ADDENDUM to the PROGRAM for RNA 2018

May 29 to June 3, 2018
University of California, Berkeley

Revised May 28, 2018

Corrections, withdrawals and new abstracts received after the program book was printed.

SESSION CHANGES

Saturday, June 2 14:00 – 17:00
Concurrent session 8: RNA Structure **Zellerbach Playhouse**

Change of Chair: Markos Koutmos, University of Michigan

POSTER CHANGES and WITHDRAWALS

Withdrawn

173 Comparative study of the adenine riboswitch in Apo and bound state using relaxation dispersion

Funding acknowledgement

181 Small nucleolar RNAs are processed to small RNAs and associate with *Saccharomyces cerevisiae* ribosomes in a stress-dependent manner

This work was supported by the Polish Ministry of Science and Higher Education under the KNOW program.

Withdrawn

190 Identification of proteins involved in the regulation of viral microRNA biogenesis

Change of presenting author

210 Exploring the Capping Code: CleanCap™ Co-transcriptional Capping Allows the Syntheses of Cap 0, Cap 1, Cap 2 and $^{m6}A_m$ Capped Messenger RNAs

Krist Azizian, Dongwon Shin, Jordana Henderson, Richard Hogrefe, Michael Houston, Alexandre Lebedev, Anton McCaffrey

Correction to author list and presenting author

212 Aminoglycoside receptors reveal patterns in RNA recognition and conformational change

Christopher Eubanks, Amanda Hargrove
Duke University, Durham, NC, USA

Withdrawn

290 Cell cycle-regulated lncRNA promotes cell proliferation by controlling HIPPO/YAP signaling.

Withdrawn

291 DDX5/p68 associated lncRNA LOC284454 is differentially expressed in human cancers and modulates gene expression

Withdrawn

299 Genome-wide screening of *NEAT1* regulators reveals mito-paraspeckle communication

Withdrawn

301 Ultrastructure of the FC/DFC organization and nascent pre-rRNA sorting in the nucleolus

Withdrawn

367 miRTrace: a tool for quality control and tracing taxonomic origins of microRNA sequencing data

Moved from Friday to Thursday

381 Prospects for recurrent neural network models to learn RNA biophysics from high-throughput data

Withdrawn

442 Capturing the Light Triggered Release of Adenine Inside Adenine Riboswitch Crystals

Withdrawn

470 Time-resolved crystallographic studies of the adenine riboswitch aptamer domain

Withdrawn

494 Stress granules are heterogeneous assemblies composed of distinct core substructures that contain unique RNA and protein compositions

Moved from Friday to Thursday

503 Association between splicing efficiency and sub-cellular localization of coding and long non-coding RNAs in human cells

Withdrawn

510 Mutant mRNA decay confers genetic robustness to mutations through triggering a transcriptional adaptation response.

Withdrawn

629 An atomic structure of the yeast Ribonuclease P

Presenter changed

647 Characterization of a group IIc intron with its intron encoded maturase protein
Presenter: Shivali Patel, Yale University

Withdrawn

653 New Insights into Spliceosome Activation using CoSMoS

Correction to author list

662 Coupled Yeast transcription and splicing *in vitro*
Hsin-I Liu, Chun-Shu Yeh, Luh Tung, Tien-Hsien Chang

Change of presenting author

665 Yeast protein Cwc2 and the N-terminal domain of Prp8 stabilize the spliceosomal catalytic center in the first-step conformation.

Katarzyna Matylla-Kulinska, Katarzyna Eysmont, Magda Konarska

Withdrawn

678 Sam68 interaction with U1A modulates mTOR pre-mRNA splicing

Withdrawn

685 Genome-wide CRISPR-Cas9 interrogation of splicing networks reveals a mechanism for recognition of autism-misregulated neuronal microexons

Correction to author list

751 Investigating function and evolution of mammalian expansion segments (ES)

Teodorus Theo Susanto, Kotaro Fujii, Maria Barna

Moved from Friday to Wednesday

755 Control of mammalian limb patterning by the ribosome

Substitute abstract and presenting author change

757 Evaluating the role of mRNP circularization by disrupting interaction of eukaryotic initiation factor G and PABP

Jay Sharma, Sarah Walker
Dept of Biological Sciences, State University of New York at Buffalo, NY, USA

Eukaryotic translation initiation involves multiple eukaryotic translation initiation factors (eIFs) binding mRNA and other binding partners to stimulate translation. The interaction between eIF4G and poly(A)-binding protein (PABP) circularizes the mRNP (messenger ribonucleoprotein), which stabilizes mRNA and is thought to promote translation. The eIF4G protein interacts with eIF4E (cap-binding protein) on the 5' cap and PABP binds the poly(A) tail on the 3' end of an mRNA. Recent reports suggest that interaction between eIF4G and PABP is not essential for cell growth in yeast, yet strong effects on translation have also been observed upon disrupting this interaction. Hence, the effects of disrupting mRNP circularization on translation remain unclear. We have generated a PABP mutant, Pab1-180p (previously characterized by Sachs and colleagues) that effectively abolishes eIF4G•PABP interaction, thereby preventing mRNP circularization. We tested three different mutations in the RRM2 domain of PABP with GST-eIF4G1 in a pull-down assay. Pab1-101p and Pab1-180p demonstrated complete disruption of eIF4G•PABP interaction. Next, we measured the RNA-binding affinities for these mutants. We found that poly(A) binding specificity was unaffected for Pab1-180p and Pab1-184p, but specificity was lost for the Pab1-101 mutant. We chose to evaluate the status of translation in the *pab1-180* mutant by ribosome profiling, since it effectively disrupted eIF4G•PABP interaction without interfering with PABP•poly(A) binding. Results of our ribosome profiling analysis will highlight the role of closed-loop formation in maintaining and possibly activating specific mRNPs for translation.

Corrected abstract due to publishing error

759 Multi-protein Bridging Factor 1(Mbf1), Rps3 and Asc1 prevent stalled ribosomes from frameshifting.

Jiyu Wang[1,2], Jie Zhou[1], Qidi Yang[1], Elizabeth Grayhack[1,2]
[1]University of Rochester, Rochester, NY, USA; [2]Center for RNA Biology, Rochester, NY, USA

To ensure the fidelity of translation, ribosomes accurately maintain the reading frame throughout the coding region, although in some cases, ribosomes execute programmed frameshifts to regulate gene expression. In bacteria, ribosomes that are slowed by some suboptimal codons are sometimes rescued by frameshifting. In the yeast Saccharomyces cerevisiae, ribosomes translate 12 inhibitory codon pairs, including CGA-CGA, slowly and inefficiently, but do not frameshift at these pairs in wild-type yeast. However, ribosomes lacking a small ribosomal protein Asc1, homolog of human RACK1, efficiently frameshift at CGA-CGA codon pairs. It is unclear how Asc1 prevents frameshifting and by what mechanism, wild-type yeast cells maintain the reading frame during slow translation.

In a selection for mutants that frameshift at CGA codon repeats in yeast, I identified mutations in the Multi-protein Bridging Factor 1 gene (MBF1) and in a specific region of the universally conserved small ribosomal protein gene RPS3. I will present evidence that these two proteins function similarly in reading frame maintenance. By contrast, Asc1, which mediates both read-through and frameshifting at CGA-CGA pairs, appears to play a related, but distinct role from Mbf1, based on two observations. First, deletions of both ASC1 and MBF1 exhibit much more frameshifting than either single mutant, indicative of distinct roles. Second, mbf1 mutants induce efficient frameshifting at the same codon pairs at which Asc1 mediates read-through, indicative of related roles. In the double mutant, frameshifting occurs at the seven most slowly translated codon pairs in yeast, evidence of the link between slow translation and frameshifting.

Substitute abstract

762 Cell Type-Specific Differential Stoichiometry of Ribosomal Protein Transcripts

Adele Xu, Maria Barna
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Each of the many cell types that comprise a metazoan organism performs a unique function, which often requires high-volume expression of one or more cell type-specific proteins. This phenomenon has been intensely investigated at the level of transcriptional regulation, and emerging evidence suggests that selective translation may also dictate specialized protein synthesis. In one proposed mechanism of translational regulation, ribosomes may become optimized for the protein synthesis needs associated with a particular biological context by altering which ribosomal proteins they contain. Examples of such ribosomal heterogeneity have been reported in yeast, and recent work has demonstrated that variations in ribosome composition can regulate translation of specific proteins in certain mammalian contexts. However, a cell type-resolved, organism-wide survey of ribosome composition has yet to be conducted. Given that the canonical mammalian ribosome contains 80 ribosomal proteins, many of which may have multiple paralogs and splice variants encoded in the genome, a vast number of permutations in ribosome composition is conceivable. I hypothesize that such variations in ribosome composition contribute to cell type-specific protein expression. I am currently analyzing publicly available single-cell RNA sequencing data and cell type-resolved proteomic data to identify cell types with unique stoichiometries of ribosomal protein expression. I intend to experimentally validate *in silico* results, and will select physiologically interesting and experimentally tractable cell types as examples to further elucidate mechanisms of translational regulation

Withdrawn

795 Single-molecule visualization of human RNA polymerase II transcription complexes assembly

ADDED POSTERS

Added Wednesday in topic area RNA Structure and Folding

801 Modeling the interaction of raloxifene, a specific RNA bulge binder selected from high-throughput screening, to the NMR high-resolution structure of the conserved 61 nt Heptatitis B virus pregenomic RNA epsilon motif

Regan M. LeBlanc[1], Wojciech Kasprzak[1], Stefano Ginnocchio[1], Fardokht A. Abulwerdi[1], Julie M. Nyman[1], Andrew P. Longhini[2], Theodore K. Dayie[2], Bruce A. Shapiro[1], Stuart F.J. Le Grice[1]
[1]Basic Research Laboratory, National Cancer Institute, Frederick, MD, USA; [2]Center for Biomolecular Structure and Organization, Dept of Chemistry and Biochemistry, University of Maryland, College Park, MD, USA

Despite the critical role RNA plays in numerous aspects of biology including signaling, gene regulation, catalysis and viral replication less than 1% of all FDA approved pharmaceutical compounds directly target RNA. Lack of progress has primarily reflected the challenges of developing and refining high-throughput drug discovery methodologies as well as the difficulties obtaining high-resolution RNA structures. In order to develop platforms for rapid discovery and characterization of novel small molecule ligands as antagonists of regulatory cellular, viral and virus-coded RNAs we chose Hepatitis B virus (HBV), a continuing global and national public health issue, as a target system for RNA-directed drug discovery. Specifically, we selected the 61-nucleotide epsilon element of pre-genomic RNA (pgRNA) for small molecule microarray screening. Five lead compounds thus discovered were further characterized by differential scanning fluorimetry, microscale thermophoresis, NMR spectroscopy and mass spectrometry. Combining these biophysical tools allowed us to classify our hits as aggregators, non-specific, and specific binding chemotypes. One hit compound, raloxifene, was previously shown to reduce HBV infection in vivo although the mechanism of action was unclear. Here we demonstrate the epsilon element of HBV pgRNA as a target for raloxifene, and related analogs. NMR spectroscopy indicates high specificity of raloxifene for the "primer bulge" of this cis-acting regulatory RNA. High-resolution structures (1.8 Å RMSD) of the epsilon motif was refined from SAXS, solvent PRE, RDC, NOE, and dihedral angle restraints. Raloxifene was docked to the high-resolution epsilon structure using chemical shift perturbations and submitted to a series of molecular dynamics simulations to generate an epsilon-raloxifene complex model to better understand the mechanism of specific RNA binding.

Added Thursday in topic area Ribosome Biogenesis

802 Quality control during late 40S ribosome maturation: Mechanistic insights into release of Dim1 and rRNA processing

Jay Rai[1], Homa Ghalei[2,3], Melissa D. Parker[2], Jason Collins[2], Katrin Karbstein[2,4], M. Elizabeth Stroupe[1]
[1]Dept of Integrative Structural and Computational Biology, The Scripps Research Institute, Jupiter, FL; [2]Dept of Biological Science and the Institute of Molecular Biophysics, Florida State University, Tallahassee, FL; [3]present address: Emory University School of Medicine, Dept of Biochemistry, Atlanta, GA; [4]HHMI Faculty Scholar

Nascent small (40S) ribosomal subunits bind large (60S) subunits to produce 80S-like ribosomes. Recently, we used genetic and biochemical experiments and showed that the essential ATPase Fap7 promotes formation of the rotated state, a key intermediate in translocation, thereby releasing the essential assembly factor Dim1 from pre-40S subunits. Our results revealed an important quality control step which tests the ability of 40S to translocate the mRNA·tRNA pair during maturation. Bypassing this quality control step produces defects in reading frame maintenance.

Here we present the structure of the 80S-like ribosome that accumulates after depletion of Fap7. The structure reveals how the subunit interface in this intermediate is remodeled to accommodate the 60S subunit and the assembly factors Tsr1 and Dim1. In this structure, Dim1 has rotated to bridge the P and E-sites, which opens up the platform to construct a new Dim1-stabilized intersubunit bridge and leaves Dim1 poised for release via Fap7 ATPase-induced subunit rotation. In addition, the opened platform also repositions the pre-rRNA in the Nob1 active site. The structure together with mutagenesis and in vivo data, provides mechanistic insights into release of Dim1 and rRNA processing and demonstrates how quality-control and 40S maturation are linked during ribosome assembly.

Added Thursday in topic area RNA Transport and Localization

803 ALYREF Links 3' End Processing of Replication-dependent histone mRNAs to nuclear export

Jing Fan[1], Ke Wang[1], Min Shi[1], Lantian Wang[1], Xudong Wu[2], Guohui Li[2], Hong Cheng[1]
[1]State Key Laboratory of Molecular Biology, Shanghai Key Laboratory of Molecular Andrology, CAS Center for Excellence in Molecular Cell Science, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China; [2]Laboratory of Molecular Modeling and Design, State Key Laboratory of Molecular Reaction Dynamics, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China

Metazoan replication-dependent histone mRNAs are the only eukaryotic mRNAs that lack polyA tails. RD histone mRNAs end instead in a conserved 26-nucleotide sequence that contains a 16-nucleotide stem-loop. The 3' processing of RD histone mRNAs require two important protein factors. One is SLBP (stem-loop binding protein) which binds to the stem loop structure; the other is the U7 snRNP which composes of U7 snRNA and seven proteins. Except for SLBP and U7 snRNP, many other factors have been identified that regulate 3' processing of RD histone mRNAs. After processing, the mature RD histone mRNAs are known to be exported to the cytoplasm via SR proteins that recruit the mRNA export receptor NXF1.

Here, our individual cross-linking and immunoprecipitations (iCLIP) data demonstrate that ALYREF, a component of the TREX mRNA export complex, binds to a region upstream adjacent to SL on most RD histone mRNAs. Consistent with this specific binding, ALYREF directly interacts with SLBP and this interaction is important for efficient ALYREF binding on RD histone mRNAs. On one side, ALYREF, independent of other TREX components, functions in efficient 3'processing of histone mRNAs by facilitating recruitment of the U7 snRNP through protein-protein interaction with Lsm11. On the other side, ALYREF, together with other TREX components, promotes nuclear export of mature RD histone mRNAs. Importantly, 3' processing significantly facilitates RD histone mRNA export. Together, our study reveals two-layer function of ALYREF in RD histone expression and raises the possibility that ALYREF might couples nuclear export to 3' processing of RD histone mRNA export.

Added Friday in topic area Emerging and High Throughput Techniques

804 Comparison of Direct RNA-Seq method

Daniel R. Garalde[1], Elizabeth A. Snell[1], Daniel Jachimowicz[1], Botond Sipos[1], Phillip James[1], Sissel Juul[2], Daniel J. Turner[1]

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Sequencing is an important tool for expanding our understanding of the biological processes mediated by RNA. Oxford Nanopore devices have been used extensively to sequence DNA and cDNA, and now, direct, electrically-based sequencing of RNA is possible using the same devices. The direct RNA-seq method has potential advantages in the study of RNA because it yields full-length, strand-specific RNA sequences and circumvents reverse transcription and amplification steps that are known to introduce biases. The direct RNA-seq method also permits the detection of nucleotide analogues in RNA. Here we present direct RNA sequencing results and comparisons to other methods of sequencing RNA.

Added Friday in topic area Interconnections Between Gene Expression Processes

805 Links between splicing and H3K4 trimethylation in Saccharomyces cerevisiae

Isabella Maudlin[1], Emanuela Sani[1], Vincent Géli[2], Jean Beggs[1]

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There is correlative and causative evidence that splicing affects chromatin structure and *vice versa.* Of particular interest to the present work are inks between splicing and Histone 3 Lysine 4 trimethylation (H3K4me3), a chromatin mark associated with promoters of active genes, that has been shown to influence and be influenced by splicing in mammalian cells [1-3]. However, the molecular basis of this is unknown. To further understand the links between splicing and H3K4me3, we make use of the auxin-inducible degron (AID) system to conditionally deplete essential splicing factors that act at different stages of the splicing cycle and analyse the effects on H3K4me3 in the budding yeast *Saccharomyces cerevisiae.* Whilst depletion of splicing factors that affect the first or second catalytic step of splicing reduces H3K4me3 on intron-containing genes, notably, depletion of the late-acting factor Prp22 reduces H3K4me3 in the absence of defects in splicing catalysis, suggesting a more direct role for Prp22. Prp22 is an RNA-dependent ATPase that proofreads to product of the second step of splicing and promotes mRNA release from the post-spliceosome [4]. Interestingly, the effect of Prp22 on H3K4me3 is dependent on its ATPase activity. Furthermore, Prp22 has been found to interact with the H3K4me3 methyltransferase Set1, and depletion of Prp22 results in reduced recruitment of Set1 to intron-containing genes. These data show a previously unknown link between Prp22, Set1 and H3K4me3 and provide mechanistic insight into this phenomenon.

[1] Sims RJ, Millhouse S, Chen CF, Lewis BA, Erdjument-Bromage H, Tempst P, Manley JL & Reinberg D (2007) Recognition of trimethylated histone H3 lysine 4 facilitates the recruitment of transcription postinitiation factors and pre-mRNA splicing. *Mol. Cell.* **28**: 665–676; [2] Luco RF, Pan Q, Tominaga K, Blencowe BJ, Pereira-Smith OM & Misteli T. (2010) Regulation of alternative splicing by histone modifications. *Science*. **327**: 996–1000; [3] Bieberstein NI, Crillo Oesterreich F, Straube K & Neugebauer KM (2009) First exon length controls active chromatin signatures and transcription. *Cell. Rep.* **2**: 62-68; [4] Schwer B (2008) A conformational rearrangement in the spliceosome sets the stage for Prp22-dependent mRNA release. *Mol. Cell.* **30**: 743-754

Added Friday in topic area RNA Synthetic Biology and Systems Biology

806 Highly Efficient RNA Circularization and an Application for Intracellular Metabolite Detection

Jacob L. Litke[1], Samie R. Jaffrey[1,2]
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In the first 25 years of aptamer development, very few aptamers have been approved for clinical use and the practical therapeutic targets of aptamers is limited to extracellular proteins. One problem with targeting intracellular components with aptamers is rapid RNA degradation. In this project, we aim to develop a eukaryotic expression system for highly stable and abundant RNA. Our strategy for protecting aptamers from degradation is to devise an expression platform for circular RNA (circRNA), which lacks 5' and 3' termini, making them resistant to exoribonucleases. We find that this system, dubbed "Tornado", can overexpress circRNAs as highly as the most abundant endogenous RNAs (5S, tRNAs) and represents a >40-fold increase in circRNA relative to the previous best expression system. We demonstrate this expression system works in a variety of commonly used cell lines and can accommodate various fluorogenic aptamers. Furthermore, we show that prior aptamers raised to inhibit NF-kB monomers block 3.5 times more of IL-1b induced NF-kB activation when expressed in Tornado as compared to a linear context. Additionally, we use this expression system to develop a circRNA-based sensor with reversible detection of S-adenosyl methionine in HEK293T. Hence, we show the first dynamic detection of intracellular metabolites using an all-RNA sensor in human cells.

Added Friday in topic area RNA Turnover

807 The Regulation of the PCA1 Transcript by the Nonsense-Mediated mRNA Decay (NMD) Pathway

Kaitlin Murtha, Bessie W. Kebaara
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The Nonsense-Mediated mRNA Decay (NMD) pathway is a highly-conserved mRNA surveillance pathway with a dual function. First, the pathway degrades mRNAs with Premature Termination Codons (PTCs). Secondly, it acts to control gene expression by degrading mRNA transcripts which do not contain PTCs. These targeted transcripts are called "natural mRNAs". Natural mRNAs are targeted by the NMD pathway by certain internal *cis* elements, for reasons largely unknown. Natural mRNAs have been found to belong to subsets of functionally related genes in both yeast and mammals. *PCA1* is one of these natural mRNAs that has been found to be a target of NMD. Putative NMD-targeting *cis* elements found in the PCA1 transcript include a -1 PRF (Programmed Ribosomal Frameshift) site and an atypically long 3' UTR. The *PCA1* gene is an alias of *CAD2*, a plasma membrane P_{1B}-type ATPase that transports cadmium. It also has a role in copper homeostasis. Peccarelli et al., 2016 showed that *PCA1* mRNA accumulates to higher levels in NMD mutants. We have determined which features of the *PCA1* transcript mark it a target of NMD. Interestingly, half-life experiments show that *PCA1* is differentially regulated by NMD in rich media versus low copper media. We aim to understand the mechanisms by which *PCA1* is regulated by NMD under different environmental conditions.

Added Friday in topic area RNA Turnover

808 Unraveling the molecular mechanism of human polynucleotide phosphorylase (hPNPase) in downregulating oxidized RNA in human cells and associated human diseases.

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Dept of Biomedical Sciences, Charles E. Schmidt College of Medicine, Florida Atlantic University, Boca Raton, FL, USA

RNA damage causes RNA dysfunction, and it is strongly implicated in the development of many age-related human diseases. Oxidation by reactive oxygen species (ROS) is the major type of damage to RNA in living organisms. In eukaryotic cells, mitochondria generate most ROS and ROS production increases dramatically under oxidative stress. In this work, we have analyzed the level of oxidation in mitochondrial and cytosolic RNA in various cultured human cells by measuring the content of an oxidized nucleoside 8-hydroxyguanosine (8-oxoG). Our results revealed that 8-oxoG level in mitochondrial RNA oxidation is normally higher than that found in cytosolic RNA, presumably due to the close proximity of mitochondrial RNA to ROS. Furthermore, when cells were exposed to oxidants, mitochondrial and cytosolic 8-oxoG levels were both increased significantly, with mitochondrial 8-oxoG being still higher than cytosolic 8-oxoG. We have demonstrated an important role for human polynucleotide phosphorylase (hPNPase), an exoribonuclease predominantly localized in mitochondria, in reducing 8-oxoG levels. hPNPase is known to form a complex with human RNA helicase (hSUV3) inside mitochondria. These proteins are both required for normal mitochondrial mRNAs turnover. Here we have examined whether hSUV3 is responsible for reducing intracellular 8-oxoG levels. Surprisingly, knocking-down hSUV3 has no effect on 8-oxoG levels in mitochondria and cytosol under normal or oxidative stress conditions. Various domains of hPNPase i.e. mitochondrial translocation signal (MTS), two catalytic domains (RPH1 and RPH2) and two RNA binding domains (KH and S1) have been shown to play differential roles in the degradation of mt- and cyt- RNA species. To further understand the molecular mechanisms of hPNPase in reducing RNA oxidation, we have studied RNA 8-oxoG levels in cells containing hPNPase mutants lacking these domains. Our data suggested specific roles for different domains of hPNPase in reducing 8-oxoG, which includes specific binding and subsequent degradation of 8-oxoG-containing RNA molecules and in protecting cells under oxidative stress. The results suggested that hPNPase plays multifaceted roles in RNA metabolism including protecting cells from deleterious effect of RNA damage.

Added Friday in topic area tRNA

809 Exceptions to conserved tRNA sequence features in eukaryotes: applications and implications for tRNA biology

Brian Lin, Todd Lowe
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Eukaryotic tRNAs are central to protein translation, and recently have been implicated in a variety of regulatory functions. Studying variation of tRNA sequence features holds promise for linking non-translation functionality to individual tRNA genes. Here, we use a comparative genomics approach, examining about 50,000 tRNA sequences across fungi, plants, insects, vertebrates, and nematodes to quantify prevalence of typical versus exceptional sequence features across clades and amino acid isotypes. We find that yeast species contain the most diversity (least constraint) in tRNA sequence features compared to all other clades combined. We highlight exceptions to features previously thought to be universal or highly conserved and show these are often recently evolved but conserved across multiple related species. For instance, all ten $tRNA^{Pro}$ genes from a fungal species violate the universal G18:Ψ55, which is a core tertiary interaction. We also show multiple instances of clade-specific conservation of base pair mismatches in the middle of stems. In human, we profile exceptional tRNAs and their atypical sequence features, including an expressed, conserved, and epigenetically active $tRNA^{Ser}$ with an apparently inviable acceptor stem. Exploration and characterization of these rare tRNA features may lead to important applications in synthetic biology or medicine.

Added Friday in topic area RNP Structure

810 Structural basis for recognition of human 7SK long noncoding RNA by the La-related protein Larp7

Catherine D. Eichhorn, Yuan Yang, Lucas Repeta, Juli Feigon
University of California, Los Angeles, CA, USA

The La and the La-related protein (LARP) superfamily is a diverse class of RNA binding proteins involved in RNA processing, folding, and function. Larp7 binds to the abundant long noncoding 7SK RNA and is required for 7SK ribonucleoprotein (RNP) assembly and function. The 7SK RNP sequesters a pool of the positive transcription elongation factor b (P-TEFb) in an inactive state; on release, P-TEFb phosphorylates RNA Polymerase II to stimulate transcription elongation. Despite its essential role in transcription, limited structural information is available for the 7SK RNP, particularly for protein-RNA interactions. Larp7 contains an N-terminal La module that binds UUU-3′OH and a C-terminal atypical RNA recognition motif (xRRM) required for specific binding to 7SK and P-TEFb assembly. Deletion of the xRRM is linked to gastric cancer in humans. We report the 2.2 Å X-ray crystal structure of the human Larp7 xRRM bound to the 7SK stem-loop 4, revealing a unique binding interface. Contributions of observed interactions to binding affinity was investigated by mutagenesis and isothermal titration calorimetry. NMR ^{13}C spin relaxation data and comparison of free xRRM, RNA, and xRRM–RNA structures demonstrate that the xRRM is pre-ordered to bind a flexible loop 4. Combining structures of the hLarp7 La module and the xRRM–7SK complex presented here, we propose a structural model for Larp7 binding to the 7SK 3′ end and mechanism for 7SK RNP assembly. This work provides insight into how this domain contributes to 7SK recognition and assembly of the core 7SK RNP.