of E.coli by reducing the -1 frameshifting and the geranylation on tRNA_{Glu}^{CU(ges2U)} increased the level of codon GAG recognition over GAA. Selenouridine-synthetase (SelU) is known to have 2 catalytic activities which are selenolation and geranylation. With the presence of geranyl-pyrophosphate (gePP) and 2-thiouridine, the geranylation at sulfur position was reported in tRNA specific for glutamine, glutamic acid and lysine naturally. We studied the SelU recognition to anti-codon stem loops to further understand the natural selection of geranyl modification of natural tRNA. In this study, SelU protein was purified from E. coli containing an over expression of SelU in a plasmid. The anti-codon stem loop oligonucleotides (ASL) containing 2-thiouridine at the wobble position was synthesized via solid phase synthesis, purified by HPLC and confirmed with mass spectrometry. The optimum condition for in vitro enzymatic catalytic of geranylation by SelU requires the presence of magnesium ion. We found that 50 mM yield higher than 100mM of Magnesium chloride. Moreover, SelU substrate is required to contain 2-thiouridine but limited by anticodon dependence. Only the presence of 2-thiouridine at wobble position could not get geranylated when the anticodon is specific for isoleucine. We further investigated on the alternative of SelU substrate using geranyl-pyrophosphate (gePP) analogues. We discovered that only geranyl from gePP can be incorporated onto the ASL containing 2-thiouridine, but not dimethylallyl-pyrophosphate or farnesyl-pyrophosphate. With the presence of SelU protein and geranyl-pyrophosphate, the geranylation occurred at different efficiency depending on the type of the ASL. SelU protein catalyzed ASL-tRNA_{Lys}^{UU(s2U)} > ASL-tRNA_{Gln}^{GU(s2U)} > ASL-tRNA_{Glu}^{CU(s2U)} respectively which correspond to the geranylated-tRNA abundance in nature. These studies provided further understanding of how selenouridine-synthetase (SelU) selection of substrate and information on what importance for enzymatic geranylation by SelU.

NOTES:
Glioblastoma multiforme (GBM) is the most common malignant primary brain tumor in adults with a mean survival of 14-15 months. 60% of GBM tumors are characterized by the dysregulation, upregulation or constitutive activation of epidermal growth factor receptor (EGFR), leading to tumor cell proliferation. With the advances in nanopore sequencing technology, we have developed a strategy to evaluate alternative splicing and polyadenylation patterns in the EGFR transcript. Our goal is to isolate alternatively spliced and polyadenylated lower abundant EGFR transcripts to characterize the expression of this oncogene. GBM cell lines, SKMG-3, U87MG, U118, and A172, were cultured in multi-layer flasks with total cell confluency of 87,500,000. RNA was isolated using the TRIzol™ Reagent RNA extraction protocol. Biotinylated DNA probes, 90 nucleotides in length, targeting EGFR mRNA exon junctions were used to capture the mRNA transcript. Captured transcripts were pulled down with streptavidin dynabeads, washed and purified. Generation of cDNA by reverse transcription with poly-dT versus EGFR specific primers were compared. PCR analysis demonstrated that biotinylated DNA probing of the EGFR mRNA transcript is a reliable method to retrieve gene specific mRNA. Repeated biotinylated DNA extraction experiments established an optimal molar ratio of 1:10:100 for our target mRNA: to DNA probe: to Dynabeads. EGFR mRNA was captured at a desired concentration of 66.7 ng/µL, polyadenylated using E. Coli Poly(A) Polymerase, purified with Agencourt magnetic beads and stored at -20°C for sequencing.

Our goal is to sequence EGFR transcripts without bias associated with reverse transcription and PCR. The Oxford Nanopore Technology MinION sequencer and accompanying cDNA-PCR sequencing kit provides VN primers that encode transcript switching oligo (TSO) sequences to the 5’ end and 3’ end of each polyadenylated molecule during reverse transcription. EGFR mRNA samples are barcoded by cell type using the PCR Barcoding Kit (Oxford Nanopore Technology). The cDNA product is amplified with strand-switching oligo primers complementary to the TSO sequence. This technique generates complete second strand synthesis of the original cDNA strand. We expect full-length cDNA reads with higher yields than traditional cDNA-synthesis. To study different EGFR mRNA isoforms resulting from alternative splicing we will use the FLAIR (Full-Length Alternative Isoform analysis of RNA) bioinformatic pipeline for the correction, isoform definition, and alternative splicing analysis of our nanopore cDNA sequencing reads. By identifying lower abundant, less common variants of EGFR transcripts and alternative isoforms of EGFR, we expect to identify candidates for induction through antisense therapeutics to activate alternative splice sites through recruitment of splicing activators or inhibit critical splicing elements through the recruitment of splicing inhibitors.
Development of \textit{mbnl} Mutant and CUG Repeat-Expressing Stable Transgenic Zebrafish that Model Molecular and Physical Phenotypes of Myotonic Dystrophy

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**Introduction**

Myotonic dystrophy (DM) is a multi-systemic genetic disorder caused by CUG/CCUG repeat RNAs that sequester MBNL RNA binding proteins. Zebrafish would complement existing mouse DM models due to their high fecundity and the external development and transparency of their embryos.

**Methods**

Zebrafish single, double, and triple homozygous \textit{mbnl1}, \textit{mbnl2}, and \textit{mbnl3} mutants were generated through CRISPR-based mutagenesis, and stable CUG-repeat expressing zebrafish were generated through Tol2 transgenesis. RNA metabolism changes were measured through RT-PCR and RNA-seq.

**Results**

Zebrafish DM models exhibit widespread changes in RNA metabolism, many of which are conserved in individuals with DM. Like mouse models, zebrafish DM models display decreased body size and impaired motor behavior. They also exhibit signs of altered digestive function.

**Conclusions**

Novel zebrafish models mimic molecular and physical aspects of DM and will be useful for drug development and studies of neglected aspects of DM biology, including developmental and digestive phenotypes.

**NOTES:**
Regulation of Arachidonic Acid Metabolism by miR-146a in Lung Cancer Cells

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One commonly dysregulated inflammatory pathway in cancer is the metabolism of arachidonic acid (AA) by cyclooxygenase-2 (COX-2) or 5-lipoxygenase (5-LO) and its activating protein (FLAP) into prostaglandins (PGs) and leukotrienes (LTs), respectively. Although COX-2, 5-LO, and FLAP inhibitors have shown promise for treating cancer, high toxicity and adverse side effects have limited their effective use in the clinic. Therefore, new methods of targeting these proteins are necessary. Our lab and others have shown that these molecules are regulated post-transcriptionally by microRNAs (miRNAs). miRNAs are small, non-coding RNAs that negatively regulate target gene expression through mRNA degradation or translational repression. They generally target multiple genes of similar biological function, making them attractive candidates for therapeutic use.

Here, we show that FLAP protein is overexpressed in lung cancer cells compared to normal lung cells. One miRNA in particular, miR-146a, is predicted to target FLAP via its 3’ untranslated region (UTR). Previous work from our lab demonstrated that miR-146a directly regulates COX-2 expression through its 3’ UTR. In this study, we have experimentally defined the specific regulation of FLAP by miR-146a. Transient and stable transfections of miR-146a in lung cancer cell lines repressed endogenous FLAP protein expression, and reporter assays indicated this regulation occurs through a direct interaction between miR-146a and the FLAP 3’ UTR. This modulation of FLAP gene expression also resulted in decreased cancer cell LTB₄ production. We have also discovered that promoter CpG methylation contributes to miR-146a downregulation in lung cancer cell lines. This work suggests miR-146a can regulate both PG and LT production in lung cancer cells by directly targeting the 3’ UTRs of COX-2 and FLAP, leading to dual inhibition of inflammatory eicosanoid production. Regulation of both arms of this metabolic pathway is significant in that it prevents shunting of AA, where blocking one pathway results in upregulation of the other due to more available substrate. Additionally, we have begun to investigate the role of specific long non-coding RNAs (lncRNAs) in AA metabolism. Overall, our data suggest an elegant mechanism of miRNA-mediated regulation of AA metabolism that further supports a tumor suppressive role for miR-146a in lung cells.

NOTES:
Drug Combination Provides Additive and Synergistic Rescue of Mis-Splicing in Myotonic Dystrophy Type 1 Models

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Myotonic dystrophy type 1 (DM1) is a multi-systemic disease that presents with clinical symptoms including myotonia, cardiac dysfunction and cognitive impairment. DM1 is caused by a CTG expansion in the 3’ UTR of the DMPK gene. The transcribed expanded CUG repeat RNA sequester the muscleblind-like (MBNL) family of RNA-binding proteins leading to global mis-regulation of RNA processing. Currently, there are no disease-targeting treatments for DM1. Given the multi-step pathogenic mechanism, combination or cocktail therapies targeting different aspects of the disease mechanism may be a viable therapeutic approach. Here, as proof-of-concept, we studied a cocktail of two previously characterized small molecules, erythromycin and furamidine, in two DM1 models. In DM1 patient-derived myotubes, additive rescue of mis-splicing was observed with little to no cell toxicity. In a DM1 mouse model, a cocktail of erythromycin and the prodrug of furamidine (pafuramidine), administered orally, displayed both additive and synergistic mis-splicing rescue. Gene expression was only modestly affected and over 40% of the genes showing significant expression changes were rescued back toward WT expression levels. Further, the cocktail treatment partially rescued the myotonia phenotype in the DM1 mouse. This cocktail treatment represents the highest degree of mis-splicing rescue coupled with the lowest off-target gene expression changes observed with small molecule treatment in a DM1 mouse model. These results indicate that cocktail therapies are a promising therapeutic approach for DM1.

NOTES:
Molecular dissection of the cancer microRNA miR-888 cluster associated with aggressive prostate disease

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Our laboratory is studying how conserved non-coding RNAs arranged within genomic clusters function as complex networks to co-regulate overlapping cancer pathways. Specifically, we are characterizing how the microRNA (miRNA) miR-888 cluster coordinates tumor suppressor TIMP2/3/4 and SMAD4 signaling in the context of prostate cancer. We identified the miR-888 cluster consisting of seven miRNA genes (mir-890, -891a, -891b, -892a, -892b, -892c) in a profiling screen as elevated in specimens and urine-derived-exosomes from patients with advanced prostate cancer as well as in metastatic, drug-resistant human prostate cancer cell lines. Our in vitro assays showed that these miRNAs control proliferation, migration, and invasion activities in human PC3 hormone-refractory and LNCaP hormone-sensitive cells. Consistent with an oncogenic role in prostate cancer, miR-888 and miR-891a accelerated prostate tumor growth in mice and induced neuroendocrine transdifferentiation in cell culture. Interestingly, this cluster resides on human chromosome Xq27.3, which maps to a genetic locus linked to hereditary PCa. Cluster members are reported to be elevated in other types of human malignancies that include breast, renal, colon, and endometrial cancers. Therefore, we likely uncovered a novel signaling cancer network with immense therapeutic potential. We hypothesize that the relationship between the miR-888 cluster miRNAs and their messenger RNA (mRNA) targets are reciprocal and these mRNAs may act in a competing endogenous RNA (ceRNA) network to regulate expression of other mRNAs in trans via microRNA response elements. We are currently testing this theory using proteomics, published HITS-clip data, and CRISPR gene edited cell lines deleted for certain miR-888 cluster members. Moving forward, we will validate the utility of miR-888 cluster anti-mir reagents to block disease progression in animal models and determine to what extent exosomal miR-888 cluster cargo controls tumorigenesis in mice. This work could lead to effective biomarkers and therapeutic targets for aggressive prostate disease.

NOTES:
Syntheses and RNAi activity of amide-modified siRNAs

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Recent developments in antisense, RNA interference (RNAi) and, most recently, CRISPR-Cas9 technologies continue driving the interest in synthetic chemistry of oligonucleotides. Our group has been developing amide linkages as replacement of phosphates in RNA. Recently, our group demonstrated that siRNA bearing amide linkages at certain positions were more active than unmodified siRNAs in RNAi mediated mRNA degradation [1,2,3]. However, a detailed investigation pertaining to the position and number of amide linkages that are required to exhibit the optimum effect required optimization of synthetic route to the monomeric building blocks and coupling of these monomers to prepare the amide-RNAs with satisfactory yields.

This presentation will discuss a novel and concise stereo selective synthesis of 3’-homologated ribonucleoside amino acids, building blocks for solid-phase synthesis of consecutive amide linkages in RNA. Our strategy utilized an atom-economical hydrogenation for the transformation of three different functional groups in a penultimate step with excellent conversion (>90%). Fine-tuning of the substrates and solvent systems led to a synthetic route that does not require separate deprotection step to reach the final building blocks [4].

This presentation will also discuss the syntheses of a series of siRNA guide strands containing up to seven consecutive amide linkages at the 3’-end. RNAi activity of the amide modified siRNAs will also be presented.

This work was supported by the US National Institutes of Health (R01 GM071461).

NOTES:
Poster #37  
Global analysis of changes in RNA-protein interactions and RNA secondary structure following excitotoxic neuronal injury in *Rattus norvegicus*.

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HIV-associated neurocognitive disorders (HAND) affect ~50% of people living with HIV, despite suppression of viral replication with antiretroviral drugs. Pathologic features of the most severe forms of HAND include synaptic loss, macrophage infiltration and neuroinflammation. In these cases, there is evidence to suggest that neuronal damage is mediated by excitotoxins, reactive oxygen species, and viral proteins. In an effort to identify mechanisms of neuronal damage and dysfunction in HAND, a number of genomic, transcriptomic, and proteomic studies have been conducted. However, extrapolating the connection between these data sets requires additional information about the post-transcriptional regulatory processes at work in these disorders and how RNA features and processing such as RNA secondary structure, RNA processing, stability, and translation are involved. Such mechanisms are particularly important in neuronal populations as it is well known that local RNA splicing, maturation, and translation play an important role in neuronal function. Furthermore, defects in RNA-binding proteins and RNA regulation have been linked to other CNS diseases.

We set out to identify global changes in RNA-protein interactions and RNA secondary structure in a simple excitotoxicity paradigm using the novel protein interaction profile sequencing (PIP-seq) method in untreated neurons (14 days *in vitro*) or neurons exposed to N-methyl-D aspartate (NMDA). Using this approach, we can identify sites within an RNA molecule that are bound by a RBP, termed protein protected sites (PPS), that are unique to either untreated- or NMDA-treated neurons. Interestingly, untreated- and NMDA- treated neurons share less than 50% of identified PPSs, suggesting that the RNA-binding proteome is highly dynamic in neurons. Furthermore, there is an over-representation of PPSs identified in intergenic regions in untreated- and NMDA-treated neurons indicating that there are novel genomic sites where RNA is transcribed currently defined as intergenic that may have unknown functions in neurons. Additionally, we have identified sequence motifs that are enriched in NMDA-specific PPSs compared to untreated. Using these motifs, we can identify RBP that are unique to NMDA treatment and may shed light on the post-transcriptional mechanisms RBPs play during excitotoxic stress.

We also observe dramatic shifts in RNA secondary structure between untreated- and NMDA-treated neurons near the start and stop codons of protein coding genes. These changes appear to be independent of RBP interactions as protein-binding density near the start and stop codon are largely unchanged between untreated- and NMDA-treated neurons. RNA secondary structure has previously been implicated in regulating mRNA translation and stability, thus these shifts in RNA secondary structure near the start and stop codons could have significant effects on translation or transcript abundance.

In total, we have globally examined RNA secondary structure and the RBP-ome of neurons that have been treated with a simple excitotoxic paradigm, providing insights into the potential role RBPs and secondary structure might have in diseases associated with excitotoxic stress, such as HIV-associated neurocognitive disorders.

NOTES:
mRNAs and IncRNAs intrinsically form secondary structures with short end-to-end distances

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The 5′ and 3′ termini of RNA play important roles in many cellular processes. Using Förster resonance energy transfer (FRET), we show that mRNAs and IncRNAs have an intrinsic propensity to fold in the absence of proteins into structures in which the 5′ end and 3′ end are ≤7 nm apart irrespective of mRNA length. Computational estimates suggest that the inherent proximity of the ends is a universal property of most mRNA and IncRNA sequences. Only guanosine-depleted RNA sequences with low sequence complexity are unstructured and exhibit end-to-end distances expected for the random coil conformation of RNA. While the biological implications remain to be explored, short end-to-end distances could facilitate the binding of protein factors that regulate translation initiation by bridging mRNA 5′ and 3′ ends. Furthermore, our studies provide the basis for measuring, computing and manipulating end-to-end distances and secondary structure in RNA in research and biotechnology.

NOTES:
Epitranscriptomic loss of Selenoprotein utilization drives mitochondrial reprogramming that is associated with ccRCC

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Over 14,000 Americans die each year from kidney cancer with clear cell Renal Cell Carcinoma (ccRCC) being the most prevalent and malignant type. Disruptions in glutathione (GSH) metabolism have emerged as predictors of poor survival in ccRCC. Critically important to the maintenance of the GSH redox cycle are the activities of many selenocysteine-containing GSH metabolizing enzymes whose translation is controlled by the tRNA methyltransferase alkylation repair homolog 8 (Alkbh8). Epitranscriptomic marks, in the form of tRNA modifications, can regulate gene expression at the level of translation. Our studies indicate that defective epitranscriptomic regulation and impairment in selenocysteine (Sec) incorporation, resulting from a deficiency in tRNA methyltransferase Alkbh8 (Alkbh8<sup>Def</sup>), alters glutathione (GSH) metabolism and triggers a gene signature that is highly predictive (Hazard Ratio 2.65, p < 0.0001) of poor ccRCC survival. We show that the Alkbh8<sup>Def</sup> adaptive gene signature drives both senescence and mitochondrial reprogramming to limit damage that results from defects in mitochondrial reactive oxygen species (ROS) detoxification. Alkbh8<sup>Def</sup> mouse embryonic fibroblasts (MEFs) increase many hallmarks of senescence, including senescence associated β-galactosidase, heterochromatic foci, the cyclin dependent kinase inhibitor p16<sup>Ink4a</sup>, mitochondrial elongation as well as the senescence associated secretory phenotype (SASP). In addition, MEFs undergo a metabolic shift that is highlighted by a striking increase in the level of uncoupling protein 2 (UCP2) which enhances oxygen consumption to reduce mitochondrial ROS burden. Our work defines how defects in Alkbh8 and Sec utilization promote senescence and mitochondrial reprogramming and unveils new mechanistic targets for prolonging ccRCC patient survival.

NOTES:
Chemical modifications of transfer RNAs (tRNAs) modulate their inherent stability and function. The methylation of cytosine at position 32 (m\(^3\)C\(_{32}\)) of several tRNA species is a ubiquitous modification found across Eukaryotes. This modification has been proposed to maintain the stability of the anticodon loop, highlighting its potential role in translational efficiency and fidelity, however, the precise role in vivo has not been uncovered.

The yeast Trm140p enzyme was found to catalyze m\(^3\)C\(_{32}\) formation on tRNA\(^{\text{Ser}}\) and tRNA\(^{\text{Thr}}\). Humans also possess m\(^3\)C\(_{32}\) on tRNA\(^{\text{Ser}}\) and tRNA\(^{\text{Thr}}\) but have evolved to harbor this modification on additional tRNA species as well, notably tRNA\(^{\text{Arg}}\) isoacceptors. Four human orthologs of yeast Trm140p have been identified by sequence homology that are encoded by the METTL2A, METTL2B, METTL6, and METTL8 genes. The diversification of this family in higher eukaryotes suggests novel substrate specificity. It has been previously shown m\(^3\)C\(_{32}\) on tRNA\(^{\text{Arg}}\) is catalyzed by METTL2A/2B, however, the mechanism by which the human METTLs target their specific tRNA substrates is unknown.

Intriguingly, we have found that METTL2A and 2B interact with an uncharacterized anticodon binding protein (DALRD3) that contains an anticodon binding domain homologous to that of class la arginyl tRNA synthetases. Notably, we find that the interaction between METTL2A/2B with DARLD3 confers binding specificity for this heterodimeric complex to tRNA\(^{\text{Arg}}\) substrates. Interestingly, purified METTL2A and METTL2B exhibit in vitro methyltransferase activity albeit only when in complex with the accessory protein. We also show that knockout of DALRD3 in humans cells causes complete loss of m\(^3\)C\(_{32}\) specific to tRNA\(^{\text{Arg}}\) isoacceptors which can be restored upon reintroduction of DALRD3. This work highlights a unique biological role for this previously uncharacterized DALRD3 protein, in vivo and reveals a novel mechanism for human tRNA methyltransferases to recognize their substrate via a tRNA synthetase-like intermediate.

NOTES:
The Epitranscriptomic Writer Alkbh8 Protects Against Reactive Oxygen Species and Environmental Stress

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Epitranscriptomic marks in the form of enzyme-catalyzed modifications to RNA nucleosides can be important regulators of translation. Using a novel animal model deficient in the epitranscriptomic writer Alkylation repair homolog 8 (Alkbh8), we have investigated the importance of translational regulation in the response to environmental stress. Alkbh8 is a tRNA methyltransferase that modifies the wobble uridine of selenocysteine tRNA (tRNAsec) to promote translation via a process of UGA stop codon recoding, to allow for the incorporation of the rare 21st amino acid selenocysteine into proteins. Corresponding selenoproteins include glutathione peroxidases (Gpx) and thioredoxin reductases (TrxR) enzymes, which play critical roles in Reactive Oxygen Species (ROS) detoxification. We have probed the importance of the epitranscriptomic writer Alkbh8 under basal conditions and in the response to naphthalene (NA), an abundant polycyclic aromatic hydrocarbon, glutathione depleter and lung toxicant found in tobacco smoke, gasoline and mothballs. Under basal conditions, lungs from the writer deficient mice have increased oxidation reduction potential (ORP), increased transcript levels for DNA damage response, oxidative stress, protein damage and inflammatory signaling pathways and increased γ-H2AX staining, which is a marker for DNA strand breaks. We have performed acute and chronic NA exposure studies on WT, Alkbh8 deficient (Alkbh8del) and Cyp2abfgs-null mice, the latter of which lack the Cytochrome P450 enzymes required for NA bioactivation. Alkbh8del mice are more sensitive to NA than WT, exhibiting greater weight loss, behavioral changes, molecular and histological changes in the lung and showing higher susceptibility to lung damage both at the cellular and molecular levels. Notably while WT mice develop a tolerance to NA after 3 days, defined as resistance to a high challenging dose after repeated exposures, Alkbh8del mice never do and die. Our data supports a model in which the Alkbh8 writer and epitranscriptomic marks allow cells to develop tolerance and adapt to chronic stress. We conclude that Alkbh8 plays a protective role against NA-induced lung dysfunction, with our work providing an early example of how epitranscriptomic writers can regulate the response to environmental stress in vivo.

NOTES:
Poster #42

Erythrosin B protects mice from lethal challenge of Zika virus

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Many flaviviruses, such as Zika virus (ZIKV), Dengue virus (DENV1-4) and yellow fever virus (YFV), are significant human pathogens. Infection with ZIKV, an emerging mosquito-borne flavivirus, is associated with increased risk of microcephaly in newborns and Guillain-Barré syndrome and other complications in adults. Currently, specific therapy does not exist for any flavivirus infections. In this study, we found that erythrosin B, an FDA-approved food additive, is a potent inhibitor for flaviviruses, including ZIKV and DENV2. Erythrosin B was found to inhibit the DENV2 and ZIKV NS2B-NS3 proteases with IC₅₀ in low micromolar range, via a non-competitive mechanism. Erythrosin B can significantly reduce titers of representative flaviviruses, DENV2, ZIKV, YFV, JEV, and WNV, with micromolar potency and with excellent cytotoxicity profile. Erythrosin B can also inhibit ZIKV replication in ZIKV-relevant human placental and neural progenitor cells. Mice treated with Erythrosin B were significantly protected from lethal challenge of Zika virus. As a pregnancy category B food additive, erythrosin B may represent a promising and easily developed therapy for management of infections by ZIKV and other flaviviruses.

NOTES:
Zinc-starved mycobacteria reprogram their ribosomes by replacing the ribosomal (r-) proteins containing the zinc-binding CXXC motif with their zinc-free (C-) paralogues. Subsequently, the reprogrammed ribosomes are targeted for hibernation by mycobacterial protein Y (MPY). Binding of MPY to the ribosome is dependent on a recruitment factor (Mrf), which is transcriptionally co-regulated with the C- r-protein paralogues by a zinc-sensing transcriptional regulator, ZurB. Our 3.46A cryo-EM structure of MPY bound ribosomes indicates that the hibernating ribosomes are functionally inactive, physically stabilized, and protected from aminoglycosides (Li et al. PNAS, 2018, vol. 115, p8191).

In pursuit of understanding the mechanisms of ribosome hibernation, we discovered that Mrf is post-transcriptionally regulated at a lower zinc concentration relative to the transcriptional induction of Mrf. In this paper, we will detail this mechanism of Mrf regulation and discuss how this could enable Mrf as a zinc sensor to orchestrate the entry and exit processes of ribosome hibernation in mycobacteria.
Dumbbell RNA structures in the Zika virus 3’ untranslated region modulate viral gene expression

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Zika virus (ZIKV) is a re-emerging mosquito-borne flavivirus. The recent ZIKV outbreak in the Americas uncovered unusual pathologies such as the association between intrauterine infections early during pregnancy and developmental and neurological anomalies such as microcephaly, as well as the development of Guillain-Barré syndrome in adults. The single stranded positive-sense RNA genome of ZIKV encodes a single open reading frame flanked by 5’ and 3’ untranslated regions (UTRs). Different RNA structures in the 3’ UTR of flaviviruses modulate distinct steps in the virus infectious cycle. In Dengue virus (DENV) for example, two dumbbell (DB) RNA structures regulate translation, and replication via long-range RNA-RNA interactions. In contrast however, the 3’ UTR of ZIKV is predicted to have a pseudo-DB (ψ-DB) and only one DB. To investigate the role of ψ-DB and/or DB RNA structures in ZIKV translation, we created a ZIKV minimal-genome (minigenome) luciferase construct. This construct contains a Nano luciferase gene flanked by the ZIKV 5’ and 3’ UTR. Using site-directed mutagenesis we deleted the ψ-DB or the DB. To investigate the effect of deleting either the ψ-DB or DB on translation, we in vitro transcribed the ZIKV minigenome and measured translation in vitro and in vivo. Excitingly, we found that deletion of the ψ-DB and DB dramatically decreased ZIKV translation in vitro and in vivo. These results suggest that the two dumbbell RNA structures are necessary for efficient viral translation. Additionally, current studies using selective 2’-hydroxyl acylation analyzed by primer extension have elucidated the local structure of the ψ-DB and DB.

NOTES:
RNA plays essential and diverse roles in living systems as genetic information carrier, functional regulator and catalyst. The structures and functions of RNA in cells are further diversified in the presence of various posttranscriptional chemical modifications. The 5-cyanomethyl-uridine (cnm$^5$U) has been discovered as a new naturally modified nucleoside at the wobble position of isoleucine tRNAs from mutant Haloarcula marismortui. Toward our goal of studying detailed working mechanisms of naturally modified RNA nucleotides. We are interested in the effects of 5-cyanomethyl-uridine (cnm$^5$U) in RNA duplexes. Herein, 5-cyanomethyluridine (cnm$^5$U) was synthesized and incorporated into RNA oligonucleotides. In addition, We also synthesized the 5-cyanouridine (cn$^5$U), the close cnm$^5$U analog with the electron-withdrawing cyano- group directly attached to the uracil, and RNA strands containing this modification. The base pairing stability and specificity studies in RNA duplexes indicated that cnm$^5$U slightly decreases the duplex stability but retains the base pairing preference. In contrast, cn$^5$U dramatically decreases both base pairing stability and specificity between U:A and other non-canonical U:G, U:U and U:C pairs. More interestingly, the cn$^5$U:G pair shows higher thermal stability than the cn$^5$U:A pair in the context of RNA duplex, implying the cn$^5$U might slightly prefer to recognize G over A. Although it has not been discovered in the natural RNA systems, our results indicate that the cn$^5$U residue might be used by certain biological systems like virus RNA to increase the base pairing diversify and induce higher rates of gene mutation, even though it decreases the overall base pairing stability.
Poster #46                                                                               Session 2

A conserved nucleolar protein acts as a ribosome concentration sensor and controls stem cell differentiation via p53 in Drosophila

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Despite the general requirement of ribosomes for cell viability, perturbations to ribosome production result in tissue specific defects and diseases known as ribosomopathies. Elucidating the molecular mechanisms that give rise to these diverse pathologies will provide insight into these disease states and the molecular mechanisms of mRNA translation that direct their etiology. We have identified three conserved RNA helicases, that we named Athos, Aramis, and Porthos, play critical roles in ribosome biogenesis in the Drosophila female germline. These helicases are required for progression through the G1/S checkpoint of cell cycle, proper cytokinesis, and germline stem cell (GSC) differentiation. Through next-generation sequencing, we have identified a distinct group of mRNAs that are highly sensitive to lowered ribosome concentration. We find that this sensitivity is governed by the presence of a Terminal Oligo Pyrimidine (TOP) motif in the 5'-UTR which is known to promote the translation of highly expressed genes. One of these targets is Novel Nucleolar 1 (Non1), a conserved p53 suppressor that we hypothesize acts as a ribosome concentration sensor, promoting cell cycle progression via p53 degradation when ribosome concentration is sufficient. We propose a model in which the differential expression of TOP-regulated mRNAs across cell types is one potential source for the tissue specific defects that manifest in ribosomopathies.

NOTES:
A member of the dosage compensation complex orchestrates meiotic commitment by coordinating transcription and translation of critical meiotic factors in *Drosophila*

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Gametes are essential for sexual reproduction. To generate gametes, germline stem cells (GSCs) must exit the mitotic program, which governs self-renewal and transit amplification, and initiate the meiotic program. The critical regulators governing the transition of GSCs from a mitotic cell cycle to meiotic cell cycle are not well understood. We have identified conserved transcriptional machinery in *Drosophila melanogaster* that allows for proper progression through meiosis. We hypothesize that this transcriptional machinery regulates the mitosis-to-meiosis transition, by licensing the expression of a germline-specific ribosomal protein that, in turn, is required for translation of meiotic-entry factors. Our data suggest that a conserved chromatin reader, Male-specific lethal 3 (MSL3), recruits the Ada2a-Containing (ATAC) histone acetyltransferase (HAT) complex to promote transcription of the germline-specific ribosomal protein small subunit (*RpS*) paralog, *RpS19b* and recombination machinery vital for meiosis. We find that *RpS19b* is expressed at high levels in the mitotic phases of *Drosophila* oogenesis and is restricted to the germline, while *RpS19a* paralog is expressed throughout oogenesis in both the germline and soma. Curiously, while *RpS19b* incorporates in actively translating polysomes early in oogenesis, *RpS19a* incorporates in polysomes throughout oogenesis, suggesting that *RpS19b* may play a role in specialized translation of proteins required for differentiation. Interestingly, we have discovered that both *RpS19a* and *RpS19b* are required for proper differentiation and expression of RNA binding protein Fox 1 (Rbfox1), a meiosis-promoting factor. Altogether, these data suggest that having appropriate levels of germline *RpS19* is essential for meiotic progression and that germ cells require a sufficient concentration of ribosomes to proceed through differentiation. We suggest that this mechanism is not just unique to *Drosophila* germ cells but may also extend to mammalian spermatogenesis, as these transcriptional regulators are conserved. Taken together, our results provide insight into transcriptional regulators of the mitotic-to-meiotic transition, mechanisms in coupled chromatin and translation regulation, and germline biology relevant to basic developmental processes.

**NOTES:**
MBNL and RBFOX are two families of RNA-binding proteins (RBPs) that regulate RNA metabolism and exhibit positional dependent-splicing regulation. Various studies have shown these RBPs cooperate to regulate groups of alternative splicing (AS) events. To study this co-regulation of AS, we created human and mouse cell lines with independently inducible expression of MBNL1 and RBFOX1. RT-PCR and RNA-Seq were used in conjunction to analyze AS events at three different concentrations of MBNL1 in the presence and absence of RBFOX1 expression. An overwhelming majority of events regulated by both MBNL1 and RBFOX1 display decreased splicing regulation by MBNL1 in the presence of RBFOX1, or in other words RBFOX1 buffers many MBNL1 regulated events. These data suggest that MBNL1 and RBFOX1 compete for the same cis-regulatory elements within the pre-mRNA. In the context of myotonic dystrophy, these results suggest that RBFOX splicing factors can buffer or compensate for MBNL sequestration by the toxic RNAs of DM.
Moonlighting function of glycogen synthase 1 in RNA metabolism

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Many enzymes have a secondary function, a phenomenon often referred to as protein moonlighting. Typically, these enzymes maintain their primary role as catalyzing agents in metabolism but also can bind to RNA or RNA:protein complexes. The association of enzymes with RNA suggest that these proteins moonlight in RNA metabolism where they regulate gene expression. One enzyme that has been proposed to have a moonlighting function is glycogen synthase 1 (GYS1) due to its phenotype in mice. Most mice lacking GYS1 are not viable and display abnormal heart morphology. However, the small population of surviving GYS1 knockout mice, exhibiting only subtle changes in cardiac metabolism and morphology, suggests that these mice were able to reprogram gene expression during development. Here, we want to investigate the possible implications of GYS1 in RNA metabolism to better understand the role of GYS1 during cardiac development. Previous research has discovered that GYS1 is found in nuclear speckles, an active site of RNA processing. Based on these findings, we hypothesize that nuclear GYS1 affects mRNA transport and/or splicing as nuclear speckles are implicated to function in mRNA processing and transport. Using the CRISPR-Cas9 genome editing system, we have created GYS1 knockout cells to investigate if nuclear GYS1 affects the efficiency of mRNA splicing and mRNA transport. In addition, we will identify nuclear proteins interacting with nuclear GYS1. These experiments will aid in the investigation of GYS1 moonlighting in various steps of RNA metabolism.

NOTES:
Characterization of G-quadruplex motifs in espB, espK and cyp51 genes of Mycobacterium tuberculosis as a potential drug targets

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G-quadruplex forming motifs are among the most studied evolutionarily conserved drug targets that are present throughout the genome of different organisms and susceptible to influencing various biological processes. Here we report highly conserved G-quadruplex structure forming motifs (PGQs) in three essential genes (espK, espB, and cyp51) among 160 strains of the Mycobacterium tuberculosis genome. Products of these genes are involved in pathways that are responsible for virulence determination of bacteria inside the host cell and its survival by maintaining membrane fluidity. The espK and espB genes are essential players that prevent the formation of mature phagolysosome and antigen presentation by host macrophages. The cyp51 is another PGQ possessing gene involved in sterol biosynthesis pathway and membrane formation. In the present study, we revealed the formation of stable intramolecular parallel G-quadruplex structures by Mycobacterium PGQs using a combination of techniques (NMR, CD and gel electrophoresis). Next, ITC and CD melting analysis demonstrated that a well-known G-quadruplex ligand, TMPyP4, binds to and stabilizes these PGQ motifs. Finally, the polymerase inhibition and RT-qPCR assays highlight the biological relevance of PGQ possessing genes in this pathogen and demonstrate that G-quadruplexes are potential drug targets for the development of effective anti-tuberculosis therapeutics.

NOTES:
Our objective is to define dysregulated post-transcriptional mechanisms in the arachidonic acid pathway relevant to cancer progression and tumor microenvironment (TME) composition. Many cancers maintain an inflammatory microenvironment to promote their growth. Besides being its own hallmark of cancer, inflammation influences other characteristics, such as proliferation, invasion, angiogenesis, and immune evasion. Lung cancer is of particular importance, as it is the deadliest cancer. Moreover, $11.9 billion is spent on lung cancer treatments in the United States annually, yet 5-year survival rates are exceedingly low. Currently, many cancer treatments focus on cancerous cells only, without considering the off-target effects on cells within the TME. Hence, resolving how inflammation is dysregulated in cancer may provide fresh opportunities for therapeutic development to more comprehensively treat tumors.

One inflammatory pathway commonly dysregulated in cancer is the metabolism of arachidonic acid by Cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LO) into potent lipid-signaling molecules called eicosanoids. Eicosanoids promote proliferation, invasion, and angiogenesis through autocrine and paracrine signaling to cancer cells and the tumor stroma. Additionally, eicosanoids stimulate an immunosuppressive response, specifically by modulating T-cell and macrophage recruitment and polarization in solid tumors. While the arachidonic acid pathway is commonly upregulated in cancer, the mechanisms governing this deregulation are not well understood. One profound regulator of expression are microRNA (miRNA), which act post-transcriptionally to suppress target mRNAs through transcript degradation or translational stalling. miRNAs are commonly misexpressed in cancer and can perform oncogenic or tumor suppressive functions. One miRNA in particular, miR-708-5p, is commonly underexpressed in cancer and has been shown to repress oncogenic signaling pathways. Interestingly, our preliminary data indicate miR-708-5p suppresses both COX-2 and 5-LO expression in lung cancer cells.

We show miR-708-5p does indeed directly target both COX-2 and 5-LO 3’ UTRs in lung cancer cells, decreasing their mRNA and protein levels. This direct targeting of COX-2 and 5-LO decreased oncogenic eicosanoid production, resulting in decreased proliferation of lung cancer cells in vitro. We are continuing to extend our research to phenotypic effects of miR-708-5p on lung cancer cells and other non-cancerous cell types commonly found within the TME.
Poster #52

Organoid Differentiation Constructed Though Collaborative Mesenchymal Signaling

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Niche signaling influences cell identity through maintenance and differentiation throughout development. Branching morphogenesis and epithelial cell differentiation are all influenced by mesenchymal signals that are critical in branching organs including lung, pancreas, sweat glands and salivary glands. However, the mesenchymal signaling pathways guiding epithelial differentiation are just being elucidated. Understanding both spatial and temporal mesenchymal-epithelial transcriptional changes are critical for development but are necessary for any regenerative therapies or disease management. Our lab previously created a mouse submandibular embryonic salivary gland organoid model allowing for both specific and systematic mesenchymal signal toggling. We previously demonstrated that the primary embryonic mesenchyme promotes proacinar organoid budding and differentiation in primary embryonic epithelium in an FGF2-dependent mechanism. We hypothesize that the mesenchyme’s transcriptional response is what allows the mesenchyme to signal proacinar differentiation. We investigated communication between epithelial and mesenchymal cells using heterotopic cell organoids. While the primary embryonic mesenchyme induces embryonic epithelial proacinar differentiation, as detected by increased AQP5 RNA, this embryonic mesenchymal messaging is less audible to developmentally older epithelium. However, the primary mesenchyme retains its inductive capacity throughout development and through short-term passaging. Long-term passaged mesenchyme loses this inductive capacity. We will classify necessary mesenchymal proacinar promoting genes using RNA and chromatin immunoprecipitation (ChIP) sequencing of cells in different temporal and spatial phases. These studies will highlight how the synergy between two different cell populations determine developmental outcomes.

NOTES:
Herpesvirus encoded microRNAs modulate host cellular functions and immune responses

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Association of various herpesviruses in pathogenesis of oral inflammatory diseases is increasingly acknowledged. However, the underlying mechanisms remain obscure. In our recent miRNA profiling of healthy and diseased human tooth pulps and inflamed gingiva (gums), elevated expression of human herpesvirus-encoded viral microRNAs (v-miRs) were identified. Based on the fold induction and significance values, we selected three v-miRs namely miR-K12-3 [Kaposi sarcoma-associated virus (KSHV)], miR-H1 [herpes simplex virus 1 (HSV1)], and miR-UL-70-3p [human cytomegalovirus (HCMV)] to further examine their impact on host cellular functions. We examined their impact on cellular miRNA profiles of primary human oral keratinocytes (HOK). Our results show differential expression of several host miRNAs in v-miR-transfected HOK. High levels of v-miRs were detected in exosomes derived from v-miR transfected HOK as well as the KSHV-infected cell lines. We show that HOK-derived exosomes release their contents into macrophages (Mφ) and alter expression of endogenous miRNAs. Concurrent expression analysis of precursor (pre)-miRNA and mature miRNA suggest transcriptional or posttranscriptional impact of v-miRs on the cellular miRNAs. Employing transcriptome analysis and bioinformatics, we identified several pathways potentially targeted by deregulated cellular miRNAs that include cytoskeletal organization, endocytosis, and cellular signaling. We validated three novel targets of miR-K12-3-3p and miR-H1 that are involved in endocytic and intracellular trafficking pathways. To evaluate the functional consequence of this regulation, we performed phagocytic uptake of labeled bacteria and noticed significant attenuation in miR-H1 and miR-K12-3-3p but not miR-UL70-3p transfected primary human Mφ. Multiple cytokine analysis of E. coli challenged Mφ revealed marked reduction of secreted cytokine levels with important roles in innate and adaptive immune responses suggesting a role of v-miRs in immune subversion. Our findings reveal that oral disease associated v-miRs can dysregulate functions of key host cells that shape oral mucosal immunity thus exacerbating disease severity and progression.

NOTES:
Human and mosquito cellular RNA modifications are altered during ZIKV infection

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Zika virus (ZIKV), a re-emerging flavivirus, has been associated with an increase in microcephaly and other neurological disorders in newborns. To date there are no licensed vaccines or antivirals. As such it is critical to understand the molecular biology of this understudied virus. To investigate the role of the epitranscriptome during ZIKV infection, numerous cell lines (Huh7, Vero, HEK293, SHSY-5Y, C6/36, and Aag2) were infected with MR766, the Uganda strain of ZIKV, or PRVABC59, the Puerto Rican isolate of ZIKV. Twenty-four or forty-eight post-infection, total RNA was isolated. Following enzymatic digestion of the RNA to mononucleotides, we analyzed the global host and viral epitranscriptome in mock- and ZIKV-infected cells by viral infection mass spectrometry (MS) approach. Our data show that the presence of ZIKV alters the RNA modification landscape of host cells. We also noted that several RNA modifications increased in host cells (m5Cm, m44C, m1I) and some modifications decreased in level (ac4Cm, ncm5U, cmo5U, chm5U) during viral infection. Of interest, two dimethylcytosine species, m5Cm, m44C, appeared only in infected cells.

We then similarly isolated RNA from whole mosquitoes which had been infected with ZIKV or unexposed to virus. Mosquitoes were collected 14 days post-infection for analysis. We found that particular dimethyl modifications were more prevalent among infected mosquitoes and may impart a role during the mosquito immune response to infection. While the localization of specific RNA modifications, the function and putative modifying enzymes remains to be identified, our study demonstrates a novel multi-host viral interaction that may present new targets for antiviral treatments.

NOTES:
Wobble Uridine Epitranscriptomic Marks Regulate Translation in Bacteria and Provide Resistance to Chloramphenicol

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tRNA modifications are epitranscriptomic marks that play roles in tRNA secondary structure, modulating mRNA decoding, nuclease cleavage protection and subcellular trafficking of tRNAs. SelU is a tRNA modification enzyme only found in bacteria and it catalyzes the modification on position 2 of the 2-thiouridine (s²U) at the wobble position of tRNAs for glutamic acid (Glu), lysine (Lys), and glutamine (Gln). SelU uses either selenophosphate (SePO₃³⁻) or geranylpyrophosphate (GPP) as the cofactor to produce 2-selenouridine (Se²U) or 2-geranylthioluridine (ges²U) from s²U at wobble position. The amino acids Glu, Lys, and Gln are encoded by 2 codons found in “split boxes”. In vitro studies provide evidences that s²U and ges²U modifications modulate canonical and non-canonical base pairing strength. We hypothesize that epitranscriptomics marks written by selU exert translational control to abate damage caused by cellular insults. We have shown that E. coli DselU cells are sensitive to the FDA approved translational inhibitor chloramphenicol (CAM). Further we have used polysome analysis to demonstrate that DselU cells have a significant reduction in the fraction of most actively translating ribosomes, with this phenotype exacerbated by CAM. Our findings support the idea that selU-modified tRNAs play an important role in regulating key aspects of translation. We propose that identifying SelU-dependent mechanisms of translational control will provide mechanistic insights into bacterial protein synthesis. In addition, selU and other tRNA modifying enzymes that are unique to the bacterial kingdom are attractive targets for the development of next generation antibiotics.

NOTES:
Poster #56                                                                               Session 2

Genomic neighborhood of bacterial group II introns

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Group II introns are mobile retroelements that can spread to new DNA sites through retrotransposition. The spread of group II introns can be influenced by a variety of host factors including the gene they interrupt. To this end, we have developed a bioinformatics pipeline where we focused on the genomic neighborhoods of introns as found in their native contexts and sought to determine the relationship between the group II intron and the region it inhabits. This work shows that group II introns are frequently associated with other mobile elements, such as transposons and their transposases. In addition, group II introns are commonly located in close proximity to conjugation-related regions, which likely contribute to their dissemination through horizontal gene transfer. We also observe group II introns around genes involved in replication, recombination and repair functions, which may be relevant to favorable conditions for their mobility within a cell through retrotransposition. Many of these observed trends are consistent with independent invasion events, suggesting functional relevance between the invasion site and intron biology. By honing in on the genomic context of group II introns in nature, we demonstrate that while group II introns are widespread, they exhibit a bias of colocalization with functional elements that are directly relevant to their biology.

NOTES:
Bin3 targets multiple mRNAs during *Drosophila melanogaster* development

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The Bin3 RNA methyltransferase is a protein that is essential for stability of 7SK RNA. 7SK is a ncRNA that forms a scaffold for proteins including Bin3, Larp7, and HEXIM, the latter of which interacts with P-TEFb to block its catalytic activity to induce promoter-proximal pausing. Bin3 adds a methyl group to the 5’ phosphate of 7SK, thus protecting it from 5’-3’ exoribonucleolytic degradation and allowing for formation of the scaffold.

We have previously demonstrated that during *Drosophila* development, Bin3 plays a role in the translational regulation of *caudal* mRNA. Consequently, we wanted to know whether Bin3 associates with this and other mRNAs, and to further demonstrate that Bin3 is a translational regulator. To these ends, we used CRISPR to epitope-tag the native *bin3* gene with 3xFLAG, and performed RNA immunoprecipitation of 3xFLAG-Bin3 from ovary and embryo extracts, followed by RNA-seq. We did not identify ncRNAs besides 7SK, but surprisingly, we identified multiple mRNA targets that were verifiable by RT-qPCR. Curiously, *caudal* mRNA was not among the hits, suggesting that Bin3 may regulate *caudal* indirectly. We are performing translating ribosome affinity purification (TRAP) on ovary extracts from wild type and *bin3*Δ flies to determine whether *bin3* affects ribosome occupancy of these mRNAs.

One of the mRNA targets from the RIP-seq experiment was *bin3* mRNA itself. Using HOMER, we identified an 8mer motif that is enriched in two regions of the *bin3* 3’UTR (termed “Bin3 Response Elements”, BRE1 and BRE2), as well as in the 3’UTRs of the other mRNA targets. We generated transgenic flies containing inducible EGFP reporters fused to the *bin3* 3’UTR, the *bin3* 3’UTR lacking BRE1, the heterologous SV40 3’UTR, or the SV40 3’UTR fused to BRE1. We have found that the EGFP expressed from *EGFP-bin3* 3’UTR mRNA accumulates to a lower level than *EGFP-SV40 3’UTR* mRNA, suggesting that the *bin3* 3’UTR inherently confers translational regulation. As we had hoped, when BRE-1 is removed from *EGFP-bin3* 3’UTR mRNA, EGFP accumulates to higher levels. Strikingly, when BRE-1 is added to *EGFP-SV40 3’UTR* mRNA, expression of EGFP is eliminated. These findings suggest that Bin3 may be a potent regulator of translation. We have crossed the 3xFLAG-*bin3* allele into these transgenic flies to address whether the BRE confers association with 3xFLAG-Bin3, and will use *bin3* RNAi and over-expression to determine whether expression of EGFP from BRE-containing mRNAs is sensitive to Bin3 levels. Furthermore, we will use TRAP to support our conclusion that the BRE confers translational regulation.

Our work has revealed that Bin3, previously only known to associate with 7SK ncRNA to regulate transcription, additionally targets several mRNAs and may regulate development through translational regulation of these targets.

**NOTES:**
Investigating the contribution of circadian clock disruption to DM1 hypersomnolence

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Myotonic Dystrophy Type 1 (DM1) is a disorder caused by CTG repeat expansion in the 3’ UTR of DMPK. Patients show a range of CNS phenotypes including hypersomnolence and sleep dysregulation. As sleep/wake rhythms are regulated by the circadian clock, we are investigating whether the clock is disrupted in DM1 using Drosophila as a model. We are examining the effects of pacemaker neuron specific expression of expanded CTG repeats on circadian activity rhythms and neuron architecture. Presence of longer CTG repeats leads to 1) weakening of locomotor activity rhythms, 2) an age-dependent loss of clock neurons preceded by 3) loss of the central clock protein, Clock (CLK), and the pacemaker neuron specific neuropeptide, PDF. Importantly, these phenotypes can be rescued by overexpression of the RNA binding protein Muscleblind (Mbl). To understand the role of Mbl in these neurons, we are investigating what transcripts are regulated by Mbl using CLIP and FISH approaches. These studies will provide key insights into the effects of microsatellite expansions on the circadian clock.

NOTES:
Structure Function Analysis of Single Domain Antibodies Against Ricin’s Catalytic (RTA) and Binding Subunit (RTB)

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Ricin toxin is an extraordinarily potent ribosome-inactivating protein (RIP) capable of inducing cell death in all mammalian cell types. Ricin’s catalytic subunit (RTA) is an RNA N-glycosidase that depurinates a conserved adenine residue within the Sarcin-ricin loop of rRNA. RTA is associated with a binding subunit (RTB) that mediates toxin attachment to mammalian cells and facilitates retrograde traffic through the trans-Golgi apparatus (TGN) and endoplasmic reticulum (ER). In an effort to engineer ricin antitoxins, we have produced and characterized a collection of alpaca-derived RTB-specific heavy chain-only antibodies (\textit{V}_{\text{H}}\textit{H}s) with toxin neutralizing activities. Using a biotinylated ricin competition assay, we identified a panel of \textit{V}_{\text{H}}\textit{H}s that cluster at the interface of RTA and RTB. Based on the \textit{V}_{\text{H}}\textit{H}s’ ability to cluster at the interface of the toxin, we have termed them as a supercluster of antibodies. We are interested in the underlying mechanisms by which supercluster \textit{V}_{\text{H}}\textit{H}s neutralize ricin toxin. To this end, we evaluated potent supercluster \textit{V}_{\text{H}}\textit{H}s’ capacity to block toxin attachment and derail intracellular trafficking, key events in ricin’s intoxication pathway. To evaluate \textit{V}_{\text{H}}\textit{H}s’ capacity to interfere with toxin attachment, we determined the amount of ricin bound to cell surfaces in the presence of antibody. Our results show that supercluster \textit{V}_{\text{H}}\textit{H}s can block up to 80% of toxin attachment to Hela cells. Next, we employed a TGN-specific sulfation assay to determine the effect of \textit{V}_{\text{H}}\textit{H} on ricin’s intracellular trafficking. In this assay, a derivative of ricin becomes sulfated upon entry into the TGN. Results show that the presence of supercluster antibodies derail 80% of ricin trafficking to the TGN. Results of our functional studies show that supercluster antibodies have dual activities, blocking toxin attachment to cell surfaces and derailing intracellular ricin trafficking. To further elucidate the mechanism by which supercluster \textit{V}_{\text{H}}\textit{H}s neutralize ricin, the crystal structures of two potent neutralizing supercluster \textit{V}_{\text{H}}\textit{H}s, V2C11 and V5E4, were co-crystallized with ricin. Crystal structures of V2C11 and V5E4 reveal their proximity to a neutralizing hotspot on RTA, a region that has been involved in interfering with ricin’s retrograde transport, and occupation of one of the two galactose binding pockets on RTB. In conclusion, our results suggest that supercluster antibodies’ potent neutralizing activity is likely a result of their abilities to block ricin at multiple steps in the intoxication pathway. Supercluster \textit{V}_{\text{H}}\textit{H}s are capable of this dual-action activity because of their unique epitope on ricin which encapsulates RTA and RTB. Ricin is a potent biothreat agent and understanding antibody-toxin interactions will afford insights into basic mechanisms for other ribosome inactivating toxins.

NOTES:
Formation of tRNA wobble inosine in humans is disrupted by a millennia-old mutation causing intellectual disability

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The formation of inosine at the wobble position of eukaryotic tRNAs is an essential modification catalyzed by the ADAT2/ADAT3 complex. In humans, a valine to methionine mutation (V144M) in ADAT3 that originated \textasciitilde1,600 years ago is the most common cause of autosomal-recessive intellectual disability (ID) in Arabia. While the mutation is predicted to affect protein structure, the cellular and molecular effects of the V144M mutation are unknown. Here, we show that ADAT3-V144M exhibits perturbations in subcellular localization and has increased propensity to form aggregates associated with cytoplasmic chaperonins. While ADAT2 co-expression can suppress the aggregation of ADAT3-V144M, the ADAT2/3 complexes assembled with ADA T3-V144M exhibit substantial defects in adenosine deaminase activity. Moreover, extracts from cell lines derived from ID-affected individuals expressing only ADA T3-V144M display a severe reduction in tRNA deaminase activity. Notably, we find that the same cell lines from ID-affected individuals exhibit decreased wobble inosine in certain tRNAs. These results identify a key role for ADAT2-dependent localization and folding of ADAT3 in wobble inosine modification that is crucial for the developing human brain.
ITPase Deficiency Causes a Martsolf-Like Syndrome with Fatal Dilated Cardiomyopathy and Increased Inosine Incorporation into RNA

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Martsolf syndrome is a rare developmental disorder characterized by congenital cataracts, postnatal microcephaly, developmental delay, hypotonia, short stature and biallelic hypomorphic mutations in either RAB3GAP1 or RAB3GAP2. Through genetic analysis of 85 unrelated “mutation negative” probands with Martsolf or Martsolf-like syndromes we identified two individuals with different homozygous null mutations in ITPA, the gene encoding inosine triphosphate pyrophosphatase (ITPase). Both probands were from multiplex families with a distinctive disorder resembling Martsolf syndrome but additionally with a lethal infantile-onset dilated cardiomyopathy. ITPase-deficiency has been previously reported to cause an infantile epileptic encephalopathy (MIM 616647). The mechanism through which ITPase deficiency causes cellular dysfunction in either of the disorders remains cryptic. ITPase prevents incorporation of inosine bases (rI/dI) into RNA and DNA. Using CRISPR/Cas9, we generated Itpa null mouse embryonic stem cells (MESCs) and embryos. Using mass spectrometry, we were unable to detect dI in genomic DNA of patient LCLs nor in Itpa null MESCs. However, rI was detectable in total RNA isolated from both patient LCLs and Itpa null MESCs. In Itpa null mouse embryonic tissue, rI was present in brain and kidney with the highest observable levels in heart (~ 1 in 385 bases) thus rI accumulation in RNA correlates with the severity of heart dysfunction in ITPase deficiency. Surprisingly, transcriptome and proteome analysis in mutant embryonic heart tissue revealed no major differences from controls. When transcripts from Itpa null embryonic heart tissues were grouped by length and compared to control heart tissues, there were modest reductions in the abundance of certain long transcripts that are important for heart development and function such as Ttn and Ryr2 whereas shorter transcripts also involved in heart development and function such as NKX2-5 or Tnnt2 did not show significant changes. Future studies will focus on characterizing rI mis-incorporation in the transcriptome and defining the mechanism(s) of cellular dysfunction resulting from ITPase deficiency.

NOTES:
Low-cost DNA nanoswitches as versatile RNA biosensors

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Detection of biomarkers is critically important for biomarker discovery and developing a mechanistic understanding of disease. While there has been an explosion in recent years of technologies for various biosensing applications, many of these methods are complex, impractical, costly, or target specific. We have developed DNA-based nanoswitches that overcome these difficulties, enabling detection and analysis of a wide range of biological molecules with a highly-sensitive yet simple and low-cost method that can be used in any lab. Our technique uses recently developed approaches in DNA nanotechnology to construct “programmable” biosensors that undergo a conformational change in the presence of a target molecule. This conformational change can be easily detected using standard gel electrophoresis, a technique already familiar to most research labs. We have used the DNA nanoswitches for microRNA and Zika viral RNA detection with multiplexed detection in each case. Due to the programmability afforded by the DNA nanotechnology approach, this can act as a nearly universal biosensor, able to detect everything from small molecules to microRNA to proteins.
Understanding myotonic dystrophy through transcriptomic analysis of humans, mice, and

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Myotonic dystrophy types I and II are multisystemic and variable diseases caused by the expansion of a CTG and CCTG repeat, respectively, that sequesters the family of MBNL proteins. MBNL proteins are master splicing regulators which when sequestered caused widespread spliceopathy that has been shown to cause the phenotypes of the diseases. Since the discovery of the mutations and the mechanisms by which the disease is caused, much time has been spent attempting to recapitulate the disease in animal models. Splicing has been widely analyzed and compared using RNA-Seq and other methods, to one or a few models, but never extensively compared between many patients and models. In addition differential expression has been widely understudied up to this point. In this study we used RNA-Seq data from 83 myotonic dystrophy type I patients and compared them to commonly used mouse DM models, DM patient derived cell lines, and newly created DM zebrafish models for alternative splicing and differential expression. We found a wide amount of variability in missplicing and differential expression depending on the tissue type, patient, or model being analyzed. The variability shows that some models recapitulate different populations of patients more accurately and discretion should be used when choosing a DM model, so that results from studies using models can be better extended to the patient populations they are trying to serve.

NOTES:
Mycobacterial HflX is a ribosome splitting factor and confers multidrug resistance

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M. abscessus is one of the most common Nontuberculous Mycobacteria (NTM) which has slowly emerged as a deadly pathogen in the recent years and thus has grabbed the attention of researchers worldwide. The major hurdle in designing potential drugs against this deadly pathogen is its intrinsic drug resistance. Although macrolides are used, bacteria become resistant due to induction of a methylase gene. So far, the only known mechanism of macrolide resistance is that conferred by the erm gene. In this study, we have investigated the role of MAB_3042c on acquired macrolide resistance. We have tested structurally different antibiotics and have found that del MAB_3042c is sensitive to azithromycin, erythromycin and clarithromycin but not to other ribosomal antibiotics like tetracycline, chloramphenicol and streptomycin. The MAB_3042c gene encodes a HflX family of translation factor related guanosine triphosphates as (TRAFAL-GTPase) that is present in bacteria, archae and eukaryotes. The CLUSTAL W alignment of M. abscessus HflX (MAB_3042c), M.tuberculosis HflX (Rv2725c) and M. smegmatis HflX (MSMEG_2736) further indicated that the gene is largely conserved in Mycobacteria. RT-PCR analysis showed that both MAB_3042c and Ms2736 was induced on exposure to macrolides. We have established that MAB_3042c is whib7 dependent and overexpression of MAB_3042c in Δwhib7 mutant restores the phenotype completely. We have shown that constitutive expression of MAB_3042c and Rv2725c in M. smegmatis caused increased resistance to macrolides. Our data shows that both MABΔ3042c and MsΔ2736 strains are highly sensitive to macrolides and this sensitive phenotype is successfully restored on constitutive expression of Mab RRF and MsRRF respectively, indicating a correlation between ribosome splitting and macrolide resistance. Also, the MsΔ2736 strain also shows selective sensitivity to kanamycin and clindamycin in contrast to the MabΔ3042c strain. We have performed biochemical assays to establish Ms2736 is a GTPase. Our study establishes that both CTD and NTD of MSMEG_2736 plays a critical role in ribosome splitting and thus both overexpressed Ms2736ΔCTD and Ms2736ΔNTD strains are defective in complementing the Δ2736 macrolide sensitive phenotype. Our in vitro studies show that Ms2736 splits the ribosome and rescues ribosome stalling caused due to exposure to macrolides. Taken together, we propose a novel function of Mycobacterial HflX in macrolide resistance.

NOTES:
Deciphering Arsenic Induced Epitranscriptomic Changes in Human Cells

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Chronic exposure to environmental pollutants may damage cellular DNA and lead to neurodegenerative disease, developmental disorders, premature aging and cancer. Cellular stress response mechanisms play important roles in cell survival and maintaining homeostasis. Cells respond to the stress by regulating gene expression. mRNAs are translated in the ribosomal complex to make proteins, and in the ribosome tRNA acts as an adapter to link codons to amino acids. The decoding ability of tRNA can be regulated by dynamic epitranscriptomic marks found in the form of RNA modifications. Previous lab studies support the idea that RNA modifications are recoded under cellular stress. Arsenic is one of the major environmental toxicants affecting millions of people across the globe. Arsenic is primarily metabolized in the liver where it generates reactive oxygen species (ROS) and induces cellular stress. We have shown that HEPG2 liver cells become sensitive to sodium arsenite when they are depleted of queuine, a required precursor to the epitranscriptomic mark queosine (Q), found at position 34 of some tRNAs. The environmentally obtained queuine is added to the tRNA by the queuine tRNA-ribosyltransferase (QTRT) complex. We hypothesize that tRNA modifying enzymes play a vital/protective role in cellular response to stress. We have shown that exposure to arsenic leads to immediate upregulation of four tRNA modifying enzymes (QTRT1, QTRTD1, ALKBH8 and TRMO) within 10 minutes of exposure. Further we have shown that the corresponding tRNA modification levels increase after arsenic exposure. We have also used polysome analysis to demonstrate that arsenic exposure affects the levels of actively translating ribosomes. Future studies will use next generation sequencing approaches to define the arsenic induced changes in total mRNA and actively translated mRNA. In addition, shRNA-based knock-down of tRNA modifying enzymes, will be done to determine their roles in preventing arsenic induced cell death. Our study will be important in understanding the role of epitranscriptomic marks and systems in the cellular response to stress.

NOTES:
Male Specific Lethal 3 (Msl3), a member of the Dosage Compensation Complex, promotes meiotic entry in *Drosophila* oogenesis

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The production of gametes is very important to initiate the development of a new organism and hence progress to the next generation. Germline stem cells (GSCs) must exit the mitotic program and initiate the meiotic program for gamete generation. We have discovered an unexpected role for the conserved Male-specific lethal 3 (Msl3) protein in regulating germline mitotic phase transcription. In *Drosophila*, MSL3 is part of the Dosage Compensation Complex (DCC) that upregulates transcription from the X chromosome in males. We find that MSL3 is expressed in the mitotic phase of the female germ line, where it acts independently of the DCC to both promote GSC maintenance and transition into meiotic fate. Our data suggest that MSL3 recruits the histone acetyltransferase (HAT) activity of the Ada2a-Containing (ATAC) complex. This specialized transcriptional complex permits the expression of a germline-specific ribosomal protein that regulates the mitotic program, which further governs GSC self-renewal and the shift to meiosis. We find that loss of MSL3 and ATAC complex components lead to reduced transcription of a ribosomal protein, RpS19b, which we propose controls translation of factors such as RNA binding protein Fox 1 (Rbfox1) to regulate the mitosis-to-meiosis switch.
Fluorescence Activated Cell Sorting, or FACS, is a method that was used to convert the Theophylline aptamer with random sequences into a riboswitch. This method could theoretically be used to convert other discovered aptamers into riboswitches, however it is a costly method and available to those with the high-tech, expensive FACS instrumentation.

We can structure a new system that would select the active Theophylline riboswitch without the use of a FACS machine. To do so, we are placing the aptamer with random sequences into a plasmid and transform the plasmid into bacteria cells. Then, replica plating along with screening selects the cells that only contain the correct riboswitch sequence. We will then evaluate the efficiency in converting aptamers into riboswitches without the need for a FACS machine.

After an aptamer has been converted into its riboswitch, the system of ratiometric fluorescence will allow for testing of the riboswitch’s function. This is because a designed plasmid containing coding for two fluorescence proteins on either side of the inserted riboswitch will give the availability to measure the riboswitch’s function through fluorescence readings.

Both of these systems are the key to innovating the next step in creating synthetic riboswitches.
Ribosomal protein RACK1 is required for both viral and cellular IRES-mediated translation

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The ribosomal protein RACK1 has been shown to be required for translation initiation of the hepatitis C virus and the 5’ IRES but not the intergenic IRES of cricket paralysis virus. Here we tested if RACK1 is also required for other viral IRESs. Indeed, we find that both encephalomyocarditis virus and poliovirus (PV) also require RACK1 for translation of the viral IRESs. The observed decrease in translation in RACK1 knockout cells can be rescued by expression of exogenous RACK1. Further, in cells lacking RACK1, we observe that PV plaques are smaller compared to wildtype cells suggesting that loss of RACK1 slows down the virus life cycle.

Since RACK1 appears to be a required ribosomal protein for translation initiation of viral IRESs, we examined if RACK1 is also required for translation initiation of cellular IRESs. Using dual luciferase reporter constructs, we observe that the cellular IRESs myb, Bag-1, L-myc, cyclin D1 and Set7 also require RACK1 for translation. Overall, we find that RACK1 function in translation initiation appears to overlap with function of eS25, previously shown by the Thompson group to be required for IRES-mediated translation.

While eS25 appears to directly interact with the IRES RNA by cryoEM, RACK1 might not directly bind the IRES RNA, but might indirectly regulate IRES translation through eIF3. To examine the mechanism of RACK1 in IRES-mediated translation, we fluorescently-labeled RACK1 with a short peptide tag called ybbR at both the N- and the C-terminus. In the future, we will probe the dynamics of the RACK1-eIF3 interaction during IRES-mediated translation.

NOTES:
Peptide tagging of Translation Initiation Factors

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Protein biosynthesis is a carefully crafted choreography, which requires precise temporal and spatial interactions of ribosomes, mRNAs, tRNAs and translation factors. Due to compositional and conformational dynamics involved in the biosynthesis pathways, there is a lack of knowledge on the exact role and mechanism of various translation factors. In recent years, single-molecule methods have been used to understand the interactions between translation components and to monitor short-lived intermediate states during translation. Single-molecule methods provides the advantages of fishing out the few signals between different molecules which are usually hidden by ensemble average analysis. In this work, we focus on tagging the eukaryotic translation initiation factors using a short peptide tag, ybbR tag, derived from the genomic library of Bacillus subtilis. Being only 11 amino acids long, the ybbR tag should have reduced steric hindrance during biosynthesis and thus is a suitable candidate to label smaller proteins of interest.

Cap-independent translation initiation of many viral genomes such as HCV, PV, and CrPV is mediated through Internal Ribosome Entry Sites (IRESs). Among the various eukaryotic initiation factors (eIFs) involved in HCV IRES-mediated translation, eIF2 was chosen for the current study because of its key regulatory role during translation initiation. EIF2 functions as the carrier of tRNA and is composed of three distinct subunits - alpha, beta and gamma. To employ single molecule studies, we have established the fusion of N-terminal end of eIF2 subunits with the ybbR- tag and successfully labeled them with a fluorescent substrate of CoA. For Total Internal Reflection Fluorescence microscopy complexes must be immobilized on the slide surface, which we are currently exploring by creating C-terminal avi-tag fusion proteins. Next, we will track the role of each subunit during translation initiation and explore the kinetics of the initiation process. If feasible, this labeling approach may be later employed for other initiation factors to explore their transition dynamics during protein biosynthesis.

NOTES:
Synthesis and Crystal Structure Studies of 2′-5′-linked RNA Duplexes

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RNA can play dual roles as a carrier of genetic information and as a catalyst of specific reactions, and it may have been the first biopolymer to have emerged on the early earth. The non-enzymatic replication of RNA was likely a key step in the evolution of simple cellular life from prebiotic chemistry. In the current model of template-directed polymerization of activated monomers, the chemical copying of RNA always generates a mixture of 3′-5′ and 2′-5′ backbone linkages due to the similar nucleophilicity and orientation of the 2′ and 3′ hydroxyl groups on the ribose. This lack of regiospecificity has been regarded as a central problem for the evolution of functional RNAs. More interestingly, it has been known for a long time that 2′-5′ linkages can reduce the melting temperature of RNA duplexes, making it easier to separate the strands. Although the detailed mechanism is still not clear, considering that strand separation is another unsolved big problem for non-enzymatic RNA replication, this feature may actually afford a selective advantage to duplexes exhibiting backbone heterogeneity. In addition, previous studies have revealed that 2′-5′ linkages in a RNA duplex are more easily hydrolyzed compared to normal 3′-5′ linkages. Thus, there is a selective advantage for the evolution of homogeneous RNA systems with more accurate replication. Altogether, the coexistence of 2′-5′ and 3′-5′ linkages may be a central feature that allowed RNA to play a central role in the original stage of life. We have previously solved four crystal structures for a 2′-5′-linked CG containing RNA 10mer duplex. In this work, we synthesized a series of new 2′-5′-linked RNA duplexes and systematically explored their thermal stability and structural features. These structures will offer general pictures about how RNA adjusts its structure to accommodate backbone heterogeneity

NOTES:
Engineering Synthetic RNA Binding Proteins to Probe the Mechanisms of Myotonic Dystrophy and Development of Potential New Therapeutics

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The MBNL proteins are master regulators of RNA processing including alternative splicing. MBNL sequestration by toxic, expanded repeats is a major disease mechanism in several microsatellite expansion disorders, including myotonic dystrophy type 1 and 2 (DM1 and DM2). The expanded repeats present in these diseases cause nuclear sequestration of MBNL proteins disrupting MBNL’s normal cellular function. Previous work showed that certain regions of MBNL’s linker are important for the splicing regulation of a few targets, it remains to be understood what about these regions are important. To study this region, we have engineered synthetic proteins that have systematic deletions of the linker region and insertions of completely synthetic linkers. Understanding the role of the linker in MBNL’s function will help to design future therapeutics and identify a minimal MBNL protein active in alternative splicing. The second part of this study will focus on the design and testing of synthetic MBNL1 proteins with enhanced binding for CUG/CCUG repeats by replacing a domain of MBNL. These engineered proteins show interesting patterns of splicing regulation and demonstrate that MBNL1 is conducive to engineering of synthetic proteins for the development of potential new therapeutics.
Exploring Hydrogen Bond Geometry in RNA With F-SAPT

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Molecular dynamics (MD) simulations with all-atom models provide important information for developing hypotheses and interpreting experimental data. MD estimates the potential energy as a function of molecular conformation using a set of classical equations and parameters called a force field. The accuracy of this force field is thus necessary but not sufficient for accurate simulations. Generally, to sample conformation energy landscapes sufficiently given current computing power, MD simulations use force fields that assume a fixed point charge model. The central importance of the structure-function relationship in RNA biology has made accurate MD simulations of RNA and RNA-protein complexes an important goal of the field. For RNA, currently available force fields with fixed point charges do not adequately model the potential energy; simulations of small RNA do not reflect the known solution conformations.

One hypothesis that may partially explain these issues is that this functional form does not adequately model the angular dependence of hydrogen bonds. Because the hydrogen bonds are represented as a partial charge interaction, they are not as able to model interactions where the natural geometry of the bond along the donor, hydrogen, and acceptor is not co-axial, such as in the case of hydrogen bonds with carbonyl lone pairs as acceptors, which should not favor a linear hydrogen bond along the axis of the double bond. In a fixed point charge model hydrogen bond they do. To test this, we are using Functional Group Symmetry Adapted Perturbation Theory (F-SAPT) to estimate the energies of hydrogen bonds as a function of their relative orientation. We compare these energies to those estimated by current force fields, and will use the results to guide the design of more sophisticated functional forms.

NOTES:
Towards direct single molecule RNA structural probing using nanopore sequencing

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RNA participates in and directs diverse functions in the cell including translation, gene regulation, small molecule sensing, host defense, and protein scaffolding. These manifold responsibilities of RNA are enabled in part by its ability to form complex structures and dynamically fold into alternative structures based on cellular conditions. Sequencing-based high-throughput RNA structure probing experiments such as SHAPE-MaP (Selective 2’-Hydroxyl acylation Analyzed by Primer Extension with Mutational Profiling) and DMS-MaPseq can be used to identify sites of nucleotide dynamics or base pairing of RNA. However, the RNA must be chemically modified, reverse transcribed into a cDNA, amplified and prepared into a library for sequencing to obtain reactivity profiles. While this approach has been successful in determining nucleotide resolution structures of various RNA molecules, these techniques operate in bulk, producing an average reactivity profile for a given molecule under study. Furthermore, SHAPE-MaP and DMS-MaPseq rely on short-read sequencing technology which obscures characterization of alternatively folded subpopulations and precludes the study of longer RNAs (> 500 bases) at the single molecule level directly. Here we employ novel and common chemical probes in the context of nanopore sequencing to measure sites of structure-induced chemical modification directly on RNA at the single molecule level. As a proof-of-principle we detect adducts on structured RNAs with single nucleotide resolution using the Oxford Nanopore miniION system. This procedure lays the groundwork for a nanopore based method to study single molecule RNA structure in combination with other modalities of interest such as alternative splicing and endogenous modifications in RNA.

NOTES:
Protein Level Catalytic Rates from Hammerhead Ribozymes- Enhanced RNA Therapeutics for Treatment of Inherited Retinal Degenerations (IRDs)

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Short Description: Minimal hammerhead ribozymes (hhRzs) were evaluated as candidate gene knockdown therapeutics for IRDs and supplanted by more potent RNAi technology. We identified unforeseen complexity in how additional RNA structural interactions can greatly facilitate catalytic rates of hhRzs into the levels characteristic of protein enzymes. Facilitated-hhRzs (F-hhRz) have potential to revitalize hhRz therapeutics as highly specific potent RNA drugs for IRDs.

Purpose: We optimized a novel F-hhRz which cleaves human rod rhodopsin (hRHO) mRNA with high catalytic activity under target (substrate) excess and physiological conditions of MgCl2 (0.5-1mM) in vitro. F-hhRz provides a novel therapeutic agent for various IRDs through hRHO suppression. F-hhRz format can be applied to arbitrary disease target mRNAs.

Approach: Ribozyme and hRHO RNAs were transcribed (T7) from templates or synthesized. In vitro hhRz cleavage assays used full length/fragment hRHO mRNAs or miniature 15-mer substrates with 5’FAM and 3’ BHQ1 (cleavage causes fluorescence). Isothermal titration calorimetry (ITC) assessed binding interactions between hhRz or F-hhRz and their substrate.

Results: The RNA facilitator (50nt sequence), when placed 3’ of a minimal “well-behaved” hhRz (CUC¯266), promotes rapid target cleavage under Michaelis-Menten conditions ([S]>>[E]) at cellular free Mg2+ levels. This behavior occurs with minimal (15nt), fractional (~500nt) and full length hRHO (1532nt) substrates and exceeds enzymatic performance of hhRzs previously embedded in scaffolds by 1-2 log orders of magnitude. Rational mutagenesis identified Facilitator regions important for enhancing catalysis. ITC demonstrates that: 1) the Facilitator must be in cis to the hhRz, 2) the Facilitator promotes (1mM Mg2+) a marked shift in Kd (1.1mM for 266-hhRz vs 10nM for 266-F-hhRz) and thermodynamic parameters (DH, DS) of 15-mer non-cleavable substrate binding; this suggests strong and specific interactions between Facilitator and hhRz:substrate complex, and 3) these interactions are not observed if substrate can be cleaved (suggesting high enzymatic turnover). We truncated the original Facilitator to a simpler structure (35nt) and find similar catalytic enhancement. A further truncated 12bp stem loop structure (derived from the original basal Facilitator) with removal of irrelevant 5’sequence enhanced cleavage to 90-100/min. A critical nt in the 5’ antisense flank of the minimal hhRz 266 (A2) (60/min) when mutated to U2 enhances rate to 115/min but when mutated to G suppresses rate to 45/min. Addition of the 12bp Facilitator at the 3’ end of the A2U hhRz enhances rate to 220/min at 0.5mM Mg2+; at 10mM Mg2+ rate is over 1,500/min!

Conclusions: F-hhRzs have properties ideal for gene therapy: enhanced catalytic turnover of substrate at low expression levels (minimize cell toxicity) at cellular Mg2+ levels. Minimal hhRzs capped at only 1-2/min rate (enzyme excess, 10mM Mg) and used only two (a,g) of four possible rate enhancement mechanisms (Emilsson and Breaker, 2003). RNaseA (50,000-80,000/min) uses all four mechanisms (a,g,b,d). This suggests that F-hhRz (>200/min, 0.5mM Mg2+) exploits more than two strategies. We expect with rational and evolutionary investigation of this novel RNA drugable space, that F-hhRzs can perform robust (protein enzyme level) catalytic functionality as site-specific designer RNA endonucleases for gene therapy.

NOTES:
A number of hereditary neurological and neuromuscular diseases are caused by the abnormal expansion of short tandem repeats, or microsatellites, resulting in the expression of repeat expansion RNAs and proteins with pathological properties. While these expansions occur in coding and noncoding regions, microsatellite sequence and repeat length diversity is more prominent in introns with different trinucleotide to hexanucleotide GC-rich, and A/AT-rich, repeats. We tested the hypothesis that these intronic microsatellite expansions selectively trigger resident intron misprocessing. Using myotonic dystrophy type 2 (DM2), \textit{C9orf72}-linked amyotrophic lateral sclerosis and frontotemporal dementia (C9-ALS/FTD), Fuchs endothelial corneal dystrophy (FECD), Friedreich’s ataxia (FRDA), and spinocerebellar ataxia type 10 (SCA10) as examples, we demonstrate that GC-rich CCTG, GGGGCC and CTG expansions lead to host intron retention in DM2, C9-ALS/FTD and FECD, respectively, while A/AT-rich expansions in FRDA and SCA10 do not. Since, this intron retention is readily detectable in affected tissues and peripheral blood lymphocytes we conclude that its screening constitutes a rapid and inexpensive intronic repeat expansion disease biomarker for both diagnostic and therapeutic trial purposes.
Identifying Sequence Determinants of Altered RNA Splicing in Myotonic Dystrophy with RNAseq

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Myotonic dystrophy is the most common adult-onset muscular dystrophy with an estimated prevalence of 1 in 2500. Myotonic dystrophy type 1 (DM1) and type 2 (DM2) are caused by expression of RNAs containing expanded CUG- or CCUG-repeat tracts. These expanded repeat RNAs bind and sequester MBNL proteins, preventing them from regulating alternative splicing of transcripts important for muscle function. Although loss of MBNL function has been shown to explain a large portion of the mis-splicing associated with CUG-repeat RNA (Du et al. 2010), other splicing factors are likely to contribute to DM1 mis-splicing. It is also likely that CUG and CCUG repeats have some distinct effects on alternative splicing. RBFOX splicing regulators colocalize with CCUG-repeat RNA in DM2 patient tissues but do not bind CUG-repeat RNA (Sellier et al. 2018). Further, myotonic dystrophy is characterized by a broad range of disease severity, with congenital DM1 (CDM) patients exhibiting life-threatening muscle dysfunction at birth. It is unknown whether disruption of the same splicing regulators affected in adult-onset DM1 is sufficient to cause CDM or if involvement of additional splicing regulators is necessary.

To compare splicing regulators implicated in different forms and mouse models of myotonic dystrophy, we performed RNAseq with skeletal muscle RNA from 8 healthy adults, 16 adult DM1 patients, 16 DM2 patients, 4 wildtype mice, 4 mice expressing CUG-repeat RNA, and 4 mice homozygous for Mbnl1 null alleles and hemizygous for an Mbnl2 null allele. RNAseq data from 3 CDM patient muscle tissues was accessed from GSE97806 (Thomas et al. 2017). Differential alternative splicing relative to respective control samples was determined using rMATS. Sequences surrounding mis-spliced exons in each group were interrogated for enrichment of all k-mers of lengths 4, 5, and 6. Consistent with previously-identified patterns of target exon regulation, conserved MBNL binding motifs (YGCY) were strongly enriched immediately upstream of exons with increased inclusion and downstream of exons with decreased inclusion in all DM patient groups and models. Adult DM1, DM2, and CDM patient groups also exhibited similarly strong enrichment of conserved RBFOX binding motifs (UGCAUG). Ranking all conserved k-mers by their capacity to predict global splicing outcomes reveals that the strongest predictors of mis-splicing in DM1, DM2, and CDM are variants of MBNL and RBFOX motifs (adjusted p < 0.05). In DM2 and CDM, RBFOX motifs are stronger predictors of splicing outcomes than any conserved MBNL motifs. These results suggest that RBFOX regulation is a component of mis-splicing in myotonic dystrophy patient muscle, and RBFOX may play a larger role in DM2 and CDM than in adult DM1.

NOTES:
sxRNA: Using RNA-binding proteins and microRNA to study and modulate the post-transcriptional regulatory code

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The wide array of vital functions that RNA performs is dependent on its ability to dynamically fold into dynamic alternative structures in response to changes in intracellular and extracellular conditions. RNA-binding proteins (RBPs) regulate much of this activity by targeting specific RNA structures or motifs often including RNA three-way junctions (3WJs). 3WJs are naturally occurring structural elements found in many functional RNA molecules, such as ribosomal RNA and ribozymes. These structures are classically characterized as resulting from an RNA molecule folding back on itself in cis to produce three separate helices that meet around a central unpaired region. However, 3WJs can also be formed in trans when a non-coding RNA binds to a messenger RNA target. 3WJs created in trans can be viewed as a new category of regulatory RNA that we have called structurally interacting RNA or “sxRNA” for short. While studying post-transcriptional gene expression using RIP-Chip and RIP-Seq, we often find multiple instances of non-coding RNA associated with various RBPs that are consistent with our sxRNA model of post-transcriptional gene regulation and our experimental work suggests that ncRNAs, including many microRNAs, regulate RBP activity by binding in trans to stabilize or disrupt potential RBP binding sites directly or indirectly. This novel regulatory mechanism may explain how complex regulation of gene multi-functionality including alternative splicing could be regulated at the post-transcriptional level. Furthermore, combinations of these interactions could be used in a modular and combinatorial manner adding a hierarchical complexity to the post-transcriptional regulatory code.

The sxRNA concept also has the potential of creating technological opportunities to utilize this regulation as an mRNA-based molecular technology. By controlling post-transcriptional mRNA activity using the unique microRNA signature of a cell, sxRNA technology can be used to express any protein of interest in a tissue specific manner. To do this, we design an sxRNA “bait” in which a natural RBP-motif is altered so it forms correctly only when a targeted “trigger” microRNA, binds in trans and stabilizes it. When engineered into a mRNA sequence, the sxRNA-bait acts as a switch to turn ON translation of the message proportional to the amount of trigger-microRNA present in the cell.

Thus sxRNA technology has the potential to increase our understanding of post-transcriptional gene regulation, while simultaneously creating technological opportunities to utilize this concept as an RNA-based molecular tool, a diagnostic or as a tissue specific mRNA therapeutic.

NOTES:
Progress on an adapted cell-free translation system using *S. cerevisiae*

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Protein translation is an essential process involving multiple factors, most importantly the ribosome. Although much progress has been made to understand ribosome functions in translation, considerably less is known about how ribosomes adapt under stress. Increasing evidence suggests that some ribosomes may undergo changes in response to stress to fine-tune protein expression, however, mechanisms by which this could occur have remained elusive. Specifically, studying the ribosome under stress in cells can be challenging, as exposing cells to stressful agents such as oxidants or heavy metals will lead to damage to virtually all biomolecules, including rRNA, a major component of the ribosome, as well as their substrates and cofactors during translation. Some of the experimental caveats of using cell model can be circumvented by taking advantage of a cell-free approach. For decades, utilization of cell-free protein translation systems has been instrumental in our understanding of basic elements involved in protein translation. Although commercial platforms do exist, they are often costly and lack room for customization. We have chosen to focus on the heavily underutilized *S. cerevisiae*-based cell-free translation system, which is considerably cheaper than commercial platforms, can be easily generated in house, and retains exceptional potential for genetic and biochemical manipulation. Here we present our progress in an adapted *S. cerevisiae*-based cell-free expression system that allows for the separation and reintroduction of enriched ribosomal fractions. Isolated ribosomes enriched from cell lysates using high-speed centrifugation can be exposed to harmful reagents such as oxidants and subsequently supplemented back into ribosome-depleted translation competent cell lysates. This approach is currently being used in the lab to investigate how a number of damaging factors may affect protein translation by directly interacting with the ribosome while limiting or eliminating off-target effects.

NOTES:
Synthesis and Biological Activity of Amide-Linked RNA

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Amide linkages are promising modifications for optimization of short interfering RNAs (siRNAs) as potential therapeutic agents as well as tools for basic science (1-3). However, the realization of their full potential requires further investigation into optimum amide placement and number (1). Progress in this area is impeded by the relatively low coupling yields observed for the amide-modified RNA monomers currently in use. Preliminary results within our group indicate that coupling must be optimized in order to allow for the efficient production of siRNA strands with more than six amide-modifications.

This presentation will discuss optimization of amide coupling efficiency by optimizing protecting groups, coupling agents and reaction time and temperature. We hypothesize that the relatively low coupling efficiency is caused by steric hindrance of the currently-used tert-butylidimethylsilyl (TBDMS) 2'-OH protecting group. Results of coupling optimization will be discussed in terms of different protection strategies aimed to decrease or move protecting group bulk away from the ribonucleoside ring. Since amide-modified RNA monomers are not susceptible to epimerization during carboxylic acid activation, we have used strong activating reagents not suitable for typical protein amino acids is possible. Coupling condition optimization results using such strong carboxylic acid activators will presented. The optimization of coupling time and temperature in concert with the strategies above will also be discussed. Such investigations are facilitated by the use of an automated microwave-capable peptide synthesizer. Through the development of efficient coupling strategies compatible with automated solid-phase-synthesis, the investigation of the biological properties of amide-linked RNAs will be greatly facilitated, and will lead to a substantial increase in their applicability as basic science tools.

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NOTES:
A ribosomal RNA methyltransferase regulates group II intron retrotransposition

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Group II introns are mobile retroelements, capable of invading new sites in DNA. They are self-splicing RNA molecules that complex with their own intron encoded protein to form a ribonucleoprotein (RNP) that targets DNA after splicing. These molecules can invade DNA site-specifically, through a process known as retrohoming, or can invade ectopic sites through retrotransposition. Retrotransposition in particular can be strongly influenced by both environmental and host factors. To investigate host factors that influence retromobility, we have used random insertional mutagenesis to generate a library of over 1,000 mutants in Lactococcus lactis, the native host of the Ll.LtrB group II intron. The insertion sites of these mutants have been identified within the L. lactis genome with Illumina sequencing. Initial screens of this library have revealed RNA modifying enzymes influencing retromobility. In particular, one mutant in an rRNA methylase (rlmH), demonstrates a 15-fold increase in retrotransposition when compared to the wild-type. In addition, our data reveal that the native, wild-type group II intron contains no post-transcriptional modifications. The increased retrotransposition could not be explained by global changes in DNA replication, transcription, intron splicing, or translation as measured experimentally. Interestingly, we observe an increase in growth rate in this mutant and that the mutant ribosomes cannot bind the intron RNA as effectively as wild-type ribosomes. We hypothesize that the specific differential modification status of the ribosome can affect group II intron retrotransposition through two mechanisms. First, the increased growth rate in the rlmH mutant creates a favorable environment for retrotransposition by generating more accessible DNA in the form of replication forks. Second, reduced ribosome binding may lead the intron in the mutant background to retrotranspose more freely. Further elucidating a mechanism of this regulation will define a new role for post-transcriptional modifications and ribosomes as regulators of mobile genetic elements.

NOTES:
B. anthracis RNA-dependent asparagine biosynthesis

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Two distinct routes for attaching asparagine (Asn) to its cognate transfer RNA (tRNA<sub>Asn</sub>), an essential step in protein synthesis, are known in Bacillus anthracis. In the direct route, asparaginyl-tRNA synthetase directly ligates Asn to tRNA. In the indirect pathway a non-discriminating-AspRS attaches aspartate to tRNA<sup>Asn</sup> which GatCAB then amidates. In this path asparagine is synthesized on the tRNA using a complex between a non-discriminating aspartyl-tRNA synthetase, tRNA<sup>Asn</sup>, and GatCAB, the transamidosome. We are purifying the components of the transamidosome in order to characterize the indirect pathway under various conditions to understand the role of the indirect route in this human pathogen and why B. anthracis acquired an archaeal aspartyl-tRNA synthetase for this purpose.

NOTES:
**Computationally Reconstructing Cotranscriptional RNA Folding Pathways from Experimental Data**

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The series of RNA folding events that occur during transcription can influence the functional roles of cellular RNAs (Kramer and Mills, 1981). However, few methods are available to generate high-resolution models of this ubiquitous cotranscriptional folding process that is important for gene expression, splicing, and macromolecular assembly (Choudhary et al., 2017). Here we present a method, Reconstructing RNA Dynamics from Data (R2D2), to uncover details of cotranscriptional folding pathways by predicting RNA secondary and tertiary structures from cotranscriptional SHAPE-Seq data (Yu et al., 2018). We applied R2D2 to the folding of the Escherichia coli Signal Recognition Particle (SRP) RNA sequence and demonstrate that this RNA navigates through non-native intermediate structures that require significant structural rearrangement to reach the functional native fold. Secondary structure folding pathway predictions and all-atom molecular dynamics simulations of folding intermediates suggest that this rearrangement can proceed through a toehold-mediated strand displacement mechanism, which can be disrupted and rescued with rationally engineered point mutations. Our results demonstrate that even RNAs with simple functional folds can undergo complex folding processes during synthesis, and that small variations in their sequence can drastically affect their cotranscriptional folding pathways.

5'-Processing of the E. coli precursor SRP RNA is mediated by RNase P, an endonuclease known primarily for its role in tRNA 5'-maturation. Available data suggest that RNase P functions post-transcriptionally. Here, we assessed the in vitro ability of E. coli RNase P to cleave the precursor SRP RNA before it has been fully transcribed, specifically at a length that is associated with a RNA polymerase pause site (Fukuda et al., 2018). We found that this truncated precursor SRP RNA, which has some of the recognition determinants present in canonical RNase P substrates, is indeed cleaved by recombinant E. coli RNase P. Overall, the R2D2 algorithm provides a powerful starting point for utilizing experimental data to gain deeper insights into cotranscriptional RNA folding and its biological impact.

NOTES:
Expanding the genetic code with pyroglutamate

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Formation of pyroglutamate in proteins is associated with diseases such as Alzheimer’s. To better understand pyroglutamate’s role in protein structure and function, an \textit{E. coli} model system was developed to directly incorporate pyroglutamate into proteins. Key to this process is the use of a modified archaeal RNA-dependent glutamine biosynthetic pathway in which pyroglutamate is synthesized on an amber suppressor tRNA. Enhanced yellow fluorescent protein was used as a reporter system to determine levels of read-through, and therefore incorporation, of pyroglutamate in response to an amber codon. As yield was poor, we are developing a new pyroglutamate system using mesophilic enzymes. Success of this system will be confirmed by mass spectrometry.

NOTES:
Deciphering the biological function of tRNA methyltransferase 1 (TRMT1) using human and mouse models

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Mutations in the tRNA methyltransferase 1 (TRMT1) gene are the cause of autosomal-recessive intellectual disability (ARID) in the human population. TRMT1 catalyzes the formation of the dimethylguanosine (m2,2G) modification in numerous tRNAs. Moreover, we have found that TRMT1 exhibits ubiquitous expression in the brain with enrichment in the hippocampus and neocortex. These results indicate that TRMT1 exhibits expression in brain areas associated with learning and memory. However, the molecular and cellular function of TRMT1 in neurodevelopment and how mutations in TRMT1 cause ARID are unknown. To begin understanding the role of TRMT1, we analyzed the functional consequences of TRMT1 knockdown in human cells. To knockdown TRMT1 expression, we used CRISPR interference (CRISPRi) to block transcription from the endogenous TRMT1 promoter. Using the CRISPRi system, we were able to deplete TRMT1 in human ReNcell neural progenitor cells (NSCs) and reduce m2,2G levels in tRNA. Notably, we find that TRMT1 knockdown caused an increase in reactive oxygen species (ROS) and the induction of superoxide dismutase (SOD1) which is responsible for detoxifying ROS. Moreover, late passage TRMT1 knockdown cells exhibited the accumulation of a possible SOD1 multimer due to increased oxidative stress. We could suppress the formation of the SOD1 multimer by restoring TRMT1 expression in TRMT1 knockdown cells. These results suggest that TRMT1 plays a role in maintaining redox homeostasis in neural progenitor cells and that increased ROS could contribute to a subset of phenotypes leading to ARID in humans.

NOTES:
Emerging evidence has shown that vascular pathology is initiated by inflammatory activities and exacerbated with a sustained and unresolved inflammation in vascular smooth muscle cells (VSMCs). However, the underlying mechanism, particularly the major long noncoding RNAs (lncRNAs) regulating inflammatory activity in VSMCs, remains to be elucidated.

Here we discovered a novel lncRNA named MKL1-interactive Inflammatory Long Noncoding RNA (KILN), which is massively induced by pro-inflammatory cytokine IL1β. KILN is selectively and abundantly expressed in cultured human VSMCs. In response to IL1β, KILN is transcriptionally activated by p65 through the NFkB binding site within its promoter. Further studies show the regulatory role of KILN on inflammatory pathways as the knockdown of KILN reduced the phosphorylated residues on p65 and p38MAP Kinase, thereby suppressing the expression of a large number of inflammatory genes.

Mechanistically, in vitro RNA pulldown assay and RNA immunoprecipitation assay reveal the physical interaction between KILN and MKL1 protein. Furthermore, RNA fluorescence in situ hybridization coupled with immunofluorescence protein staining confirmed such physical interaction and unraveled it occurs predominantly in the cytoplasmic compartment of VSMCs. The regulatory role of MKL1 in inflammatory activity was reported recently and was further confirmed in our lab (unpublished data). Knockdown of MKL1 in VSMCs decreased the expression of pro-inflammatory genes, suggesting KILN regulates inflammation through effects on MKL1. We further explored that IL1β triggers the nuclear translocation of MKL1 and increases MKL1 protein stability, both of which are hindered by the knockdown of KILN. Protein immunoprecipitation assay further revealed the knockdown of KILN breaks down the p65-MKL1 physical interaction and reduces the luciferase activity of NFκB reporter.

Collectively, this novel lncRNA KILN plays a pivotal role in VSMC inflammation, likely through stabilization of MKL1 protein as well as MKL1-p65 interaction and thus potentiates p65 transactivity.
**Poster #86**

**Mini DNA-RNA Hybrid Origami Nanostructure**

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DNA nanotechnology has been a popular approach to create nanostructures with arbitrary 2D and 3D geometries by using DNA strands as materials. However, those DNA based nanostructures tend to have low stability in body fluids, such as blood and urine, which limits their applications as drug vehicles or biosensors. Here we present the design and characterization of a small DNA-RNA hybrid origami brick (~12 nm × 11 nm × 9 nm) that we expect to have improved resistance to nucleases present in biofluids due to its small size and the hybrid composition. Preliminary tests on the structure suggest that it does have improved stability. Currently, we are working to attach drug or fluorophore molecules to this structure and make it function as a reliable drug vehicle or biosensor. Also, its design and fabrication method can be used to create any other well-controlled shapes of DNA-RNA hybrid origami nanostructures according to the requirements of applications.

**NOTES:**