Invited External Speakers

RNAs as Global Metabolic Regulators
Martha Fedor, The Scripps Research Institute

Abstract:

Riboswitches are RNA motifs that respond to the binding of small molecules to control gene expression. Riboswitches were originally discovered in bacteria but have since been found across all domains of life. Each riboswitch was once thought to respond to just a single cognate ligand. However, we learned that a single riboswitch can integrate information from an array of metabolites and coordinate gene expression in response to the overall metabolic state of the cell. Building on this discovery, we applied a combination of biochemical, structural and genetic approaches to investigate whether RNAs can also integrate signals from central components of cellular energy metabolism. Energy balance is fundamental to life and the protein that sense ATP and work to coordinate cells' activities in response to energy availability have been well studied. We have identified the first natural ATP-sensing RNA that regulates gene expression in response to intracellular ATP levels, evidence that RNAs also play a role in this regulatory network. This discovery highlights an entirely new aspect of RNA-mediated gene regulation and global regulation of metabolism.

"Mechanisms and Functions of RNA Silencing Pathways."
Phillip Zamore, University of Massachusetts Medical School

Abstract:

N/A

"Chromatin modifications for signaling and transcription"
Jerry Workman, Stowers Institute for Medical Research

Abstract:

All DNA-dependent nuclear processes are affected by post-translational modification of histone. Such modifications can affect chromatin structure but also serve as signals and information on chromosomes. This presentation will explore the use of histone modifications to restore chromatin structure behind elongating RNA polymerase II. A second topic will discuss the interface between a histone modifying complex and cell signaling pathways.

Invited Internal Speakers

Mixed signals; translational quality control is critical for bacterial responses to amino acid stress

Tammy Bullwinkle

Abstract:

Aminoacyl-tRNA synthetase (aaRS) editing is an important fidelity step in translation that helps prevent misincorporation of amino acids during protein synthesis. Outside of directly impacting the integrity of the proteome, there is the potential for tRNA mischarging to have additional impacts on various cellular pathways. In bacterial systems that lack aaRS editing, growth is often limited in the presence of a noncognate substrate, amino acid starvation, or environmental stress, however the precise means by which cellular growth is inhibited remains unclear. Here we demonstrate several amino acid starvation sensors at the level of transcription and stringent response activation are misread in the absence of aaRS accuracy due to modest yet significant changes in the tRNA:aminoacyl-tRNA ratio within the cell. These targets of tRNA mischarging impact the global response to starvation and stress, therefore differences in cellular growth in the absence of aaRS proofreading are not simply due to protein synthesis errors. These findings also suggest variations in aaRS accuracy likely influenced how tRNA sensing mechanisms for gene regulation and stress signaling emerged.

Keywords: tRNA, aminoacyl-tRNA synthetase, stringent response

Targeting CDKs with PHA-848125 in estrogen receptor negative breast cancer

Douglas G. Cheung

Abstract:

The cell cycle is frequently dysregulated in breast cancer, resulting in the uncontrolled division of cells. It is tightly controlled by cyclins, cyclin-dependent kinases (CDKs), and their downstream target, retinoblastoma protein (RB). We recently tested the efficacy of a multi-CDK inhibitor, PHA-848125, in breast cancer cells and in mice. Cell proliferation assays on a panel of breast cancer cell lines showed that ER-negative breast cancer cell lines are more sensitive to PHA-848125 than ER-positive cell lines. Cell cycle analysis showed a specific G1 arrest with a concomitant reduction of the phosphorylation of RB, agreeing with the CDK2 inhibitory activity of the drug. We also observed the anti-proliferative activity *in vivo* after PHA-848125 treatment of mice xenotransplanted with the ER-negative MDA-MB-231 cell line. A two-week treatment with PHA-848125 on MMTV-PyVT transgenic mice that normally develop multifocal mammary adenocarcinoma and lung metastatic lesions resulted in tumor growth inhibition and a strong reduction in metastatic colonization of lungs. We further confirmed the effect of PHA-848125 by orthotopic transplantation of MDA-MB-231 in NOD-SCID mice. These experiments demonstrated that PHA-848125 specifically targets ER-negative breast cancer both *in vitro* and *in vivo*. These data lay out the rationale for the clinical evaluation of PHA-848125 as a potential targeted therapy for ER-negative breast cancer.

Keywords: breast cancer, cell cycle, CDK inhibitor

Listen to the cells: tRNA subcellular trafficking

Anita K. Hopper

Abstract:

tRNAs are essential components in all domains of life to translate genetic information into proteins. tRNAs serve additional roles such as targeting proteins for degradation, signaling the general amino acid control pathway, and regulation of apoptosis by binding cytochrome C. We employ the yeast model system to study tRNA biology. Although for decades it was thought that tRNA movement from its nuclear site of synthesis to its cytoplasmic site of function was unidirectional, it is now known that, in yeast and vertebrate cells, tRNAs in the cytoplasm can be imported into the nucleus via the tRNA retrograde pathway and be returned to the cytoplasm via the tRNA re-export process. We learned that bi-directional tRNA trafficking between the nucleus and cytoplasm serves as a tRNA quality control mechanism to assure that tRNAs in the cytoplasm are appropriately matured and modified, important because tRNAs are long lived and aberrant tRNAs can cause errors in translation. The retrograde tRNA pathway functions in parallel with the previously described Rapid tRNA Turnover pathway that monitors 3-d structure in the nucleus and cytoplasm. In an effort to identify the gene products that function in all steps of tRNA biology, we undertook a genome-wide approach to query the consequences of every yeast gene upon tRNA biology and identified scores of novel genes. To date, we identified new regulators of transcription and 5' processing, new components of the tRNA export process and delivery to tRNAs to appropriate cytoplasmic sites, and discovered the mechanism of tRNA intron turnover.

References:

Kramer, E.B., A.K. Hopper. Retrograde tRNA nuclear import provides a new level of tRNA quality control in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. 110:21042-21047 (2013).
Wu, J., A.K. Hopper. Healing for destruction: tRNA intron degradation in yeast is a two-step cytoplasmic process catalyzed by tRNA ligase Rlg1 and 5'-to-3' exonuclease Xrn1. Genes Dev. 28:1556-1561 (2014)
Huang, H.-Y., A.K. Hopper. In vivo biochemical analyses reveal distinct roles of β-importins and eEF1A in tRNA subcellular traffic. Genes Dev. 29:772-783 (2015).

Keywords: tRNA biology, nuclear export, quality control

Characterization and Selection of Biophysically Optimized Antibody Fragments for Enhanced in vivo Tumor Imaging

N. Emerson Long

Abstract:

Antibody fragments have great potential for clinical application as cancer theranostics. Their small size compared to full-length IgG's allows for faster blood clearance, potentially decreased immunoreactivity, better tumor penetrance, and easier engineering and production. The smallest possible fragment of an IgG that still binds to its antigen, called the single-chain variable fragment (scFv), can be created by fusing the variable light and variable heavy domains together with a peptide linker. Along with switching domain orientations, altering the length and amino acid sequence of the linker can significantly change the biophysical characteristics such as binding, stability, and quaternary structure. Comprehensive studies of these attributes have not been reported in the literature, making design and optimization of antibody fragments challenging. Here we constructed linker and orientation libraries of 3E8, an antibody specific to TAG-72, a mucinous glycoprotein overexpressed in 80% of adenocarcinomas.

Our studies have confirmed dramatic differences based on linker and orientation choices regarding biophysical properties and *in vivo* imaging. Specifically we have cloned, expressed, and characterized scFVs, diabodies, and higher order multimer constructs with varying linker compositions and sizes and domain orientations. These constructs were characterized by surface plasmon resonance (SPR) to test for antigen binding, by differential scanning fluorimetry (DSF) to test for thermal stability, and by gel chromatography to test for quaternary structure and homogeneity. We then optimized expression and purification of two biophysically favorable constructs, 3E8.scFV and 3E8.G_4S. Both constructs were subjected to mouse biodistribution and pharmacokinetic studies. From this analysis, we selected 3E8.G_4S as a lead candidate for cancer imaging and detection. This hypothesis was confirmed with successful PET and SPECT imaging of cancer xenograft mice at 24 hours.

Keywords: Antibody fragments, Cancer diagnostics, Protein engineering

Unexpected connections of aminoacyl-tRNA synthetases to HIV

Karin Musier-Forsyth

Abstract:

All retroviruses use specific host cell tRNAs to prime reverse transcription of their retroviral RNA genomes into DNA. The primer for reverse transcription in HIV-1, human $tRNA^{Lys}$, is selectively incorporated into virions during viral assembly. Surprisingly, we find that a specific $tRNA^{Lys}$ binding protein, human lysyl-tRNA synthetase (LysRS), is also specifically packaged into HIV-1 leading to the enrichment of $tRNA^{Lys}$ in virions. A highly conserved region of the HIV-1 RNA genome is responsible for regulating many steps of the retroviral lifecycle. We show that part of this region mimics the L-shaped fold of tRNA, providing a structural basis for understanding how this genomic RNA coordinates interactions with a tRNA-binding host factor to facilitate initiation of reverse transcription. Cytoplasmic LysRS is normally localized to a dynamic mammalian multisynthetase complex (MSC). In addition to their normal function in translation, many tRNA synthetases have been shown to be mobilized from the MSC and to function in a wide variety of non-translational pathways including inflammation, immune activation, and metastasis. Using immunofluorescence and confocal microscopy we find that LysRS localization is dramatically altered upon HIV-1 infection. In uninfected cells, the majority of LysRS is in the MSC, as expected, whereas LysRS is released from the MSC and traffics to the nucleus following HIV-1 infection. These findings have implications for the potential development of novel anti-retroviral therapies.

Keywords: HIV-1, Aminoacyl-tRNA synthetases, tRNA

Function and regulation of the Bacillus subtilis tyrZ gene

Rebecca N. Williams-Wagner

Abstract:

Misincorporation of D-tyrosine (D-Tyr) into cellular proteins due to mischarging of $tRNA^{Tyr}$ with D-Tyr by tyrosyl-tRNA synthetase inhibits growth and biofilm formation of *Bacillus subtilis* (1, 2). D-Tyr is produced by *B. subtilis* during stationary phase, which influences peptidoglycan biosynthesis (3) and for incorporation into antibiotic peptides (4). Furthermore, many *B. subtilis* strains lack a functional gene encoding D-aminoacyl-tRNA deacylase (2), which prevents misincorporation of D-Tyr in most organisms (1). *B. subtilis* has two genes that encode tyrosyl-tRNA synthetase; *tyrS* is expressed under normal growth conditions, and *tyrZ* is known to be expressed only when *tyrS* is inactivated by mutation (5). We hypothesized that *tyrZ* encodes an alternate tyrosyl-tRNA synthetase, expression of which allows the cell to grow when D-Tyr is present. We show that TyrZ is more selective for L-Tyr over D-Tyr compared to TyrS; however, TyrZ is less efficient overall. We also show that expression of *tyrZ* is required for growth and biofilm formation in the presence of D-Tyr. Both *tyrS* and *tyrZ* are preceded by a T box riboswitch (6), but *tyrZ* is found in an operon with *ywaE*, which is predicted to encode a MarR-family transcriptional regulator (7). Expression of *tyrZ* is repressed by YwaE and is regulated at the level of transcription attenuation by the T box riboswitch. We conclude that expression of *tyrZ* may allow growth when excess D-Tyr is present.

References:

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Keywords: tyrosyl-tRNA synthetase, T box riboswitch, MarR regulator

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1) The Role of Tax-1 and the Alternative NF-kB and Akt Signaling Pathways in HTLV Transformation

Jacob Al-Saleem (Center for Retrovirus Research, The Ohio State University, Columbus, OH, USA), **Mathew Cherian** (Division of Oncology, Washington University, St Louis, MO, USA), **Hicham Baydoun** (Division of Oncology, Washington University, St Louis, MO, USA), **Mamuka Kvaratskhelia** (Center for Retrovirus Research, The Ohio State University, Columbus, OH, USA), **Lee Ratner** (Division of Oncology, Washington University, St Louis, MO, USA), **Patrick L. Green** (Center for Retrovirus Research, The Ohio State University, Columbus, OH, USA)

Abstract:
Human T-cell Leukemia Virus Type-1 (HTLV-1) is a complex retrovirus infecting 15-25 million people worldwide, and is the etiological agent of a malignancy of CD4+ T cells termed Adult T-Cell Leukemia. By contrast, HTLV-2 is non-pathogenic in humans. Both HTLV-1 and HTLV-2 express related Tax proteins termed Tax-1 and Tax-2, respectively. Studies have revealed that Tax-1 contains a C-terminal PDZ (post synaptic density protein) domain binding motif (PBM) and a central leucine zipper region (LZR), which are absent in Tax-2. Previous studies indicated that these two domains are important for the ability of Tax-1 to activate alternative the NF-kB signaling pathway. Since Tax-2 is incapable of activating alternative NF-kB signaling we proposed that Tax-1 activation of the alternative NF-kB pathway is important for the HTLV-1 pathogenesis. We set out to identify binding partners of Tax-1 that are important for activation of alternative NF-kB. Using Tax-1 mutants that do not possess the PBM or LZR we identified several potential candidates via a proteomic screen. We plan to utilize siRNA knockdowns to screen these candidates for importance in Tax-1 driven alternative NF-kB activation. During our analyses we found that deletion of the PBM from Tax-1 did not cause a deficiency, but resulted in an enhancement of alternative NF-kB activation. With further analysis, we found that Tax-1 PBM is important for the ability of Tax-1 to activate Akt. Tax-1 diminishes the function of PTEN (Phospatase and Tensin homologue), which inhibits the PI3K-Akt-mTOR pathway. We found that Tax-1, but not PBM deleted Tax-1, competes with PTEN for binding to DLG-1 (Drosophila disk large tumor suppressor), which leads to an increase in Akt activation. These studies suggest that alternative NF-kB and Akt signaling pathways may explain the differences in HTLV-1 and HTLV-2 pathogenesis. Moreover, these findings suggest a new approach to therapeutics for HTLV-1 diseases.

Keywords: HTLV, NF-kB, AKT

2) Thinking outside the box: an investigation of an unusual S box family member

George M. Allen (Department of Microbiology, Center for RNA Biology, The Ohio State University), **Vineeta A. Pradhan** (Department of Microbiology, Center for RNA Biology, The Ohio State University), **Frank J. Grundy** (Department of Microbiology, The Ohio State University), **Tina M. Henkin** (Department of Microbiology, Center for RNA Biology, The Ohio State University)

Abstract:
S box riboswitches are regulatory RNA elements located in the 5' untranslated (leader) region of genes involved biosynthesis or acquisition of methionine or SAM. Binding of *S*-adenosylmethionine (SAM) to the riboswitch results in repression of downstream gene expression via transcriptional attenuation. *Bacillus subtilis* contains 11 S box regulated transcriptional units[1]. The *B. subtilis metK* gene, which encodes SAM synthetase, is an atypical member of the S box regulon. Unlike most S box regulated genes, which have been shown to be induced under methionine starvation conditions that result in low SAM levels, *metK* is expressed only when methionine is high and SAM concentrations are low. The *metK* leader region contains additional conserved sequence elements 5' and 3' of the S box riboswitch, designated the Upstream (US) and Downstream (DS) boxes, respectively. These sequences are highly conserved in *Firmicutes*, which include many pathogenic organisms. The US and DS box elements were shown to base-pair in the absence of SAM, and mutations in these sequences result in reduced transcript stability *in vivo*[2]. We hypothesize that an unknown factor binds to paired US and DS boxes when SAM concentrations are low and methionine is high to increase transcript stability. Crosslinking with a 4-thiouridine labeled, biotin tagged US-DS RNA to *B. subtilis* cell extract is being used to identify and purify the unknown factor responsible for increasing *metK* transcript stability.

References:
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Keywords: S-adenosymethionine, riboswitch, transcript stability

3) An adenosine deaminase and methyltransferase act co-dependently to edit and modify tRNA in Trypanosoma brucei.

Katherine M. Anderson (Department of Microbiology and OSU Center for RNA Biology, The Ohio State University, Columbus, 43210 Ohio, USA), **Ian M.C. Fleming** (Department of Microbiology and OSU Center for RNA Biology, The Ohio State University, Columbus, 43210 Ohio, USA), **Kirk W. Gaston** (Department of Chemistry, Rieveschl Laboratories for Mass Spectrometry, University of Cincinnati, Cincinnati, OH 45221, USA), **Pat A. Limbach** (Department of Chemistry, Rieveschl Laboratories for Mass Spectrometry, University of Cincinnati, Cincinnati, OH 45221, USA), **Mary Anne Rubio** (Department of Microbiology and OSU Center for RNA Biology, The Ohio State University, Columbus, 43210 Ohio, USA), **Juan D. Alfonzo** (Department of Microbiology and OSU Center for RNA Biology, The Ohio State University, Columbus, 43210 Ohio, USA)

Abstract:
Precursor transfer RNAs (tRNAs) acquire various modifications and editing that fine-tune their structural and decoding properties. These processing events can be indispensable for translation and consequently for survival. In *T. brucei*, $tRNA^{Thr}$ undergoes hydrolytic deamination from adenosine (A) to inosine (I) at position 34. This deamination event expands the decoding capacity of $tRNA^{Thr}$ by permitting wobble basepairing with the third position of the cognate codon. Along with the A to I editing, $tRNA^{Thr}$ in *T. brucei* is edited from cytosine (C) to uridine (U) at position 32 of the anticodon loop. Both deamination events are uniquely catalyzed by the same enzyme, TbADAT2/3. Besides the deamination editing, the $tRNA^{Thr}$ is further modified to 3-methylcytidine (m^3C) at position 32. Our laboratory has identified two homologs of the Saccharomyces cerevisiae m^3C methyltransferase in *T. brucei*, one of which has been validated to be the m^3C methyltransferase (Tbm^3C MTase). Interestingly, in vitro assays have shown that recombinant Tbm^3C MTase methylates C32 of a synthetic tRNA substrate only when TbADAT2/3 is in the reaction. Furthermore, deamination of m^3U is observed in vitro only upon prior formation of m^3C. These unprecedented results demonstrate that the methytransferase and deaminase depend on each other to carry out their respective functions. To this end we also show that the two enzymes act cooperatively and that the deaminase enhances the affinity of the methylase for its substrate. This interaction supports the idea that some modification enzymes act in a concerted manner to shape tRNA structure and function.

Keywords: Trypanosoma, tRNA editing, Methylation

4) The Effect of SAMHD1 on HIV-1 Gag Protein Synthesis and Virion Release

J. Antonucci (Microbiology, OSU), **S. de Silva** (Veterinary Biosciences, OSU), **C. St. Gelais** (Veterinary Biosciences, OSU)

Abstract:
ABSTRACT

Background: Restriction factors are cellular proteins defined by their ability to inhibit viral infection. Sterile alpha motif and HD domain-containing protein 1 (SAMHD1) is a mammalian protein identified as an HIV-1 restriction factor. Through the hydrolysis of deoxynucleotide triphosphates (dNTPs), SAMHD1 reduces the amount of intracellular dNTPs required for efficient HIV-1 reverse transcription. Recent studies have identified SAMHD1 as a 3' to 5' exonuclease, capable of degrading single-stranded RNAs (ssRNA) including in vitro synthesized transcripts of HIV-1 RNA, and blocking HIV-1 infection through its ribonuclease activity. Based on these studies, we hypothesize that SAMHD1 may restrict HIV-1 protein synthesis and virion release through cleavage of viral mRNA.

Methods and Results: We transfected HIV-1 proviral DNA into HEK293T cells alongside a plasmid expressing SAMHD1 or an empty vector control. To determine HIV-1 protein production in the cell and virion release into the supernatant, we measured the levels of HIV-1 Gag and p24 proteins by ELISA and immunoblotting. Our results show that SAMHD1 expression has no effect on intracellular Gag production and viral release from virus producing cells. To test the effect of SAMHD1 expression on the infectivity of newly produced HIV-1, we infected an HIV-1 reporter cell line with viruses generated from HEK293T cells in the presence or absence of SAMHD1. Our results show SAMHD1 expression does not alter the infectivity of newly produced HIV-1.

Conclusions: Overall, our results show that SAMHD1 does not reduce HIV-1 protein synthesis and virion release or affect the infectivity of HIV-1 produced in the presence of SAMHD1. Our data suggest that the exonuclease activity of SAMHD1 may not be responsible for HIV-1 restriction. These results will extend the knowledge of the mechanism by which SAMHD1 restricts HIV-1 infection.

Keywords: SAMHD1, HIV-1, Viral Restriction

5) Identification and characterization of S-box riboswitches predicted to regulate at the level of translation initiation

Divyaa Bhagdikar (Department of Microbiology, The Ohio State University), **Frank J. Grundy** (Department of Microbiology, The Ohio State University), **Tina M. Henkin** (Department of Microbiology, The Ohio State University)

Abstract:
The S-box riboswitches regulate the expression of genes involved in methionine and cysteine metabolism. These riboswitches consist of an aptamer domain that binds *S*-adenosylmethionine (SAM) and an expression platform that undergoes a SAM-dependent conformational rearrangement to regulate the expression of downstream genes. When intracellular levels of SAM are high, SAM binds to the aptamer domain and causes stabilization of a terminator helix that leads to premature termination of transcription. When SAM levels are low, an alternate antiterminator helix forms, which allows transcription of the downstream genes. *In silico* analyses have suggested that a rarer class of S-box riboswitches may regulate at the level of translation initiation. We identified two leader RNAs that have the potential for SAM-dependent sequestration of the Shine-Dalgarno (SD) region of the mRNA. The aptamer domains of both of these RNAs bound SAM with affinities comparable to that of the well-characterized *Bacillus subtilis yitJ* S-box riboswitch, and with similar selectivity against *S*-adenosylhomocysteine (SAH). A G11C mutation in *yitJ* was previously shown to disrupt SAM binding. An equivalent mutation in the candidate aptamer domains also resulted in loss of SAM binding. These results demonstrate that the aptamer domains have SAM binding properties similar to that of the previously characterized S-box riboswitches. SD regions of both the leader RNAs exhibited SAM-dependent structural rearrangements consistent with our hypothesis that regulation occurs at the level of translation initiation.

Keywords: S-box, Translation initiation, SAM

6) Distinctions in HIV-1 RNA structure and host components between the Translation RNP and the Packaging RNP

Ioana Boeras (Veterinary Biosciences, The Ohio State University), **Kathleen Boris-Lawrie** (Veterinary Biosciences, The Ohio State University)

Abstract:
HIV-1 encodes a single, multifunctional transcript that serves as mRNA template for synthesis of viral structural proteins and as virion RNA template for reverse transcription. Overlapping within the 5' untranslated region (UTR) are molecular determinants of ribonucleoprotein particles (RNPs) that carry-out the processes of translation, encapsidation, or balanced splicing of the unspliced transcript. A major focus of our studies is to define the distinct form and function of the HIV-1 RNPs because they are fundamental aspects of HIV-1 infection and persistence.
RNA affinity/proteomic analysis of HIV-1 5'UTR and validation studies identified host protein components of the translation RNP and the packaging RNP. Co-immunopreciptiation and domain analysis demonstrated N-terminal residues (N-term) of DHX9/RHA specifically and selectively recognize structural features of the 5' RNA terminus corresponding to RU5 and PBS [TAR-poly(A)]. The specific recognition of TAR-pol(A) tethers ATP-dependent activity that stimulates cap-dependent ribosome scanning and polyribosome accumulation on the gag-pol open reading frame.
Semiquantative analysis determined that RHA is incorporated into HIV-1 in stoichiometry similar to the virion RNA, ~2 on average. Virions made from RHA-deficient cells exhibit normal genome RNA content, but diminished reverse transcription activity. RHA's incorporation requires its N-term RNA binding domain and HIV-1 leader nucleotides that overlap the packaging signal (psi). While ATPase activity was necessary for HIV-1 mRNA translation, nonenzymatic chaperone activity was sufficient for RHA incorporation and rescue of viral infectivity; thus implicating a distinct molecular basis for activity of RHA at TAR-poly(A) or distal gag mRNA leader. Biophysical studies had defined structural conformers of the 5' UTR that mimic thermodynamic equilibrium between AUG-exposed and AUG-constrained 5' UTR. These were introduced into HIV-1 provirus and studied in transfected cells. The efficiency of gag translation was significantly different between the isoforms and validated the projection of distinct structural conformation of the HIV-1 translation RNP. Our results are defining fundamental tenets of HIV-1 RNA biology and therapeutic options.

Keywords: HIV-1, Translation, RHA

7) Germline-restricted repression of CycB mRNA in Drosophila embryos

Nathanael P. Braun (MCDB, The Ohio State University), **Tammy H. Wharton** (MVIMG, The Ohio State University), **Robin P. Wharton** (Molecular Genetics and MVIMG, The Ohio State University)

Abstract:
Maintenance of germ cell identity during embryonic development is critical for the future fertility of adult organisms. One player in this maintenance is the translational repressor Nanos (Nos). Nos genes are highly conserved from invertebrates to mammals and many have functions in primordial germ cells (PGCs). In *Drosphila* embryos, Nos contributes not only to PGC identity maintenance, but also to mitotic arrest of the cells during a period in development. Nos and another translational repressor, Pumilio (Pum), cooperate to repress translation of the cell cycle regulator *CycB* in the PGCs. Pum is present throughout the embryo, but Nos is restricted to the posterior of the embryo in the PGCs. Previous work in our lab showed that ectopic expression of Nos in the anterior of the embryo does not repress *CycB* mRNA, suggesting that one or more germline specific co-repressors are required in addition to Nos. One candidate factor is CG8939, which was identified in a yeast 3-hybrid screen for *CycB* mRNA-binding proteins. In this screen, CG8939 binds specifically to a sequence in the CycB 3'UTR adjacent to Nos and Pum binding sites. We have found that CG8939 interacts with Nos in yeast 2-hybrid assays. In ongoing research we are working to overexpress or knock out CG8939 and then examine if modifying the levels of CG8939 causes deregulation of CycB as in *nos* or *pum* mutant embryos. These studies on CG8939 will further define the partners of Nanos in its function in the germline.

Keywords: Nanos, Drosophila, development

8) SnRK1 Phosphorylates eIF4E/eIFiso4E to Regulate Translation

Sizhun Li (Molecular Genetics), **Aaron Bruns** (OSBP), **David Bisaro** (Molecular Genetics)

Abstract:
Plant SNF1-Related Kinase (SnRK1) belongs to a conserved protein family that includes animal AMP-activated Kinase (AMPK) and yeast SNF1. These kinases sense the AMP:ATP ratio in cells and play important roles in maintaining energy homeostasis through a variety of pathways. In these studies, we have demonstrated that SnRK1 interacts with Eukaryotic Initiation Factor 4E (eIF4E) and its isoform (eIFiso4E) and phosphorylates it. In addition, we have found that phosphorylation of eIF4E/eIFiso4E inhibits protein synthesis. This represents a previously unknown regulatory pathway of translation.

Keywords: SnRK1, Translation, eIF4E

9) Identification of novel proteins involved in nuclear export of pre-tRNAs in Saccharomyces cerevisiae

Kunal Chatterjee (Center for RNA biology, Department of Molecular Genetics, The Ohio State University), **Jingyan Wu** (Center for RNA biology, Department of Molecular Genetics, The Ohio State University), **Hsiao-Yun Huang** (Department of Biology, Indiana University), **Anita K. Hopper** (Center for RNA biology, Department of Molecular Genetics, The Ohio State University)

Abstract:
tRNAs perform essential role of delivering amino acids to the cytoplasmic protein synthesis machinery. To execute this role in protein production, eukaryotic tRNAs have to be escorted out of the nucleus, their site of synthesis, to the cytoplasm, their site of function. By the primary nuclear export, newly transcribed, end matured, partially modified tRNAs are shuttled to the cytoplasm, where, in yeast, but not vertebrate cells, the tRNA splicing machinery is located. tRNAs are also constitutively imported to the nucleus by a process known as retrograde tRNA import. These tRNAs are then shuttled back to the cytoplasm by tRNA re-export mechanisms. Genetic and biochemical studies have identified a set of RanGTPase binding proteins called β-importins, that are either directly or indirectly implicated in this bidirectional tRNA movement. Recent in vivo biochemical data in yeast have shown two such members of β-importin family, Los1 (Exportin-t in vertebrates) and Msn5 (Exportin 5), serve overlapping but distinct functions in tRNA export. Los1 forms complexes with RanGTP and both intron-less and intron containing tRNAs, regardless of their aminioacylation status. Hence Los1 is thought to participate in both primary export as well as re-export of tRNAs to the cytoplasm. In contrast, Msn5 preferentially binds only spliced aminoacylated tRNAs documenting its role to tRNA re-export. Although Los1 and Msn5 both participate in tRNA nuclear export, they cannot be the only nuclear exporters for tRNAs in yeast as los1Δ msn5Δ double mutant cells are viable. A comprehensive screening representing ~90% of the total yeast proteome conducted recently in our laboratory uncovered novel genes involved in tRNA subcellular localization. Two such proteins Mex67 and Mtr2 when inactivated, accumulate end-matured unspliced tRNAs. Interestingly, the Mex67:Mtr2 complex is the principal yeast nuclear export factor for bulk mRNA and also contributes to ribosomal subunit nuclear export. Hence it is possible that this complex can bind tRNAs as well and may constitute the missing third pathway for nuclear export. Genetic and biochemical studies are currently underway to elucidate the roles of this protein complex in tRNA subcellular dynamics and, if proven, will provide a novel Ran pathway-independent mechanism for tRNA export.

Keywords: tRNA export, tRNA subcellular dynamics, Yeast

10) Role of MicroRNA-122 in Drug Induced Liver Injury

Vivek Chowdhary (Department of Pathology, College of Medicine, The Ohio State University), **Kalpana Ghoshal** (Department of Pathology, College of Medicine, The Ohio State University), **Xiaoli Zhang** (Center of Biostatics, College of Medicine, The Ohio State University), **William Lee** (Division of Digestive and Liver Diseases, University of Texas, Southwestern), **Laura James** (College of Medicine, University of Arkansas)

Abstract:
Approximately 2000 cases of acute liver failure occur annually in the United States and drugs account for over 50% of them (39% are due to acetaminophen, 13% are idiosyncratic reactions due to other medications). Acetaminophen (APAP) or Tylenol is the most commonly used hepatotoxic drug (1-3). Although circulating miR-122, the most abundant liver-specific miRNA (4), is a biomarker of APAP toxicity in humans (5) and mice (6), its role in DILI has not been investigated. To address this question, I treated liver-specific miR-122 knock out (aka LKO) mice generated in our lab (7) to APAP and monitored pathophysiology. Results showed significantly higher mortality rate of LKO mice compared to the age-matched wild type littermates. Increased liver toxicity (high serum ALT and hepatocyte necrosis) in APAP-treated LKO mice at sub-lethal dose correlated with higher basal expression of Cyp2e1 and Cyp1a2, two key enzymes that convert APAP to the toxic metabolite, NAPQI (N-Acetyl-Parabenzo-Quinone Imine) that causes liver damage. Western blotting and qRT-PCR analysis of unspliced-mRNA and mRNA revealed increased basal level of these proteins in LKO livers compared to that in the WT livers is due to transcriptional upregulation of these genes. Based on ENCODE Chip-seq data demonstrating association of CTCF (CCCTC-binding factor) with human and mouse Cyp2e1 promoters, I hypothesized that upregulation of CTCF, a potential transcriptional activator of Cyp2e1, and a validated target of miR-122, enhances Cyp2e1 expression in LKO livers. Indeed, preliminary results showed that siRNA-mediated CTCF knockdown reduced Cyp2e1 expression in cultured hepatocytes from LKO mice. In contrast, CTCF indirectly increases Cyp1a2 expression by inducing expression of Ahr (Aryl hydrocarbon receptor), the key transcriptional activator of Cyp1a2 (8). Taken together, these results show that miR-122 protects mice from acetaminophen-induced liver injury by regulating expression of two key enzymes involved in drug metabolism. My next goal is to test if miR-122 delivery using lipid nanoparticles or a viral vector can protect mice from acetaminophen/Tylenol toxicity.

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Keywords: Acetaminophen, microRNA-122, DILI

11) Splicing Factor SRSF2 Positively Regulates the Alternative Splicing of MDM2

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Abstract:
Murine Double Minute 2 (MDM2) is an E3 ubiquitin ligase and negative regulator of the tumor suppressor protein p53. Under normal conditions, MDM2 is constitutively spliced to generate a full-length protein. However, under stress MDM2 undergoes alternative splicing, generating splice variants that are unable to bind and regulate p53. Despite this tumor-protective activity, alternatively-spliced variants of MDM2 have been linked to cancer including soft tissue sarcomas, breast, colon, bladder and ovarian cancers. MDM2-ALT1, which consists of only the two terminal coding exons 3 and 12, is the most frequently observed of these splice isoforms. Despite these functional studies, there is very little known about the regulators of that govern this alternative splicing event.

In order to study the alternative splicing of MDM2 we have developed a damage-inducible minigene system. The MDM2 3-11-12 minigene recapitulates the splicing of the endogenous gene by excluding exon 11 under genotoxic stress. Using ESEfinder 3.0 we identified conserved consensus sequences for splicing regulator SRSF2 in exon 11 of MDM2.

We report that SRSF2 promotes the inclusion of exon 11 under damage both in vitro and in vivo using our MDM2 minigene. Our binding data has demonstrated that upon mutation, binding of SRSF2 is attenuated. Also, overexpression of SRSF2 promotes the inclusion of MDM2 exon 11 under damage, whereas knockdown induces the skipping of MDM2. Furthermore, antisense oligonucleotides (ASOs) targeting SRSF2 binding sites push endogenous MDM2 splicing toward MDM2-ALT1.

In summary, we have identified a critical regulator of MDM2 alternative splicing. Therefore by titrating the amount of MDM2-ALT1 endogenously, we hope ASOs can be utilized as an attractive strategy for anticancer therapy.

Keywords: MDM2, SRSF2

12) Putative histone readers EML1 and EML3 regulate geminivirus infection

Tami Coursey (Molecular Genetics, Ohio State University), **Milica Milutinovic** (ABRC, Ohio State University), **Jelena Brkljacic** (ABRC, Ohio State University), **David Bisaro** (Molecular Genetics, Ohio State University)

Abstract:
Similar to their host counterparts, plant DNA viruses are subject to chromatin formation. Bound around histone octamers, DNA accessibility is regulated by post-translational modification (PTM) of exposed histone tails. While previous work has suggested the deposition of PTMs is important for plant defenses, we have not explored the role of proteins recognizing/reading these PTMS. Binding specific histone modifications, histone reader proteins are thought to alter gene expression by recruiting targeted nucleosome remodeling complexes and transcription factors. Using plant DNA geminiviruses as a model viral pathogen, we are testing viral interaction with two putative, Agenet domain-containing histone readers (Emsy-like, EML proteins 1 & 3) sharing homology to Royal Family histone readers (e.g. Tudor domains). Surprisingly eml plants inoculated with Cabbage leaf curl virus (CaLCuV) exhibited hypersusceptibility (eml1) or increased tolerance (eml3) not observed in wild-type plants. We hypothesized EML proteins may read histone marks on the geminivirus that repress (EML1) or enhance (EML3) viral infection. These phenotypes were supported by differences in symptomatic plant stunting and viral DNA levels (qPCR). Quantitative RT-PCR confirmed the presence of EML1 and EML3 expression in wild-type symptomatic tissue where EMLs are usually lowly expressed. Additionally, transiently expressed EML1 and EML3 bind CaLCuV (chromatin immunoprecipitation, ChIP) and histone H3 (co-immunoprecipitation), supporting their role as histone readers and their interaction with viral chromatin. We are currently further testing the impact of EML proteins on CaLCuV infection by measuring changes in the amount of viral chromatin and transcripts in eml plants. These results suggest Agenet domain-containing EML proteins are putative histone readers and play specific roles in promoting virus infection or bolstering plant antiviral defense pathways.

Keywords: histone readers, geminivirus

13) Determinants of specific recognition of mischarged Ala-tRNAPro by a bacterial trans-editing domain

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Abstract:
Aminoacyl-tRNA synthetases (ARS) catalyze the attachment of specific amino acids to cognate tRNAs. Mistakes in this process lead to errors in protein synthesis that can be deleterious to cells. Prolyl-tRNA synthetase (ProRS) mischarges $tRNA^{Pro}$ with Ala; this aberrant product is hydrolyzed by a *cis*-editing domain (INS) in most bacteria. However, some bacteria lacking the INS domain encode a homologous free-standing *trans*-editing domain known as ProXp-ala that functions to clear Ala-$tRNA^{Pro}$. Previous studies showed that specific nucleotides in the acceptor stem of $tRNA^{Pro}$ (G72 and A73) are critical for ProXp-ala activity, and that a small RNA stem-loop containing these elements, $microhelix^{Pro}$, is a good substrate for Ala deacylation. To define the elements in ProXp-ala that confer acceptor stem specificity, NMR mapping studies were carried out with an uncharged $microhelix^{Pro}$ and with a non-hydrolyzable, amide-linked Ala-$microhelix^{Pro}$ mimic. We observe similar but significantly stronger chemical shift perturbations in the presence of the charged microhelix, which also displays 5-fold higher affinity for binding to ProXp-ala, as measured by analytical ultracentrifugation (AUC). The largest chemical shift perturbations were mapped to three main regions: helix α2 at the top of the active site pocket (aa 27-30), β-strands β2 (aa 43-49) and β6 (aa 128-134) within the active site, and β-strand β4 (aa 80-84) that we propose is the G72/A73 interacting domain. Site-directed mutagenesis and AUC studies are consistent with the critical nature of residues 80-83 for substrate binding. Mutation of conserved active site residues K45 and N46 also caused severe losses in activity. Additionally, ^{15}N NMR relaxation experiments revealed that the helix α2 exhibits significant dynamics at the ps-ns timescale. These results allow us to propose a mechanism for recognition of Ala-$microhelix^{Pro}$ that involves induced-fit binding, specific protein-RNA contacts, and key contributions from the Ala moiety.

Keywords: tRNA, NMR, dynamics

14) bPNA's as fluorescent reporters of RNA loop-loop interactions.

Christopher DeSantis (Chemistry and Biochemistry, The Ohio State University), **Xin Xia** (Chemistry and Biochemistry, The Ohio State University)

Abstract:
We have previously reported that synthetic melamine-displaying α-peptides, termed bifacial peptide nucleic acid (bPNA), can simultaneously dock two T/U strands to form a triplex hybrid stem via a designed molecular recognition interface. When T/U-rich domains are separated by an intervening oligonucleotide sequence of 4–10 nt, a hairpin triplex stem-loop structure is formed with high affinity (Kd ~2 nm) [1]. We have demonstrated that these high melting temperature complexes (Tm = 57°C) are competitive with protein enzymes whose native substrates are DNA or RNA, such as transcriptase, exonuclease and reverse transcriptase [2]. We have also demonstrated, in three nucleic acid systems, complete structure-function rescue upon refolding of a T/U loop into a bPNA triplex hybrid stem [3]. In this work, we use a bPNA triplex hybrid to template RNA-RNA interactions. Homodimerization of genomic RNA of the human immunodeficiency virus type 1 (HIV-1) is initiated by a "kissing loop" interaction between stem-loop structures of the dimerization initiation site (DIS) within the dimer linkage structure (DLS) [4,5]. Integration of a bPNA triplex hybrid into the stem-loop sequence of the DIS renders dimerization of the RNA as a pair of "kissing loops" dependent on bPNA. Dyes covalently linked to the bPNA's are predicted to serve as fluorescent reporters of RNA loop-loop interactions.

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Keywords: Kissing Loop, bifacial nucleic acid, FRET

15) Heat shock response alters SMN alternative splicing

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Abstract:
Spinal Muscular Atrophy (SMA) is a neurodegenerative disease and one of the greatest sources of genetic mortality in infants. Motor neuron degeneration leads to muscle weakness, which characterizes SMA. Over 90% of SMA cases are characterized as severe, which often results in respiratory distress and death. SMA is caused by low levels of the protein, SMN, which is encoded by two genes, SMN1 and SMN2. These two genes are nearly identical, but a key difference is a translationally silent single nucleotide switch in exon 7. This difference causes SMN2 to mis-splice and prevents exon 7 inclusion in the majority of its transcripts during RNA splicing.
While no therapy currently exists, efforts to combat SMA revolve around: (1) stabilizing the SMN protein, (2) increasing transcription rate or stability of SMN2 mRNA transcripts, or (3) altering splicing of SMN2. Alteration of SMN2 splicing is a particularly appealing method, as it alters the ratio of full length to $\Delta 7$ transcripts without altering the cell's natural transcription and degradation process of SMN.
In mid to late disease stage in SMA, there are increased levels of cellular stress. To examine if these stresses are capable of exacerbating SMA condition through increased SMN2 skipping (as in hypoxia) we tested additional stresses. Surprisingly, we found that heat shock (42° for 24hr) dramatically improves splicing in SMN2 transcripts. In addition, we found that SMN protein levels were also increased in multiple cell types. We determined that one key splicing factor (Tra2beta) is responsible for this response.
Therefore, we have determined that heat shock improves molecular outcomes for SMA on an RNA and protein level. Additionally, we are looking to test small molecule heat shock response co-inducers as a potential therapy for SMA in the future.

Keywords: Splicing, SMA, heat

16) Examination of Human Lysyl-tRNA Synthetase/tRNALys Primer Recruitment and Packaging into HIV-1

Alice Duchon (Department Chemistry and Biochemistry, Center for RNA Biology, Center for Retroviral Research), **Nathan Titkemeier** (Department Chemistry and Biochemistry, Center for RNA Biology, Center for Retroviral Research), **Corine St. Gelais** (Center for RNA Biology, Center for Retroviral Research, Department of Veterinary Biosciences), **Li Wu** (Center for RNA Biology, Center for Retroviral Research, Department of Veterinary Biosciences), **Karin Musier-Forsyth** (Department Chemistry and Biochemistry, Center for RNA Biology, Center for Retroviral Research)

Abstract:
The primer for reverse transcription in HIV-1, human $tRNA^{Lys3}$, is selectively packaged into virions along with $tRNA^{Lys1,2}$. Human lysyl-tRNA synthetase (LysRS) , the only cellular factor known to interact specifically with all three tRNALys isoacceptors, is also packaged into HIV-1. Selective packaging of $tRNA^{Lys}$ depends on the ability of the tRNA to bind to LysRS and the presence of both host cell factors is required for optimal viral infectivity. LysRS is part of a dynamic mammalian multisynthetase complex (MSC) and has been shown to be mobilized from the MSC and to function in a wide variety of non-translational pathways. While some aspects of tRNA primer packaging into HIV-1 particles are now understood, the mechanism by which the LysRS/tRNA complex is diverted from its normal function in translation and recruited into viral particles is unclear. Here, we show that the expression of LysRS is unaltered upon HIV-1 infection, suggesting that the LysRS species packaged is recruited from an existing pool of LysRS. Using immunofluorescence and confocal microscopy with both HEK293T and HuT/CCR5+ cells, we find that LysRS trafficking is altered upon HIV-1 infection with more LysRS localized to the nucleus. LysRS-Gag and LysRS-genomic RNA co-localization studies are currently underway. Our studies also indicate that HIV infection results in phosphorylation of LysRS on Ser. We hypothesize that LysRS phosphorylation results in release from the MSC and nuclear entry. Studies to understand the significance of these findings for HIV infectivity are in progress.

Keywords: HIV, LysRS, tRNA

17) The DExH-box helices RHA/DHX9 selectively regulates pathogenic retroviral gene translation by a novel cap-dependent mechanism.

Sarah Fritz (Veterinary Biosciences, College of Veterinary Medicine), **Kathleen Boris-Lawrie** (Veterinary Biosciences, College of Veterinary Medicine)

Abstract:
Retroviruses are known to exploit complex posttranscriptional mechanisms to facilitate their infectivity and pathogenicity. We identified the DExH-box helicase RNA helicase A (RHA/DHX9) as a critical host factor important for the translation control of pathogenic retroviruses that infect animal and human hosts, including avian, simian, and the human pathogenic retroviruses HIV-1 and HTLV-1. This targeted translation control results from the select recognition and binding of RHA to a distinct RNA element, termed the posttranscriptional control element (PCE), in the 5' leader of these viral mRNAs. Together RHA-PCE association facilitates robust ribosome loading and viral protein production. An outstanding question is the mechanistic role of RHA in this process: how is RHA interacting with the translation machinery to facilitate efficient viral protein production?
Our preliminary data supported a role for RHA in cap-dependent initiation, the first and rate-limiting step of translation. Cap-dependent initiation is a highly choreographed process dependent upon cap-associated protein factors, either the predominant eIF4F or the non-canonical CBP80/20 protein complexes, to recruit the ribosome and stimulate translation. We hypothesized that the association of RHA with these cap-associated initiation factors was important for its mechanistic role in this process. Through the use of extensive cellular co-immunoprecipitation assays we identified a novel cellular interaction between RHA and the non-canonical CBP80/20 cap initiation complex. Polysome profiling validated a functional role for RHA-CBP80/20 association in targeted translation control. Further translation initiation analysis revealed significance for RHA-CBP80/20 association in engaging RHA within the mechanistic process of translation initiation. Our identification of a RHA-CBP80/20 translation complex distinguishes RHA from related DExH-box helicases DHX29 and DDX3, which function in translation initiation of other viral families via interaction with eIF4F. Together the results from this study have identified a novel RHA-CBP80/20 cap translation complex that is selectively co-opted by pathogenic retroviruses to facilitate their protein production.

Keywords: RNA helicase A (RHADHX9), CBP8020 cap-complex, translation regulation

18) Homology recognition and expression dependent methylation of an exogenous transposable element

Dalen Fultz (Molecular Genetics, The Ohio State University), **R. Keith Slotkin** (Molecular Genetics, The Ohio State University)

Abstract:
Transposable elements (TEs) are mobile, parasitic DNA elements that make up large portions of most organism's genomes. In plants and animals, it is well understood that pathways that lead to small RNA production, DNA methylation, and chromatin modification protect the genome by maintaining these elements in a silent state. However, little is known about how TEs are originally recognized by the host - integral to the fundamental biological question of how an organism deciphers self from non-self. In order to understand the molecular mechanism that a host uses to identify fragments of DNA as TEs, I have stably transformed foreign TEs into a new host, Arabidopsis thaliana (a powerful model genetic system with expansive resources and tools). I have found that both a yeast and a tobacco retrotransposon are quickly silenced in the first generation of transformants. Full silencing appears to be dependent on the RNA-directed DNA Methylation (RdDM) pathway, previously known to be involved in maintenance of silencing. I have shown that this pathway is key to recognizing new TEs that contain even limited homology to endogenous TEs. However, when this pathway cannot recognize incoming TEs, non-canonical pathways perform this function de novo, generating a unique pattern of small RNAs and DNA methylation. In this state, histone methylation cannot be established unless RNA Polymerase II (Pol II) expression is triggered. Simlarly, when a fragment of a foreign TE is introduced into wildtype plants, de novo silencing is fully dependent on transcription by Pol II. Through this work, we are elucidating a detailed understanding of the earliest steps of TE recognition which occurs either through homology to endogenous TEs or through a Pol II-expression dependent pathway. This has also led us to a greater understanding of the transition from this state to epigenetically-maintained transcriptional silencing that provides immunity to further TE insertions.

Keywords: Epigenetics, Transposable element, small RNA

19) Pnrc2 targets oscillating transcripts for rapid degradation via 3' UTR recognition

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Abstract:
Vertebrate segmentation is controlled by the segmentation clock. The segmentation clock regulates oscillating gene expression and cycles rapidly, having a period of 30-120 minutes, depending upon the vertebrate model. At the core of the segmentation clock are a family of transcriptional repressors encoded by the *hairy/Enhancer of split-related* (*her* or *Hes*) genes, which auto-inhibit their own expression. Although many studies have elucidated transcriptional mechanisms important for oscillatory expression, fewer have investigated mechanisms of post-transcriptional processing that promote rapid clearance of *Hes/her* transcripts. In a zebrafish screen for genes involved in cyclic transcript regulation, we uncovered the *tortuga* mutation. *tortuga* ^{b644} mutants accumulate cyclic transcripts post-splicing. The gene affected in *tortuga* mutants is *pnrc2*, which encodes a proline-rich nuclear receptor co-activator implicated in promoting mRNA decay. We hypothesize that Pnrc2 is part of an mRNA decay complex that recognizes elements and/or structural features of developmentally-regulated transcripts that must be cleared rapidly during early development. In support of this hypothesis, we observe that *pnrc2* genetically interacts with *upf1*, a core component of the nonsense-mediated decay (NMD) complex.
In order to define the instability feature(s) recognized by Pnrc2, we have developed an inducible in vivo reporter system to observe transcript stability in zebrafish embryos. We observe that the 3' UTR of *her1* confers instability to an otherwise stable heterologous transcript in a Pnrc2-dependent manner. Furthermore, tethering of Pnrc2 to a stable transcript is sufficient to trigger rapid decay. Our goal is to define the regulatory logic controlling targeted degradation of oscillating transcripts.

Keywords: Pnrc2, mRNA decay, Upf1

20) Dissecting the requirement of Arabidopsis RanGAP1 subcellular targeting and GAP activity for its cellular and developmental functions

Anna H. N. Griffis (Department of Molecular Genetics, Center for RNA Biology, The Ohio State University), **Joanna Boruc** (Department of Plant Systems Biology, VIB, Ghent, Belgium), **Thushani Rodrigo-Peiris** (Department of Molecular Genetics, The Ohio State University), **Xiao Zhou, Bailey Tilford** (Department of Molecular Genetics, The Ohio State University), **Daniel Van Damme** (Department of Plant Systems Biology, VIB, Ghent, Belgium), **Iris Meier** (Department of Molecular Genetics, Center for RNA Biology, The Ohio State University)

Abstract:
The Ran GTPase activating protein (RanGAP) is important to Ran signaling involved in nucleocytoplasmic transport, spindle organization and post-mitotic nuclear assembly. Unlike vertebrate and yeast RanGAP, plant RanGAP has an N-terminal WPP domain, required for nuclear envelope association and several mitotic locations of *Arabidopsis* RanGAP1. A double null mutant of the two *Arabidopsis RanGAP* homologs is gametophyte lethal. Here, we have created a series of mutants with various reductions in RanGAP levels by combining a *RanGAP1* null allele with different *RanGAP2* alleles. As RanGAP level decreases, severity of developmental phenotypes increases but nuclear import is unaffected. To dissect whether the GAP activity and/or the subcellular localization of RanGAP are responsible for the observed phenotypes, this series of *rangap* mutants were transformed with RanGAP1 variants carrying point mutations abolishing the GAP activity and/or the WPP-dependent subcellular localization. The data show that plant development requires the GAP activity of RanGAP and is susceptible to reductions in RanGAP protein level, while the subcellular positioning of RanGAP is dispensable. In addition, our results indicate that nucleocytoplasmic trafficking can tolerate both partial depletion of RanGAP and delocalization of RanGAP form the nuclear envelope.

Keywords: RanGAP, Arabidopsis, Cell Biology

21) Building a consensus co-expression network for CNS genes relevant to pharmacogenomics

Samuel K. Handelman (MVIMG, The Ohio State University), **Michal Seweryn** (Mathematics and Computer Science, Uniwersytet dzki), **Amy Webb** (Pharmacology, The Ohio State University), **Ryan M. Smith** (Pharmacology, The Ohio State University), **Andrzej Kloczkowski** (Battelle Center for Mathematical Medicine, Nationwide Childrens Hospital), **Wolfgang Sadee** (Pharmacology, The Ohio State University)

Abstract:
In studies associating genetic variants with efficacy or side-effects of psychiatric medications (for schizophrenia, depression and bipolar disorder), key variants in less than 40 CNS-well-expressed genes are repeatedly implicated. Using next generation sequencing expression measurements from: the Genotype-Tissue Expression project (GTEx); five human studies deposited in the gene expression omnibus; and, from brain transcriptomes produced internally for the use of Expression Genetics in Drug Therapy (XGEN), co-expression networks including these repeatedly-implicated genes are identified. Several alternative methods are utilized: Weighted Gene Coexpression Network Analysis (WGCNA), Algorithm for the Reconstruction of Accurate Cellular Networks (ARACNE), and variations on ARACNE incorporating alternative information measures. Different methods produce divergent results in terms of reproducibility within and between the available expression experiments and tissues, assessed using random nested resampling of the original transcriptomes and data series. The sparse and parsimonious (not-highly connected) consensus co-expression network has relevance both to biological interpretation and to the identification of potential statistical epistasis between gene variants for CNS pharmacotherapy.

Keywords: Pharmacogenomics, Transcriptomics, Networks

22) Investigating the disassembly pathway of the Anti-TRAP dodecameric complex by surface induced dissociation mass spectrometry (SID-MS).

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Abstract:
Cellular homeostasis requires regulation of many cellular processes. In *Bacillus*, production of the enzymes responsible for biosynthesis of the amino acid tryptophan (Trp) is tightly regulated. When there is abundant tryptophan in the cell, transcription of the genes responsible for the synthesis of Trp, encoded in the *trp* operon, is inhibited the trp RNA-binding attenuation protein (TRAP). To balance the repression by TRAP, another protein, Anti-TRAP (AT) binds and inhibits RNA binding by TRAP, allowing the Trp biosynthesis genes to be expressed. AT is an oligomeric protein that exists in equilibrium between a trimer AT_3 and a dodecamer AT_{12}, while only the trimeric form can bind TRAP. AT_{12} is composed of a tetrahedral arrangement of four AT_3, i.e., tetramers of trimers, whose mechanism of assembly and disassembly represents a fascinating puzzle. To understand how inactive AT_{12} dissociates into active AT_3 we use surface-induced dissociation mass spectrometry (SID-MS). In SID-MS, dissociation of complexes is observed upon collision with a surface of selected ions corresponding to the “parent” complex, then detecting the resulting “daughter” ions. The SID-MS approach is similar to that of CID (collision induced dissociation), in which daughter ions are observed after collision with an inert gas, but has the advantage that higher activation energies can be overcome, which means more native structure occur for dissociation products. Results thus far suggest that dissociation of trimers is not a favored pathway, with major daughter ions corresponding to monomers and their counterparts (undecamers, decamers). Because formation of AT_{12} is dependent on pH and proper deformylation of the protein’s N-terminus, we will explore these and other variables to understand how inactive AT 12-mers can dissociate to form trimers that are able to bind and reverse the inhibitory effect of TRAP.

Keywords: Anti-TRAP, mass spectrometry, surface induced dissociation

23) Probing the physiological function of prolyl-tRNA synthetase-related trans-editing domains

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Abstract:
Although organisms can tolerate some mistakes in translation without a substantial fitness cost, cellular conditions that increase the rate of mistranslation above this threshold can result in toxicity in bacteria and pathologies in mammalian cells. Aminoacyl-tRNA synthetases (ARSs) attach each amino acid (aa) to its cognate tRNA, but errors can occur in the presence of similar aa's. About half of the ARSs have been shown to possess proofreading capabilities *in vitro*, which clear errors before (pre-) or after (post-transfer editing) the formation of aa-tRNA. Post-transfer editing may occur in *cis* (via specialized domains appended to the ARS) or in *trans* (via free-standing editing domains). We have identified a family of *trans*-editing factors homologous to the bacterial prolyl-tRNA synthetase *cis*-editing domain (INS), including YbaK and ProXp-ST1 in *E. coli*. These domains have been shown to edit Cys-tRNA^{Pro} and Ser/Thr-tRNA^{Lys}, respectively, *in vitro*. Although deletion of these factors does not result in a growth defect under normal conditions, we hypothesize that INS homologs become critical under conditions where the balance of aa's and aa-like metabolites is perturbed, generating mischarged aa-tRNAs and an altered protein pool. To test this hypothesis, we have developed two different reporters for measuring misincorporation at Pro codons (YbaK) and Lys codons (ProXp-ST1) in the deletion strains. A gain-of-function GFP reporter (expressed in tandem with mCherry) takes advantage of a hydrogen bonding interaction between residues 65 and 222 that is crucial for fluorophore formation. Purification of His-tagged EF-Tu expressed in the null strains under a variety of growth conditions will allow us to observe changes at all possible codons to either Cys or Ser/Thr by mass spectrometry analysis. Preliminary data using both of these reporter proteins will be presented.

Keywords: tRNA, aminoacyl-tRNA synthetase, translational fidelity

24) Alternative RNA processing leads to decreased DNA topoisomerase IIα in etoposide (VP-16) resistant human leukemia K562 cells

Ragu Kanagasabai

Abstract:

Acquired resistance to the DNA topoisomerase II (topo II)-targeting agent VP-16 is associated with decreased levels of topo II mRNA and protein in a K562 clone, K/VP.5 (Br. J. Cancer 68: 687-697, 1994). qPCR revealed a 2-fold decrease in topo II mRNA expression in K/VP.5 compared to K562 cells when probes were downstream of exon 19.

RNA stability studies 4 qPCR probes, targeted to 3' and 5' domains, revealed similar topo II mRNA half-lives in K562 cells but an increase in topo II mRNA half-lives in K/VP.5 cells suggesting alterations in exo- or endo-nuclease expression/function in resistant cells and/or alternative RNA processing events generating transcripts of different size and stability characteristics.

3'-RACE showed loss of ~3 kb of the total 5.8 kb topo IIα in K/VP.5 cells. PCR products derived from this truncated transcript were sequenced, revealing that the 5' splice site of intron 19 is suppressed, with retention of ~300 nt of intronic sequence in the mature mRNA, and the use of an alternative polyadenylation site. The retained portion of intron 19 contains an in-frame stop codon, as well as the consensus AAUAAA hexamer of the poly(A) signal. As a result of alternative 3' end processing, exons 20-35 of the topo II gene are not included in the truncated transcript. Surprisingly, the truncated transcript is also present in K562 cells, albeit at a greatly reduced level compared to K/VP.5, suggesting that alternative polyadenylation is a normal mechanism to regulate topo IIα expression.

Using a custom topo IIα antibody raised against intron 19 sequences as well as an extreme N-terminal and an extreme C-terminal topo IIα antibody, immunoblot results from cell lysates indicated that truncated alternatively processed topo II was expressed in K/VP.5 and less so in K562 cells. To further validate results, a 2431-base pair fragment coding for the 810 amino acid truncated topo II (97 kDa) was cloned into the polylinker region of pcDNA3.1(+) followed by transfection into both K562 and K/VP.5 cells. Immunoblot results confirmed enforced expression of the 97 kDa truncated form of topo IIα.

Taken together results indicate that posttranscriptional alterations in topo IIα may account, in part, for acquired resistance to VP-16, and potentially for regulation of topo IIα expression that may dictate intrinsic chemosensitivity as well.

25) Cap homeostasis is independent of poly(A) tail length

Daniel L. Kiss (Biological Chemistry and Pharmacology, The Center for RNA Biology, The Ohio State University), **Kenji Oman** (Department of Physics, The Center for RNA Biology, The Ohio State University), **Julie A. Dougherty** (Biological Chemistry and Pharmacology, The Center for RNA Biology, The Ohio State University), **Chandrama Mukherjee** (Biological Chemistry and Pharmacology, The Center for RNA Biology, The Ohio State University), **Ralf Bundschuh** (Department of Physics, The Center for RNA Biology, The Ohio State University), **Daniel R. Schoenberg** (Biological Chemistry and Pharmacology, The Center for RNA Biology, The Ohio State University)

Abstract:
Cap homeostasis is a cyclical process of decapping and recapping that maintains the cap on a subset of the cytoplasmic transcriptome. Interfering with cytoplasmic capping results in the redistribution of target transcripts from polysomes to non-translating mRNPs, where they accumulate in an uncapped but nonetheless stable form. It is generally thought that decapping is preceded by shortening of the poly(A) tail to a length that can no longer support translation. Therefore recapped target transcripts would either have to undergo cytoplasmic polyadenylation or retain a reasonably long poly(A) tail if they are to return to the translating pool. We find that in cells that are inhibited for cytoplasmic capping there is no change in the overall distribution of poly(A) lengths or in the elution profile of oligo(dT)-bound cytoplasmic capping targets. Consistent with this observation there is little difference in poly(A) tail length of uncapped target transcripts recovered from non-translating mRNPs and capped forms of the same mRNAs on translating polysomes. Finally, an in silico analysis of cytoplasmic capping targets found significant correlations with genes encoding transcripts having uridylated or multiply modified 3'-ends, and with genes having multiple 3'-UTRs generated by alternative cleavage and polyadenylation.

Keywords: cytoplasmic capping, cap homeostasis, poly(A)

26) Investigating an additional tRNA binding specificity determinant of the glyQS T box riboswitch

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Abstract:
The T box riboswitch regulates expression of many amino acid-related genes in Gram-positive bacteria at the level of transcription attenuation. The nascent transcript, or leader RNA, of T box family genes binds to a specific tRNA and detects its aminoacylation status, which determines if an intrinsic transcription terminator helix forms upstream of the coding region[1]. Binding of uncharged tRNA stabilizes a mutually exclusive antiterminator helix that allows RNA polymerase to transcribe the downstream gene. The primary specificity determinant of the T box leader RNA-tRNA interaction is a three nucleotide Specifier Sequence that base pairs with the anticodon of the cognate tRNA. An additional base pairing interaction occurs between the acceptor arm of uncharged tRNA and a bulge within the antiterminator element. A third leader RNA-tRNA interaction has been described for the *glyQS* T box riboswitch that consists of a stacking interaction between the Stem I terminal region and the D/T-loops of tRNA[2,3]. The terminal region of Stem I contains two highly conserved sequence motifs that interact to form a docking platform for the tRNA elbow. Glycyl T box genes (e.g., *glyQS*, encoding glycyl tRNA synthetase) contain a specific pattern of nucleotide identity and spacing in both Stem I motifs, including residues that comprise the core of the tRNA docking platform and interact with the tRNA, that differs from the majority of T box RNAs. These differences in Stem I that are specific to glycyl T box RNAs suggest the potential for a base pairing-independent mechanism of tRNA binding specificity. Here, we reveal that changes to the *Bacillus subtilis glyQS* Stem I terminal region that match the consensus pattern improve binding and *in vitro* antitermination for non-cognate tRNA. Additionally, this Stem I variant is less able to discriminate against non-cognate tRNA during transcription *in vitro*. We propose that the wild-type *glyQS* Stem I terminal region screens the available cellular tRNA pools and discriminates against non-cognate tRNAs before the Specifier Sequence is transcribed.

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2. Zhang JW & Ferre-D'Amare AR. 2013. Co-crystal structure of a T-box riboswitch stem I domain in complex with its cognate tRNA. Nature 500:363-366.

3. Grigg JC & Ke AL. 2013. Structural determinants for geometry and information decoding of tRNA by T box leader RNA. Structure 21:2025-2032.

Keywords: tRNA, Riboswitch, RNA-RNA interaction

27) Mechanism of a multifunctional tRNA methyltransferase family.

Aiswarya Krishnamohan (Department of Chemistry and Biochemistry, The Ohio State University), **Jane Jackman** (Department of Chemistry and Biochemistry, The Ohio State University)

Abstract:
The tRNA methyltransferase (Trm10) methylates N1 of guanosine residues at the 9th position (G_9) of multiple tRNAs using S-adenosyl methionine (SAM) as the methyl group donor. Trm10 was originally discovered in *Saccharomyces cereviseae* but is nearly ubiquitous in Archaea and Eukarya, and familial mutations in a human homolog of Trm10 (TRMT10A) are associated with numerous abnormalities, including defects in glucose metabolism and neurological dysfunction. Despite classification of Trm10 enzymes as members of the SpoU-TrmD (SPOUT) family of methyltransferases, the molecular mechanism of these enzymes is not completely understood, and cannot be inferred directly due to limited sequence similarity to other more well-studied family members. Moreover, biochemical analysis of Trm10 homologs identified in human mitochondria and some Archaea revealed the surprising ability of some Trm10 family members to catalyze m^1A_9 methylation, either in addition to, or instead of the prototypical m^1G_9 activity. This flexibility in terms of substrate nucleotide is particularly remarkable in light of the different pKa values expected for the N1 atom in G_9 vs. A_9-containing tRNA substrates. For all of these reasons, we hypothesize that Trm10 enzymes exhibit an atypical mechanism of N1 methylation, and aimed to determine the molecular features of catalysis utilized by this unusual and important enzyme family.
Here we use site-directed mutagenesis to alter conserved residues implicated in Trm10 catalytic activity based on their positions in a recently solved crystal structure and utilize single-turnover kinetic assays to evaluate activities of the resulting proteins. Although the SAM-binding site predicted by the crystal structure is supported by this analysis, these data argue against an important role for a previously proposed general base in the reaction. Analysis of additional Trm10 variants is underway, with the ultimate goal of elucidating the complete catalytic mechanisms exhibited by members of this methyltransferase enzyme family.

Keywords: tRNA methylation, enzymatic mechanism, tRNA modification

28) Characterization and Selection of Biophysically Optimized Antibody Fragments for Enhanced in vivo Tumor Imaging

N. Emerson Long (Ohio State Biochemistry Program), **Brandon J. Sullivan** (Ohio State Biochemistry Program), **Thomas J. Magliery** (Ohio State Department of Chemistry and Biochemistry)

Abstract:
Antibody fragments have great potential for clinical application as cancer theranostics. Their small size compared to full-length IgG's allows for faster blood clearance, potentially decreased immunoreactivity, better tumor penetrance, and easier engineering and production. The smallest possible fragment of an IgG that still binds to its antigen, called the single-chain variable fragment (scFv), can be created by fusing the variable light and variable heavy domains together with a peptide linker. Along with switching domain orientations, altering the length and amino acid sequence of the linker can significantly change the biophysical characteristics such as binding, stability, and quaternary structure. Comprehensive studies of these attributes have not been reported in the literature, making design and optimization of antibody fragments challenging. Here we constructed linker and orientation libraries of 3E8, an antibody specific to TAG-72, a mucinous glycoprotein overexpressed in 80% of adenocarcinomas.
Our studies have confirmed dramatic differences based on linker and orientation choices regarding biophysical properties and *in vivo* imaging. Specifically we have cloned, expressed, and characterized scFVs, diabodies, and higher order multimer constructs with varying linker compositions and sizes and domain orientations. These constructs were characterized by surface plasmon resonance (SPR) to test for antigen binding, by differential scanning fluorimetry (DSF) to test for thermal stability, and by gel chromatography to test for quaternary structure and homogeneity. We then optimized expression and purification of two biophysically favorable constructs, 3E8.scFV and 3E8.G_4S. Both constructs were subjected to mouse biodistribution and pharmacokinetic studies. From this analysis, we selected 3E8.G_4S as a lead candidate for cancer imaging and detection. This hypothesis was confirmed with successful PET and SPECT imaging of cancer xenograft mice at 24 hours.

Keywords: Antibody fragments, Cancer diagnostics, Protein engineering

29) Identification of alternate exon junction complexes in mammalian cells

Justin W. Mabin (Molecular Genetics, Ohio State University), **Lauren A. Woodward** (Molecular Genetics, Ohio State University), **Kate M. Ehrensberger** (Molecular Genetics, Ohio State University), **Guramrit Singh** (Molecular Genetics, Ohio State University)

Abstract:
In mammalian cells, the exon junction complex (EJC) is deposited during pre-mRNA splicing ~24 nt upstream of most exon-exon junctions in a sequence-dependent manner. The EJC core is comprised of a stable tetrameric protein complex consisting of eIF4AIII, Y14:Magoh heterodimer, and MLN51. This stable core travels with mRNA to the cytoplasm, and serves as a platform for recruitment of more dynamic peripheral/adapter proteins that direct mRNA export, localization, translation and nonsense-mediated mRNA degradation (NMD). To date, it is unknown if peripheral proteins interact with all EJC cores. In EJCs purified from cultured human cells, we found several peripheral proteins levels to be sub-stoichiometric to those of the core suggesting that they are part of only a subset of EJCs. Surprisingly, we discovered that the so-called core protein MLN51 is also sub-stoichiometric to the other three core proteins. Thus, it may act more like an adapter protein rather than as an integral core component. Spurred by these findings, we have now discovered at least two stable alternate EJCs that are distinguished by the presence of either MLN51 or RNPS1. The presence of these mutually exclusive complexes suggest sorting of peripheral proteins into distinct EJCs and raises the possibility of parallel post-transcriptional gene regulatory branches rooted in alternate EJCs. In support of such a possibility, we find that translation-enhancing adapter protein SKAR primarily associates with RNPS1-containing EJCs. Furthermore, alternate EJCs also differentially associate with NMD proteins Upf2 and Upf3b, and could lie at the root of previously documented Upf2- or Upf3-independent alternate NMD branches. We have now initiated efforts to identify RNAs and proteins specific to alternate EJCs to uncover the nature of post-transcriptional regulatory networks regulated by them, and reveal the underlying mechanisms.

Keywords: Exon Junction Complex, post-transcriptional gene regulation

30) Investigating the molecular basis for substrate recognition by Thg1

Ashanti Matlock (Department of Chemistry and Biochemistry; Ohio State University), **Jane E. Jackman** (Department of Chemistry and Biochemistry; Ohio State University)

Abstract:
Eukaryotic members of the tRNAHis guanylyltransferase (Thg1) enzyme family catalyze non-templated 3'-5' addition of a single guanine (G-1) to the 5' end of tRNAHis. The addition of G-1 is necessary for the recognition of tRNAHis by its cognate aminoacyl-tRNA synthetase (aaRS) and like aaRS, Thg1 uses the histidine anticodon, GUG, to recognize tRNAHis. The strict tRNAHis substrate specificity exhibited by eukaryotic Thg1 enzymes is largely absent in Thg1-like proteins (TLPs) from Bacteria and Archaea, where TLPs participate in repair of multiple 5' truncated tRNA substrates. A recent crystal structure of Candida albicans Thg1 (CaThg1) bound to an engineered tRNAHis substrate identified conserved residues involved in anticodon recognition and highlights several contacts with the 5'-end of the acceptor stem, the site of catalysis. Previous mutational analysis; however, suggests that there are other interactions between tRNAHis and Thg1 that are important for substrate specificity. For example, mutating the conserved Thg1 residue D68 to alanine results in loss of selective G-1 addition activity with tRNAHis by catalyzing G-1 addition to non-substrate tRNAs, tRNAPhe and tRNALeu. Interestingly, D68 is located near the TΨC loop, but due to poor resolution, visualization of the exact interaction between D68 and tRNAHis is not clear. Determining interactions that confer substrate specificity upon Thg1 could provide insight into the hypothesized evolution of the essential Thg1 enzyme from an ancestral non-tRNA selective 3'-5' polymerase to a specialized enzyme whose nucleotide addition is highly specific for a single tRNA. To determine how Thg1 differentiates substrate and non-substrate, we are using chemical probing to compare the footprints of Thg1 bound to substrate (tRNAHis) and non- substrate (tRNAPhe). This approach will help distinguish interactions required for substrate specificity from general contacts used for binding tRNA.

Keywords: tRNA modification, RNA-Protein Interactions

31) Using resonance raman spectroscopy to elucidate metal incorporation of R2lox, a novel heterobimetallic oxidase

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Abstract:
One of the most common bioinorganic motifs present in nature is the non-heme, bimetallic cofactors coordinated within an α-helical protein. These proteins carry out interesting and diverse chemistry, from iron storage to 1- and 2-electron redox reactions. However, until recently, it was thought that these proteins contained only Fe/Fe bimetallic sites; it wasn't until recently that both a functional Mn/Mn and heterobimetallic Mn/Fe protein was identified. Within the Mn/Fe group, R2-like ligand-binding oxidase (R2lox) represents a new class of protein that utilizes the Mn/Fe moiety to do catalysis. R2lox has been identified in *Mycobacterium tuberculosis* as well as several other pathogenic bacteria, and is particularly noteworthy due to its upregulation in the virulent strain of *M. tuberculosis*. The above considerations make R2lox an attractive target for tuberculosis therapeutics. However, very little is known about R2lox. The heterobimetallic cofactor spontaneously assembles *in vitro* in the presence of air, running counter to the well-established Irving-Williams series for metal-binding affinities; furthermore, while the protein is isolated with a fatty acid bound to the active site, the specific function of R2lox remains unknown. One outstanding question we seek to address is the mechanism of metal incorporation. To do this, we are making use of a state-of-the-art resonance Raman (rR) spectroscopy setup combined with isotopic substitution and multi-wavelength studies to probe the vibrational and electronic structure of the R2lox active site.

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Andersson, C. and Högbom. A Mycobacterium tuberculosis ligand-binding protein reveals a new cofactor in a remodeled R2-protein scaffold. *PNAS* **2009**, *106*, 5633-5638.

Griese, J. G. et al. Direct observation of structurally encoded metal discrimination and ether bond formation in a heterodinuclear metalloprotein. *PNAS* **2013**, *110*, 17189-17194.

Keywords: R2lox, metal incorporation, resonance Raman spectroscopy

32) ARGONAUTE 6 bridges the RNAi of transposable element mRNAs to the establishment of DNA methylation

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Abstract:
Because transposable elements (TEs) can generate mutations, mechanisms exist in eukaryotes to both degrade transcriptionally active TE mRNAs into small interfering RNAs (siRNAs) via RNAi and to repress TE transcription through RNA-directed DNA methylation (RdDM) and chromatin modification. However, the mechanism through which transcriptionally active TEs transition from post-transcriptional to epigenetic silencing has remained unclear. Through a combination of genetics, small RNA deep sequencing, and bisulfite sequencing, we discovered a pathway in Arabidopsis that functions to direct DNA methylation to transcriptionally active TEs, which is partially responsible for the initiation and corrective reestablishment of TE silencing. We genetically identified the Argonaute 6 (AGO6) protein as the key AGO effector protein in this expression-dependent TE DNA methylation pathway. Then with particular focus on AGO6, I identified the molecular mechanism of this pathway. Utilizing AGO6 immunoprecipitations and subsequent small RNA deep sequencing, I demonstrate that 21-22nt siRNAs from the RNAi of TE mRNAs are directly incorporated into the AGO6 protein. Additionally, I found that the 21-22nt siRNAs that derive from TE mRNAs then direct AGO6 to TE chromatin in a PolV-dependent manner to guide its function in RdDM, as determined by AGO6 chromatin immunoprecipitation experiments in several genetic backgrounds. Further analysis of AGO6-bound siRNAs indicated that long centromeric high-copy TEs are the main targets for this expression-dependent DNA methylation pathway. Lastly, bisulfite sequencing and expression analysis in various tissues revealed that this pathway is active only in reproductive precursor cells, likely to target active TEs for RdDM prior to gametogenesis. Overall, these data provides a direct mechanism for a pathway that bridges the gap between the TE post-transcriptional regulation by RNAi and the establishment of chromatin modification.

Keywords: small RNA, epigenetics, transposable elements

33) Recognition of SAM by SMK box (SAM-III) in the ligand-free state

Orlando McEwan (Molecular, Cellular and Developmental Biology MCDB)

Abstract:
N/A

References:
N/A

Keywords: NA

34) PheRS editing activity limits conditional cytotoxic mistranslation of the genetic code.

Adil Moghal (OSBP), **Tammy Bullwinkle, Noah Reynolds, Medha Raina, Eleftheria Matsa** (Department of Microbiology, The Ohio State University), **Andrei Rajkovic** (MCDB), **Huseyin Kayadibi, Farbod Fazlollahi, Christopher Ryan, Nathaniel Howitz** (University of California, Los Angeles, CA), **Kym Faull, Beth Lazazzera** (University of California, Los Angeles, CA), **Michael Ibba** (Department of Microbiology, The Ohio State University)

Abstract:
Translational adherence to the standard genetic code requires accurate pairing of amino acids and tRNAs by aminoacyl-tRNA synthetases. Recent observations of tolerated and beneficial deviations from classical decoding support an emerging view of translational fidelity as variable in both environmental and evolutionary contexts. Bacterial phenylalanyl-tRNA synthetase (PheRS) bears post-transfer editing activity, which hydrolyzes mispaired aminoacyl-tRNAPhe species. This editing activity is dispensable under typical growth conditions, but serves to protect the cell from mistranslation of the genetic code under oxidative stress. This is due to the accumulation of meta-Tyrosine (m-Tyr), a product of spontaneous phenylalanine (Phe) oxidation. m-Tyr is efficiently utilized as a substrate by PheRS, and presents a challenge to typical growth in an oxidatively stressed PheRS post-transfer editing Escherichia coli mutant. m-Tyr and other hydroxylated phenylalanine derivatives are a similar challenge in a Saccharomyces cerevisiae (S. cerevisiae) cytoplasmic PheRS editing mutant. Taken together, these observations suggest that PheRS editing activity has been evolutionarily conserved to protect the cell from cytotoxic stress-dependent mistranslation of Phe codons. Analysis of the effects of oxidative stress in S. cerevisiae reveals evolutionary similarities and unexpected differences in stress-dependent deviations from translational fidelity, as well as the role of quality control mechanisms in proteome stability and cell survival.

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T. Bullwinkle, et al. Oxidation of cellular amino acid pools leads to cytotoxic mistranslation of the genetic code. eLife (2014), p. e02501

Keywords: aminoacyl-tRNA synthesis, cellular stress, mistranslation of the genetic code

35) Capping enzyme is a nucleocytoplasmic shuttling protein whose cytoplasmic pool is maintained by Nck1 binding

Chandrama Mukherjee (Center of RNA Biology and Department of Molecular and Cellular Biochemistry), **Daniel R Schoenberg** (Center of RNA Biology and Department of Molecular and Cellular Biochemistry)

Abstract:
Cytoplasmic capping is catalyzed by a complex that contains capping enzyme (CE) and a kinase that converts RNA with a 5'-monophosphate end to a 5' diphosphate for subsequent addition of GMP (1). Prior to our identification of the cytoplasmic capping complex CE was thought to be restricted to the nucleus. We therefore sought to determine the source of the cytoplasmic pool of protein. This was accomplished by fusing human U2OS cells expressing epitope-tagged forms of CE with mouse 3T3 cells, and visualizing the nuclear distribution of CE in the resulting heterokaryons. The appearance of CE in 3T3 cell nuclei identified it as a nucleocytoplasmic shuttling protein. Treatment of U2OS cells prior to fusion with Leptomycin B had no impact on the appearance of CE in 3T3 cell nuclei, thus indicating CE nuclear export is independent of Crm1. We showed previously that the cytoplasmic capping complex assembles on Nck1, a cytoplasmic SH2/SH3 adapter protein (2). Loss of the cytoplasmic pool of CE following Nck1 knockdown identified a second role for this protein in maintaining the cytoplasmic pool of CE. This finding also affirms the importance for cap homeostasis of CE binding to Nck1.
Supported by NIH grant GM084177 to DRS and American Heart Association postdoctoral award 13POST17270021 to CM.

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Keywords: Capping Enzyme, Nucleocytoplasmic shuttling, Heterokaryon assay

36) Base pair recognition by the Thg1 family 3'-5' polymerases

Krishna Patel (Chemistry&Biochemistry,The Ohio State University), **Paul Yourik** (Chemistry & Biochemistry,The Ohio State University)

Abstract:
The tRNAHis guanylyltransferase (Thg1) was originally discovered in Saccharomyces cerevisiae where it catalyzes 3'-5' addition of a single non-templated guanosine (G-1) to the 5' end of tRNAHis. Interestingly, in addition to this activity, S. cerevisiae Thg1 (SceThg1) also catalyzes 3'-5' polymerization of Watson-Crick (WC) base pairs (bp), utilizing nucleotides in the 3'-end of a tRNA as the template for addition. Subsequent investigation revealed an entire class of enzymes related to Thg1 (Thg1-like proteins, or TLPs). TLPs are found in all three domains of life and exclusively catalyze 3'-5' polymerase activity, utilizing this unusual activity to repair tRNA during 5'-tRNA editing, among other functions. Although both Thg1 and TLPs utilize the same basic mechanism involving two metal ion catalysis, the molecular basis for differences between WC bp-dependent (catalyzed by both Thg1 and TLPs) and non-WC bp-dependent (catalyzed exclusively by Thg1) reactions remains unknown. Here we describe an investigation into the mechanism of base pair recognition by 3'-5' polymerases, and the first identification of protein residues that play a role in the discrimination between WC and non-WC base pairs. First, transient kinetic assays were used to measure the maximal rate and NTP affinity (kntrans and KD, respectively) for each possible WC and non-WC nucleotide incorporation during 3'-5' addition catalyzed by SceThg1. These assays revealed that the ability to add a non-WC base pair, the hallmark of eukaryotic Thg1-type activity, is driven by a unique kinetic preference for GTP as the nucleotide donor for 3'-5' addition. These results open the door to identification of specific residues involved in non-WC GTP recognition, and next we sought to identify these through a comparison of conserved residues that differ between Thg1 and TLPs. Site-directed mutagenesis was used to alter several candidate residues and perform kinetic analysis of WC vs. non-WC reactions catalyzed by the resulting variants. Through these studies we provide new insights into the mechanism of catalysis utilized by these unique reverse polymerases

Keywords: Thg1, tRNAHis

37) Structural identification by Mass Spectrometry of Cyclic Rhamnosylated EF-P

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Abstract:
Elongation factor P (EF-P) is a ubiquitous bacterial protein that is required for the efficient synthesis of poly-proline motifs during translation. In Escherichia coli and Salmonella enterica the post-translational β-lysylation of Lys34 by the PoxA protein is critical for EF-P activity. PoxA is absent from many bacteria such as Pseudomonas aeruginosa, prompting a search for alternative EF-P post-translation modification pathways. ETD-HCD MS3 analyses of P. aeruginosa EF-P revealed the attachment of a single cyclic rhamnose moiety to an Arg residue at the equivalent position where β-Lys is attached to E. coli EF-P. Analysis of the genomes of organisms that both lack poxA and encode an Arg34 containing EF-P (efpR) revealed a highly conserved glycosyltransferase (EarP) encoded adjacent to efpR. EF-P proteins isolated from P. aeruginosa ΔearP, or from a ΔrmlC::aac1 strain deficient in dTDP-L-rhamnose biosynthesis, were unmodified. In vitro assays confirmed the ability of EarP to use dTDP-L-rhamnose as a substrate for the post-translational glycosylation of EF-P. The role of rhamnosylated EF-P in translational control was investigated in P. aeruginosa using a (Pro)4-GFP in vivo reporter assay, florescence of which was significantly reduced in Δefp, ΔearP, and ΔrmlC::acc1 strains. ΔrmlC::acc1, ΔearP and Δefp strains also displayed significant increases in their sensitivities to a range of antibiotics including ertapenem, Polymyxin B, cefotaxim, and piperacillin, consistent with a role for modified EF-P in virulence. Taken together our findings indicate that post-translational rhamnosylation of EF-P plays a key role in P. aeruginosa gene expression and virulence.

Keywords: Elongation factor P, Glycosylation, tRNA-mimic

38) Role of LptFG in LPS transport across the cell envelope of Escherichia coli.

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Abstract:
Gram-negative bacteria contain a cell envelope characterized by an asymmetric outer membrane with lipopolysaccharide (LPS) in the outer leaflet. The selective permeability of the LPS layer provides an intrinsic resistance to detergents and hydrophobic compounds including many antibiotics. In Escherichia coli, assembly of this asymmetric layer requires a seven-protein Lpt complex (LPS transport). The Lpt complex spans every compartment of the cell forming a bridge between the inner and outer membranes. Energy for LPS transport comes from ATP hydrolysis by an LptB2 dimer. LptB2 further forms an ABC (ATP-binding cassette) transporter with inner membrane components LptFG. LptFG are predicted to have a similar architecture of six transmembrane domains and a large periplasmic domain. We are interested in understanding the role of LptFG and how they interact within the Lpt complex. Targeted mutagenesis of the periplasmic domains of LptFG suggests they have both structural and functional roles. In addition, within their cytoplasmic domains we have identified putative coupling helices with a shared critical Glu. Site-directed cross-linking demonstrates interaction of the putative coupling helix of LptF with LptB. These findings provide new insight into how energy harnessed from ATP hydrolysis by LptB is transferred to the membrane components LptFG to power LPS transport.

Keywords: lipopolysaccharide, permeability barrier, membrane biogenesis

39) Structural characterization of a RNA motif required for cell-to-cell trafficking

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Abstract:
Double-stranded RNA (dsRNA) mediates many critical processes within the cell. Among a variety of functions, non-canonical base pairing in these nucleic acids, such as the A+•C wobble base pair, have been suggested to be structural indicators for regulatory and metabolic molecules to act upon. We aim to characterize a potential A+•C wobble base pair present in the Potato Spindle Tuber Viroid (PSTVd)—Loop 19. Viroids are infectious, single-stranded, noncoding RNA molecules that are covalently circularized and display structural homology to dsRNA. PSTVd is a 359 nucleotide RNA that causes a disease state in food crops through RNA-mediated gene regulation. The secondary structure of PSTVd has been characterized by the functional significance of 27 looped regions. Through mutagenesis, Loop 19 was shown to be significant for viroid trafficking between cell types through an unknown mechanism. Loop 19 entails a single nucleotide mismatch pairing of A with C flanked by stable G-C base pairs. Recent studies on model RNAs suggest that adenosines in this context experience a substantial shift in pKa, resulting in protonation at physiological pH, thus enabling hydrogen bonding to cytidine. We utilize UV and NMR spectroscopy to monitor the ionization of the adenosine residue during acid titration. Preliminary UV results support protonation of this residue at physiological pH while NMR experiments do not indicate stable base pairing at this motif. We continue to explore the higher order structure and characteristics of Loop 19, thus contributing to our understanding of the function of Loop 19 and the mechanisms by which A-C mismatch base pairings are recognized by cellular factors in dsRNAs.

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Keywords: noncoding RNA, NMR

40) Potential Role for RNA Polymerase II in Repressive Histone Methylation

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Abstract:
Epigenetic modifications are influential in organizing and controlling gene expression. One such example of epigenetic control is histone and cytosine methylation that leads to transcriptional gene silencing of potentially harmful DNA, such as transposable elements and viruses. Geminiviruses are a family of plant viruses that are small, circular, and single-stranded DNA, and form double-stranded DNA intermediates that associates with histones to form minichromosomes. As such, these viruses are subject to, and repressed by epigenetic modifications leading to transcriptional gene silencing. Arabidopsis encodes two plant-specific polymerases RNA polymerase IV (Pol IV) and RNA polymerase V (Pol V), currently modeled to establish repressive cytosine and histone methylation leading to transcriptional gene silencing. Using geminiviruses as a *de novo* model to more precisely define the role of Pol IV and Pol V in initiating chromatin methylation, it was determined that these polymerases were required for the establishment of repressive histone modification dimethylated histone 3 lysine 9 (H3K9me2), but were not required for initiating cytosine methylation. This suggests that another polymerase is required for the establishment of cytosine methylation. These two polymerases are multi-subunit enzymes shown to be related to, and share subunits with, RNA polymerase II (Pol II), an enzyme that orchestrates the formation of heterochromatin and transcriptional gene silencing in S. pombe. Plants deficient for Pol II showed susceptibility to geminivirus infection, as well as a requirement of Pol II in the establishment of H3K9me2 on the viral genome. This leads to the hypothesis that Pol II is required to recruit Pol IV and V to geminivirus genomes to established H3K9me2, and also to establish repressive cytosine methylation.

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Zheng, B. et al. (2009) Intergenic transcription by RNA polymerase II coordinates Pol IV and Pol V in siRNA-directed transcriptional gene silencing in Arabidopsis. Genes Dev. 23(4):2850-2860

Keywords: RNA polymerase II, RNA-directed DNA methylation, geminivirus

41) Uncovering the shared and distinct roles for the transcription factor gene duplicates hmg-3 and hmg-4 during C. elegans development

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Abstract:
In the nematode *Caenorhabditis elegans*, several key developmental decisions are governed by transcription factor (TF) pairs resultant from a simple duplication event: GATA TFs *end-1* and *end-3* specify the intestine; T-box genes *tbx-37* and *tbx-38* promote formation of the ABa-derived anterior pharynx, and *tbx-8 and tbx-9* generate C-lineage muscle and hypodermis. For each of these pairs, double mutants are embryonic lethal, whereas single mutants are not. We hypothesize that duplication of embryonic development genes is important in the evolution of C. elegans. We screened additional TF pairs to uncover other pairs exhibiting the same behavior. Of 13 tested pairs, only pair *hmg-3* and *hmg-4* showed embryonic lethality when both were depleted using RNAi. In addition, *hmg-3*(RNAi) confers sterility; *hmg-4*(RNAi) arrest during early larval development, indicating this pair has both unique and shared developmental functions. To determine the specific functions of these genes, we used markers for various developmental milestones to assist in determining any defects. In *hmg-3*(RNAi); *hmg-4*(RNAi) embryos, while enclosure occurs properly and the intestine and posterior portion of the pharynx develop normally, the anterior portion of the pharynx appears to be absent, suggesting that *hmg-3* and *hmg-4* are acting in/on the ABa lineage to promote the formation of that structure. *hmg-3* and *hmg-4* encode SSRP1-related proteins that are part of the histone chaperone FACT complex. Our findings suggest that there are specific developmental genes that are especially dependent on this complex in C. elegans.

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Keywords: C elegans, Gene duplication, Embryogenesis

42) Structural and Functional Characterization of Yeast Argonaute Proteins Reveals a Unique RNA-binding Activity of the Extended N-terminal Domain

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Abstract:
MicroRNAs (miRNAs) are non-coding RNAs capable of regulating gene expression in eukaryotic system. Many miRNAs have been discovered in extracellular regions of our bodies, though their functions are not very clear yet. Recently it has been revealed that microorganisms infecting humans secrete their miRNAs in the cardiovascular system as a ribonucleoprotein complex with Argonaute proteins (AGOs) to cause severe diseases. Given that human body is naturally inhabited by a large number of microorganisms including some budding yeasts, we believe that those inhabitants also secrete their miRNAs, likely affecting our health condition. We studied several budding yeast AGOs and discovered that some of them possess another RNA binding domain in their extended N-terminal domain (exN), in addition to well characterized MID and PAZ domains responsible for the guide-RNA binding at the 5' and 3' termini, respectively. Our crystal structure of the full-length budding yeast AGO shows the exN running along the exterior of the N-PAZ lobe towards the entrance of the nucleic acid-binding channel where the seed nucleotides of guide are exposed to the solvent for scanning of the target mRNAs. Such N-terminal extended AGOs are also found in worm, plant, fly etc., albeit with less identity in their sequences. In contrast, human AGOs lack an exN and locate their N-terminus at the distal end of the channel. These observations shed the light on the versatile functions of budding yeast AGOs with their exN in miRNA biogenesis or unidentified different biological processes beyond RNA interference.

Keywords: RNA interference, Argonaute proteins, budding yeast

43) Tandem repeats are implicated in both the establishment and maintenance of paramutation at *Pl1-Rhoades*

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Abstract:
Paramutations represent meiotically heritable epigenetic changes facilitated by trans-homolog interactions (THI). The maize *purple plant1* locus (*pl1*) regulates anthocyanin production, and one of its alleles, *Pl1-Rhoades*, is a model for understanding the paramutation mechanism. Paramutant *Pl1-Rhoades* states (*Pl'*) are stable, but can revert to a strongly expressed form (*Pl-Rh*) in the absence of required to maintain repression (RMR) proteins responsible for 24 nucleotide (nt) RNA biogenesis. THIs also stabilize *Pl'* states since hemizygous conditions facilitate reversion to *Pl-Rh*. Other *pl1* alleles are classified by whether they stabilize or destabilize *Pl'* in heterozygous conditions, and these differences are predicted to reflect underlying structural diversity. We used Pacific Biosciences long read sequences to assemble a 200 kb contig representing the *Pl1-Rhoades* haplotype to identify important structural features related to paramutation behaviors. A series of five 2,092 bp tandem repeats (TR) were found coincident with the genetic placement of an essential paramutation element and transcriptional enhancer ~14 kb downstream of the *Pl1-Rhoades* coding region. Another TR previously found ~100 kb upstream of the *B1-Intense* coding region has similar genetic properties, although paramutant *B1-Intense* states do not revert. The *B1-Intense* TR consists of 7 repeats of unique sequence, while the *Pl1-Rhoades* TR includes both LTR retrotransposon, DNA transposon and helitron fragments in addition to a unique 390 bp region. Sequence alignments with *pl1-B73* (stabilizing) and *pl1-Mo17* (destabilizing) implicate the TR unique sequence in maintaining *Pl'* states *in trans*. The presence of 24 nt RNAs at this TR unique region indicate that RMR-based machinery - including RNA polymerase IV - operate on this feature. Together, these new data focus molecular attention to a small region potentially modulated by the THIs responsible for paramutation behaviors at *Pl1-Rhoades*.

Keywords: paramutation, RNA polymerases, epigenetics

44) Rapid clearance of oscillating transcripts during somitogenesis requires the decay adapter Pnrc2

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Abstract:
Vertebrate segmentation is controlled by the segmentation clock, a biological oscillator that controls periodic formation of embryonic segments, or somites. This molecular oscillator generates cyclic gene expression in the presomitic mesoderm (PSM) and has the same periodicity as that of somite formation. Core cyclic components of the segmentation clock include the *hes/her* family of transcriptional repressors, but additional transcripts also cycle. Maintenance of the oscillation period requires that transcriptional activation and repression, RNA turnover, translation, and protein degradation are all very rapid as somite pairs quickly develop in all vertebrates, just 30 minutes in the zebrafish model we study. Our lab isolated a zebrafish segmentation clock mutant, *tortuga*^{b644}; mutant embryos express elevated levels of cyclic mRNAs such as *her1* and *rhov*. We have demonstrated that loss of Proline-rich nuclear receptor coactivator protein Pnrc2 is responsible for cyclic transcript accumulation in *tortuga*^{b644} mutants. In human cell culture systems, Pnrc2 has been implicated in mRNA decay through interactions with Dcp1a and Upf1. Data from our lab supports a similar mRNA decay function in zebrafish since partial depletion of both Pnrc2 and Upf1 increases *her1* and *rhov* accumulation.

We hypothesize that in *pnrc2* mutants, oscillating transcripts accumulate due to inefficient removal of the 5' cap, thus causing mRNA perdurance. In order to test this hypothesis, we are examining if Pnrc2 regulates the localization, decapping, or deadenylation of *her1* and *rhov* transcripts and if these accumulating transcripts are actively being translated. While the mechanisms of aberrant mRNA decay are well studied, less is understood about pathways regulating decay of non-aberrant transcripts. Our goal is to define mechanisms critical for the rapid turnover of oscillating genes during somitogenesis.

Keywords: Pnrc2 , her1 , somitogenesis

45) Studies of proteins required for proper function and localization of tRNA splicing endonuclease in Saccharomyces cerevisiae

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Abstract:
tRNAs function to bring amino acids to ribosomes during protein synthesis. In yeast, splicing of pre-tRNAs is essential for the production of 10 families of tRNAs. Intron removal is catalyzed by the heterotetrameric tRNA splicing endonuclease (SEN) complex, which is located on the cytoplasmic surface of mitochondria. However, how and why SEN subunits (Sen2, Sen15, Sen34, and Sen54) assemble on the surface of mitochondria is unknown. Recently our lab completed a genome-wide screen to search for all yeast gene products involved in tRNA biology. We identified two interesting mitochondrial outer membrane proteins Tom70 and Sam37. Deletion of TOM70 or SAM37 causes pre-tRNA splicing defects and the accumulation of end-matured, intron-containing tRNAs. We first hypothesized that TOM70 or SAM37 mutations may cause impaired oxidative phosphorylation by mitochondria which results in pre-tRNA splicing defect. To test this idea, we isolated petites that are defective in respiratory metabolism and showed that they spliced pre-tRNAs as well as wild-type cells, showing that loss of oxidative phosphorylation has no affect upon SEN activity. As Tom70 and Sam37 function in targeting proteins to the mitochondrial outer membrane and initial import steps of proteins into mitochondria, we then hypothesized that deletion of TOM70 or SAM37 causes mislocalization of SEN subunits, preventing formation of the heterotetramer on the mitochondrial surface. To test this, each SEN subunit was tagged with GFP at its endogenous locus in wild-type, tom70Δ and sam37Δ strains. Employing live cell confocal microscopy, we learned that the distribution of SEN subunits on mitochondria was reduced and the cytoplasmic pools were increased in the mutant cells compared with wild-type cells. We will conduct cell fractionation to determine the ratio of SEN subunits localizing between mitochondria surface and cytoplasm. Thus far our data indicate that via direct or indirect interactions, Tom70 and Sam37 are required for the proper localization, assembly, and function of the SEN subunits on mitochondria and that appropriate assembly of the SEN complex proteins on mitochondria is necessary for efficient pre-tRNA splicing.

Keywords: intron-containing tRNA, tRNA splicing endonuclease, mitochondrial outer membrane protein

46) The effect of phosphorylation of mouse SAMHD1 on restriction of HIV-1 and murine leukemia virus infection

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Abstract:
Human SAMHD1 (hSAMHD1) functions as a retroviral restriction factor in non-dividing cells mainly by limiting intracellular dNTP levels to complete viral reverse transcription. Phosphorylation of hSAMHD1 at T592 by cyclin dependent kinase 1 (CDK1) and CDK2 impairs its restriction of HIV-1 infection. As mouse SAMHD1 (mSAMHD1) and hSAMHD1 are highly conserved proteins, it is conceivable that similar mechanisms may regulate their anti-retroviral functions in cells. Our previous work identified that both hSAMHD1 and mSAMHD1 interact with CDK1/2 and cyclin A2. However, it remains unknown whether phosphorylation of mSAMHD1 regulates its restriction of retroviral infection. In this study, we identified phosphorylation of residue T634 in mSAMHD1 by mass spectrometry, and confirmed the phosphorylation in dividing cells using a phospho-specific SAMHD1 antibody. Using dominant-negative mutants, siRNA-mediated knockdowns, or specific inhibitors of CDK1 and CDK2 to treat mSAMHD1-expressing cells, we found decreased levels of T634 phosphorylated mSAMHD1 in cells. Furthermore, we examined the effect of T634 phosphorylation on mSAMHD1-mediated restriction of HIV-1 in differentiated human monocytic U937 cells and murine leukemia virus (MLV) in dividing mouse fibroblast NIH3T3 cells. These cell lines were transduced with lenti-viral vectors to stably express similar levels of mSAMHD1 wild-type (WT), phospho-ablative (T634A) or phospho-mimetic (T634D) mutants. We are confirming the effect of mSAMHD1 T634 phosphorylation on HIV-1 restriction. Interestingly, we found that MLV infection was reduced in NIH3T3 cells expressing mSAMHD1 WT or the mutants (T634A/D) compared to control cells. Our results indicate that phosphorylation of mSAMHD1 at T634 is regulated by CDK1 and CDK2 in cells. MLV infection is restricted by mSAMHD1 in dividing NIH3T3 cells independent of T634 phosphorylation of mSAMHD1, suggesting a novel mechanism of mSAMHD1-mediated MLV restriction.

Keywords: SAMHD1, HIV-1, MLV

47) EGL-38/PAX functions in communicating cells of the Caenorhabditis elegans egg-laying system to coordinate signaling and differentiation

Allison Webb (OSBP), **Ryan Johnson** (OSU Molecular Genetics), **Helen Chamberlin** (OSU Molecular Genetics)

Abstract:
Paired-box (Pax) transcription factors are essential regulators of coordinated development responsible for controlling the growth and differentiation of diverse cells into organ systems. Pax transcription factors rely on cellular context to initiate their cell-specific activity through poorly understood mechanisms. To investigate PAX function, we are utilizing the PAX2/5/8 *Caenorhabditis elegans* ortholog EGL-38 as a simplified model system. EGL-38 functions in the hermaphrodite egg-laying system to coordinate anchoring of the vulva to the uterus. To initiate creation of this vulval-uterine connection, a LIN-3/Epidermal Growth Factor (EGF) signal is sent from the vulval vulF cells to the LET-23/EGFR receptor on a subset of neighboring uterine cells. These cells are specified as uv1 and are characterized by non-migration and by expression of the neuropeptide *nlp-2*. EGL-38 is required for *lin-3/egf* expression in the vulF cells[1,2], thereby initiating this signaling pathway. EGL-38 may function in response to the LIN-3/EGF pathway in the uv1 cells, as we have confirmed that EGL-38 can bind the *nlp-2* promoter *in vitro*, and that both uv1 non-migration and *nlp-2* expression are significantly decreased in *egl-38* mutants as well as in *lin-1* mutants. LIN-1 is an ETS-family transcription factor; ETS proteins often serve as PAX co-factors to confer DNA binding specificity[3]. To identify if an interaction with LIN-1 is the source of EGL-38 cell-specificity, we are investigating LIN-1 and its role in the creation of the vulval-uterine connection. We have discovered that a loss-of-function mutation in *lin-1* significantly decreases *lin-3* expression in the vulF cells, and further experiments are being conducted to examine the *in vivo* interaction of EGL-38 and LIN-1. Additionally, we are investigating the activation and role of EGL-38 in the uv1 cells to determine how PAX proteins may function discriminately in communicating cells.

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Keywords: coordinated development, signaling, cell-specificity

48) Tuning RNA Structure and Function Through Bifacial Nucleic Acid (bPNA)

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Abstract:
We have previously reported that synthetic melamine-displaying α-peptides, termed bifacial peptide nucleic acid (bPNA), can simultaneously dock two T/U strands to form a triplex hybrid stem via a designed molecular recognition interface. When T/U-rich domains are separated by an intervening oligonucleotide sequence of 4–10 nt, a hairpin triplex stem-loop structure is formed with high affinity (Kd ~2 nm) [1]. We have demonstrated that these high melting temperature complexes (Tm = 57 8C) are competitive with protein enzymes whose native substrates are DNA or RNA, such as transcriptase, exonuclease and reverse transcriptase [2]. Here, we demonstrate, other than the inhibitory effects, that the bPNA triplex hybrid functionally substitutes for native duplex structures in three distinct nucleic acid folds. In all three nucleic acid systems, complete structure-function loss was inflicted by replacement of an essential duplex structure with an unstructured T/U loop, and functional rescue was observed on refolding of the T/U loop into a bPNA triplex hybrid stem. Indeed, bPNA could be used as a turn-on switch for protein and small molecule binding/luminescence in an (IgE) binding DNA aptamer and the RNA aptamer Spinach, respectively. Furthermore, native-like catalytic splicing rates could be induced by bPNA triplex stem refolding of crippled type I hammerhead ribozyme folds. These studies indicate bPNA may have general utility as an allosteric trigger for a wide range of function in non-coding nucleic acids.

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Keywords: Bifacial Nucleic Acid, Ribozyme, Aptamer

49) Analysis of allostery within the 30S ribosomal subunit

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Abstract:
During decoding, ribosome selects the correct (cognate) aminoacyl-tRNA (aa-tRNA) from a large pool of incorrect aa-tRNAs. In the initial selection stage, interaction between codon and anticodon in the 30S A site leads to activation of GTPase domain of elongation factor EF-Tu and GTP hydrolysis, which allows release of aa-tRNA from EF-Tu. The mechanism by which codon recognition stimulates GTPase activation of EF-Tu remains unclear. In previous work, a number of ribosomal ambiguity (*ram*) mutation in 16S rRNA were isolated, largest subset mapping to helices h8 and h14. These helices interact with each other and with the 50S subunit to form bridge B8. Biochemical and structural studies have shown that disruption of bridge B8 is an important aspect of GTPase activation, and mutation G299A in h12 allosterically destabilizes B8 from 80 Å away.
Here, we investigate the functional dependence of various *ram* mutations on B8, using genetic epistasis. We combined mutation h8Δ2bp (truncation of h8, disrupting B8) with each *ram* mutation and measured miscoding rates of the double-mutant ribosomes *in vivo*. The miscoding rates of most double-mutant ribosomes are substantially higher than the corresponding single-mutant ribosomes. These data suggest that *ram* mutations located in h12, h16, h21, h27, h34 and h44 act, at least in part, independently of B8.
To further explore allostery within the 30S subunit, we tested the impact of two B8 destabilizing mutations, G347U (at B8) and G299A (in h12), on A-tRNA binding. The results show that mutations G347U and G299A stabilize tRNA in the 30S A site. These findings suggest conformational coupling not only between B8 and h12, but also between B8 and the 30S A site and between h12 and the 30S A site. Taken together, these data shed light on the role of the 16S rRNA in the decoding process.

Keywords: decoding, allostery, ribosome

50) Discrete polyacrylate hybridization with DNA and RNA

Zhun Zhou (Department of Chemistry and Biochemistry), **Xin Xia** (Department of Chemistry and Biochemistry), **Dennis Bong** (Department of Chemistry and Biochemistry)

Abstract:
We report herein discrete triplex hybridization of DNA and RNA with polyacrylates. Length-monodisperse triazinederivatized polymers are prepared on gramscale by reversible addition fragmentation transfer (RAFT) polymerization. Despite stereo-regio backbone heterogeneity, the triazine polymers bind T/U rich DNA or RNA on mixing, in a 1:1 ratio with nanomolar affinity, as judged by thermal melts, CD, gel shift assays and fluorescence quenching. We call these polyacrylates “bifacial polymer nucleic acid” (bP_oNA). Hybridization with amphiphilic diblock bifacial polymer nucleic acids enables formation of DNA loaded bP_oNA nanoparticles that are readily internalized by cells in culture. Complexation of siRNA with sterol-modified bP_oNA further enables RNA delivery and silencing of luciferase expression in HeLa cells. It is anticipated that bP_oNAs will have utility in both bio and nanotechnology.

Keywords: siRNA delivery, polymer nucleic acid

51) Tobramycin derivatives with enhanced ribosome-targeting activity

Hongkun Zhu (Department of Microbiology, The Ohio State University), **Marina Fosso** (Department of Pharmaceutical Sciences, University of Kentucky), **Keith D. Green** (Department of Pharmaceutical Sciences, University of Kentucky), **Sylvie Garneau-Tsodikovab** (Department of Pharmaceutical Sciences, University of Kentucky), **Kurt Fredrick** (Department of Microbiology, The Ohio State University)

Abstract:
With the increased evolution of aminoglycoside-resistant bacterial strains, the need to develop aminoglycosides with (i) enhanced antimicrobial activity, (ii) the ability to evade resistance mechanisms, and (iii) the capability of targeting the ribosome with higher efficiency, is more and more pressing. The chemical derivatization of the naturally occurring tobramycin (TOB) by attachment of 37 different thioethers groups at the 6

Keywords: aminoglycosides, ribosome