Oral Presentations

Identification of Novel Factors Required for the Initiation of Epigenetic Silencing

Sarah G. Choudury (MCDB), Andrea D. McCue (MG), Kaushik Panda (MG), Alissa Cullen (MG)

Abstract:
In order to maintain genome integrity, fungi, plants, and animals modify transposable element (TE) chromatin to epigenetically repress TE activity. DNA methylation is critical for this epigenetic repression. Once established at TEs, DNA methylation is propagated through cell divisions by methyltransferases. However, the mechanism by which DNA methylation and epigenetic silencing are originally targeted to TEs is not well understood. Our lab has identified a pathway in Arabidopsis directs the initiation of silencing through targeting de novo cytosine DNA methylation to active TEs. This pathway utilizes TE transcript degradation into endogenous 21-22 nucleotide (nt) small interfering RNAs (siRNAs). The siRNAs direct Argonaute (AGO) proteins to chromatin which then triggers de novo DNA methylation. Previous studies from our lab genetically identified AGO6 as the key effector protein in this pathway. Because of AGO6’s key role in the initiation of silencing, we have focused on it and its protein interactors to characterize and control the initiation of silencing mechanism. Previous experiments in our lab utilized immunoprecipitation (IP) of AGO6 followed by mass spectrometry (MS) to identify AGO6-interacting proteins. We set out to discover novel silencing factors by screening through AGO6-interactors identified by IP-MS. For those factors that were found to play an important role in silencing, I aimed to understand their mode of action by performing molecular characterizations including whole genome methylation analysis, siRNA production assays, and AGO siRNA-loading assays. Using this approach I have identified two novel AGO6-interacting proteins that load siRNAs into AGO6 and target the initiation of epigenetic silencing.

Keywords: Epigenetics, Transposable Elements, DNA Methylation
A deaminase and a methyltransferase that act co-dependently to edit and modify tRNA at a single site

Katherine M. McKenney (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 Microbiology and OSU Center for RNA Biology, The Ohio State University, Columbus, 43210 Ohio, 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: All types of nucleic acids in cells undergo naturally occurring chemical modifications, including DNA, rRNA, mRNA, snRNA, and most prominently tRNA. Over 100 different modifications have been described and every position in the purine and pyrimidine bases has been seen modified; often the sugar is also modified. However, despite recent progress, the mechanism for the synthesis of most modifications is not fully understood partly due to difficulty in reconstituting modification enzyme activity in vitro. Cytosine to uridine (C to U) editing of tRNAs in eukaryotes was first discovered over 25 years ago, yet due to the lack of an in vitro assay, the editing enzyme is still at large and the editing mechanism remains unsolved. In this work we show that cytosine 32 in the anticodon loop of tRNA<sup>Thr</sup> is methylated to 3-methylcytosine (m<sup>3</sup>C) as a prerequisite for C to U deamination. Formation of m<sup>3</sup>C in vitro requires the presence of both TbTRM140 (the T. brucei homolog of the yeast m<sup>3</sup>C methyltransferase) and the TbADAT2/3 A to I editing deaminase. Once m<sup>3</sup>C is formed the same set of enzymes are required for further deamination from m<sup>3</sup>C to form 3-methyluridine (m<sup>3</sup>U). At the molecular level these enzymes bind the tRNA substrate synergistically whereby the deaminase enhances the affinity of the methyltransferase for its substrate and vice versa. In our previous work, we showed that TbADAT2/3 was a highly mutagenic enzyme randomly deaminating DNA. Here we also provide evidence that when co-expressed with the methylase its mutagenic ability is kept in check via complex formation with TRM140. This helps explain how T. brucei escapes “wholesale deamination” of its genome while harboring both enzymes in its nuclear compartment. This observation has implications for the control of another mutagenic deaminase, human AID, and provides a rationale for its regulation.

Keywords: RNA editing, methylation, trypanosomes
RNA guanine-7 methyltransferase catalyzes the methylation of cytoplasmically recapped RNAs

Jackson B. Trotman (Center for RNA Biology, The Ohio State University), Andrew J. Giltmier (Center for RNA Biology, The Ohio State University), Chandrama Mukherjee (Center for RNA Biology, The Ohio State University), Daniel R. Schoenberg (Center for RNA Biology, The Ohio State University)

Abstract:
Cap homeostasis is a cyclical process of decapping and recapping that impacts a portion of the mRNA transcriptome. The metastable uncapped forms of recapping targets redistribute from polysomes to non-translating mRNPs, and recapping is all that is needed for their return to the translating pool. Previous work identified a cytoplasmic capping metabolon consisting of capping enzyme (CE) and a 5’-monophosphate kinase bound to adjacent domains of Nck1. The current study identifies the canonical cap methyltransferase (RNMT) as the enzyme responsible for guanine-N7 methylation of recapped mRNAs. RNMT binds directly to CE, and its presence in the cytoplasmic capping complex was demonstrated by pulldown assays, gel filtration, and proximity-dependent biotinylation. The latter also identified the RNMT cofactor RAM, whose presence is required for cytoplasmic cap methyltransferase activity. These findings guided development of an inhibitor of cytoplasmic cap methylation whose action resulted in a selective decrease in levels of recapped mRNAs.

Keywords: cytoplasmic capping, cap methylation, RNA metabolism
Building the great barrier: plasma membrane deposition during cytokinesis

Jian-Qiu Wu (Dept. of Molecular Genetics, OSU), Ning Wang, I-Ju Lee, Galen Rask

Abstract:
The cleavage-furrow tip adjacent to the actomyosin contractile ring is believed to be the predominant sites for plasma-membrane insertion through exocyst-tethered vesicles during cytokinesis. Here we found that most secretory vesicles are delivered by myosin V on linear actin cables in fission yeast cytokinesis. Surprisingly, by tracking individual exocytic and endocytic events, we found that vesicles with new membrane are deposited to the cleavage furrow relatively evenly during contractile-ring constriction, but the rim of the cleavage furrow is the main site for endocytosis. Fusion of vesicles with the plasma membrane requires vesicle tethers. Our data suggest that the transport particle protein II (TRAPP-II) complex and Rab11 GTPase help to tether secretory vesicles or tubulovesicular structures along the cleavage furrow while the exocyst tethers vesicles at the rim of the division plane. We conclude that the exocyst and TRAPP-II complex have distinct localizations at the division site but both are important for membrane expansion and exocytosis during cytokinesis. Besides delivering lipids for the plasma membrane expansion, secretory vesicles also transport extracellular matrix or the enzymes that synthesize them. The extracellular matrix are essential for cytokinesis in both animal cells and fungi, where the septum serves as the extracellular matrix. Proper synthesis of the trilaminar septum is essential for cytokinesis and cell integrity in fungi, so the participating enzymes are attractive targets for anti-fungal drugs with minimum impacts on fungal hosts. Conserved glucan synthases synthesize the glucans of the septum and cell wall in fission yeast as well as in fungal pathogens. We will present our investigation on how several novel proteins regulate delivery and functions of glucan synthases at the division site. These novel proteins and their binding partners that we discovered are conserved in fungal pathogens and can be targeted for new anti-fungal drugs.

Keywords: cytokinesis, exocytosis, extracellular matrix
List of Posters
(Alphabetical by Presenter Number/Abstracts)

1) Identification and Characterization of Novel Tax-1 Interacting Protein, SNX27, and its Role in HTLV-1 Pathobiology

Jacob Al-Saleem (Center for Retrovirus Research, The Ohio State University, Columbus, OH, USA; Department of Veterinary Biosciences, OSU, Columbus, OH, USA), Mamuka Kvaratskhelia (Center for Retrovirus Research, OSU, Columbus, OH, USA; Department of Pharmaceutics and Pharmaceutical Chemistry, OSU, Columbus, OH, USA), Lee Ratner (Division of Oncology, Washington University, St Louis, MO, USA), O John Semmes (The Leroy T Canoles Jr. Cancer Research Center, Eastern Virginia Medical School, Norfolk, Virginia, USA), Patrick L. Green (Center for Retrovirus Research, OSU, OH, USA; Department of Veterinary Biosciences, OSU, Columbus, OH, USA)

Abstract:
Background: HTLV-1 and HTLV-2 are highly related viruses, with differential pathogenic outcomes in humans. While HTLV-1 is associated with several diseases, such as adult T cell leukemia, HTLV-2 is not associated with disease. The trans-activator of HTLV-1, Tax-1, has higher transforming potential than its HTLV-2 homolog, Tax-2. It is believed that this difference in transforming capacity plays a pivotal role in HTLV-1 pathogenesis. We propose that Tax-1 interacts with cellular gene products via domains lacking in Tax-2, and that these interactions contribute to pathogenesis.

Methods: We performed proteomic screens of Tax-1 binding partners utilizing Tax-1 mutants to identify these interactions. Novel interactions were confirmed and mapped by co-immunoprecipitation studies and further characterized by biochemical and biologic assays.

Results: We identified a novel interacting partner of Tax-1, Sorting Nexin 27 (SNX27). SNX27 regulates the localization and expression of transmembrane proteins via interactions with its PDZ domain. SNX27 has been demonstrated to regulate glucose transporter 1 (GLUT1); SNX27 knock down in HeLa cells results in a dramatic redistribution of GLUT1 from the cell surface to the lysosome. GLUT1 serves as one of three receptor molecules for HTLV-1. We propose that Tax-1 alters GLUT1 localization post-infection via its interaction with SNX27. We demonstrate that Tax-1 and SNX27 interact via their PDZ domain binding motif (PBM) and PDZ domains, respectively. We further show that SNX27 expression levels are inversely related to virus release and that GLUT1 surface localization is reduced by Tax-1 overexpression in a dose-dependent manner. Other biologic effects of the Tax-1/SNX27 interaction and implications will be discussed.

Conclusion: This work demonstrates a novel mechanism by which HTLV-1 regulates a surface receptor molecule post-infection and this interaction could serve as a target to inhibit viral spread. Keywords: HTLV, Tax-1, SNX27
2) SAMHD1 suppresses HIV-1 gene expression and reactivation of viral latency in CD4+ T-cells

Jenna M. Antonucci (Center for Retrovirus Research, Center for RNA Biology, Department of Veterinary Biosciences, Department of Microbiology), Alice A. Duchon (Center for Retrovirus Research, Center for RNA Biology, Department of Chemistry and Biochemistry), Olga Buzovetsky (Department of Molecular Biophysics and Biochemistry, Yale University), Yong Xiong (Department of Molecular Biophysics and Biochemistry, Yale University), Karin Musier-Forsyth (Center for Retrovirus Research, Center for RNA Biology, Department of Chemistry and Biochemistry), Dr Li Wu (Center for Retrovirus Research, Center for RNA Biology, Department of Veterinary Biosciences)

Abstract:
The cellular dNTP hydrolase SAMHD1 restricts HIV-1 replication in non-dividing cells by degrading intracellular dNTPs to a level that limits efficient viral reverse transcription. Recombinant SAMHD1 binds HIV-1 DNA and RNA fragments in vitro, but the functional significance of the binding remains unclear. SAMHD1 is highly expressed in cell types that contribute to HIV-1 latent reservoirs, such as resting CD4+ T cells and myeloid cells. Transcriptional suppression of proviral DNA gene expression contributes to HIV-1 latency. However, it is unknown whether SAMHD1 regulates HIV-1 proviral gene expression in latently infected cells. Here, we investigate the effect of SAMHD1 on HIV-1 gene expression and the underlying mechanisms. We found that overexpression of SAMHD1 in HEK293T cells suppressed HIV-1 LTR promoter-driven luciferase expression in a dose-dependent manner at the level of transcription. We hypothesize that SAMHD1 may bind to the HIV-1 LTR to transcriptionally suppress viral gene expression in latently infected CD4+ T-cells. To study the effect of SAMHD1 on gene expression of integrated HIV-1 proviral DNA, we utilized the HIV-1 latently infected J-Lat cell line. We expressed exogenous SAMHD1 in J-Lat cells and observed that SAMHD1 reduced reactivation of HIV-1 gene expression. Additionally, a chromatin immunoprecipitation assay followed by qPCR revealed that SAMHD1 binds preferentially to the LTR in J-Lat cells, though it also binds to other HIV-1 gene sequences such as gag, rev, and vpr. We further investigated the in vitro binding affinity of recombinant SAMHD1 to single-stranded HIV-1 LTR and gag DNA fragments by fluorescence anisotropy. Binding assays performed over a range of salt concentrations are consistent with more specific binding of SAMHD1 to the LTR-derived DNA fragment relative to the gag fragment. Our data suggest that SAMHD1-mediated suppression of HIV-1 gene expression likely contributes to viral latency in CD4+ T-cells.

Keywords: HIV-1, SAMHD1, Latency
3) Driving lipopolysaccharide out of its membrane via an unusual ABC transporter

Blake R. Bertani (Department of Microbiology, Ohio State University), Natividad Ruiz (Department of Microbiology, Ohio State University)

Abstract:
With antibiotic resistance on the rise, the need for new antimicrobials is growing. Gram-negative organisms are particularly problematic due to their intrinsic resistance to many antibiotics. This resistance is mediated, in part, by a potent permeability barrier generated by lipopolysaccharide (LPS) at the surface of the cell. This feature makes LPS biogenesis an attractive target for antimicrobial development, as inhibiting this process could sensitise Gram-negative organisms to existing antibiotics. Additionally, LPS is essential in many organisms, and thus sufficient inhibition of LPS biogenesis could also kill directly. The synthesis of LPS is well characterized. However, how this molecule, once synthesized, traverses the cell envelope to assemble at the cell surface is not entirely understood. The process is mediated by a seven-protein complex, termed the Lpt complex (Lipopolysaccharide transport), which forms a proteinaceous bridge spanning all compartments of the cell. This complex is powered by an unusual ATP-binding cassette (ABC) transporter, LptB2FG. LptB is the ATPase of the system, but the functions of the transmembrane subunits LptF/G are not well characterized. We now report evidence suggesting that LptG interacts directly with LPS during transport. Specifically, we have identified a positively charged region in LptG critical for function. Amino acid substitutions in this region confer defects in LPS biogenesis, particularly when adding more negative charge to this region of LptG. Moreover, these defects can be suppressed by activation of the BasSR system, a two-component system which controls chemical modification of LPS. This suppression by activation of BasSR requires the enzymes EptA and ArnT, which modify negatively-charged phosphates on LPS with positively charged moieties. These results suggest a direct, charge based interaction between this newly identified region of LptG and LPS. We propose LptFG directly extract LPS from the inner membrane.

References:
1. Okuda et al. (2012): Cytoplasmic ATP hydrolysis powers transport of lipopolysaccharide...
2. Malojčić et al. (2014): LptE binds to and alters the physical state of LPS...
3. Hamad et al. (2012): Aminoarabinose is essential for lipopolysaccharide export...
7. Moran et al. (1991): Structural analysis of the lipid A component of Campylobacter jejuni...
8. Wetzler et al (2015): Structural Relationship of the Lipid A Acyl Groups to...
9. Ovchinnikov et al (2015): Large-scale determination of previously unsolved protein structures...

Keywords: Escherichia coli, Lipopolysaccharide Transport, Envelope Biogenesis
4) Identification and characterization of S-box riboswitches that regulate at the level of translation initiation

Divyaa Bhagdikar (Department of Microbiology and Center for RNA biology, The Ohio State University, Columbus, OH 43210 ), Frank J. Grundy (Department of Microbiology and Center for RNA biology, The Ohio State University, Columbus, OH 43210 ), Tina M. Henkin (Department of Microbiology and Center for RNA biology, The Ohio State University, Columbus, OH 43210)

Abstract:
The S-box riboswitches regulate the expression of genes involved in methionine and cysteine metabolism. Most S-box riboswitches operate at the level of transcription attenuation, such that a terminator helix is stabilized when S-adenosylmethionine (SAM) binds the aptamer. In silico analyses have suggested that a rarer class of S-box riboswitches may regulate at the level of translation initiation. We identified two different leader RNAs, metI from Desulfurispirilum indicum and metA from Rhodanobacter denitrificans, that have the potential for SAM-dependent sequestration of the Shine-Dalgarno (SD) region. 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 mutation at a position previously shown to disrupt SAM binding in other S-box RNAs resulted in loss of SAM binding in metI and metA. These results demonstrate that these aptamer domains have SAM binding properties similar to those of previously characterized S-box riboswitches. The SD regions of both of these RNAs exhibited SAM-dependent structural rearrangements consistent with the hypothesis that regulation occurs at the level of translation initiation. Additionally, binding of 30S subunits to metI RNA was reduced in the presence of SAM, demonstrating that the riboswitch regulates at the level of translation initiation. These riboswitches will be used to compare the characteristics of transcriptional and translational riboswitches in the same class. We hypothesize that the translational riboswitches can perform multiple reversible regulatory decisions whereas transcriptional riboswitches cannot reverse their regulatory decisions. Such a comparative study will provide insight into the functional role of transcriptional vs. translational regulation of the same gene by the same class of riboswitch in different organisms.

Keywords: Riboswitch, S-box, translation
5) Establishing the role of SINE proteins in regulating stomatal dynamics during environmental stresses in Arabidopsis thaliana

Alecia M. Biel (Department of Molecular Genetics, The Ohio State University, Columbus, OH 43210), Iris Meier (Department of Molecular Genetics, Center for RNA Biology, The Ohio State University, Columbus, OH 43210)

Abstract:
Drought is the main contributor to agricultural losses worldwide. The consequences of drought on plants are exacerbated by water loss through the opening of epidermal pores known as stomata. Stomata are formed by pairs of specialized plant cells called guard cells, which regulate pore opening and closing by increasing and decreasing intracellular turgor pressure, respectively. Additionally, stomatal opening allows for the uptake of carbon dioxide (CO2). This CO2 uptake is necessary for photosynthesis, biomass accumulation, and for the release of oxygen. When unregulated, stomatal dynamics can be detrimental to plant health and development.

SINE1 and SINE2 are two outer nuclear envelope proteins forming Linker of Nucleoskeleton to Cytoskeleton (LINC) complexes with Arabidopsis SUN inner nuclear envelope proteins. In wild type plants, the guard cell nuclei predominantly localize at the center of each cell whereas in mutants containing the homozygous sine1-1 allele, the position of the nuclei skew toward a greater distance from the center of the guard cells. Here, we present data that suggest a role for SINE1 and SINE2 in regulating stomatal aperture control under abiotic stress but not biotic stress. Plants lacking either protein show an inability to fully close stomata during ABA-induced drought response but have unimpaired closure during pathogen exposure. Moreover, stomatal opening appears to be compromised under standard growth conditions but can be rescued with exogenous applications of potassium and calcium, suggesting hyposensitivity to these ions. Finally, sine1 mutant plants may have a decreased biomass accumulation. These results implicate that SINE proteins play an important role in regulating stomatal dynamics during abiotic stress. Conversely, biotic stress response may have a separate pathway leading to stomatal closure. Gaining a better understanding of how plants can become drought tolerant and better adapt to environmental stress can be useful not only to improve sustainable food production but also to increase biofuel production on drought susceptible land.

References:

Keywords: LINC complex, ABA, Guard Cells
6) RNA Nanotechnology for the Specific Targeting and Delivery of miRNA for Inhibition of Prostate Cancer

Daniel W. Binzel (College of Pharmacy; College of Medicine/Department of Physiology Cell Biology/Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University, Columbus, Ohio, USA.), Yi Shu (Nanobiotechnology Center; Markey Cancer Center; Department of Pharmaceutical Sciences; University of Kentucky, Lexington, KY 40536, USA), Hui Li (College of Pharmacy; College of Medicine/Department of Physiology Cell Biology/Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University, Columbus, Ohio, USA.), Meiyan Sun (Department of Pharmaceutical Sciences, North Dakota State University, Fargo, ND 58108, USA), Bin Guo (Department of Pharmaceutical Sciences, North Dakota State University, Fargo, ND 58108, USA), Peixuan Guo (College of Pharmacy; College of Medicine/Department of Physiology Cell Biology/Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University, Columbus, Ohio, USA.)

Abstract:
Both siRNA and miRNA can serve as powerful gene silencing reagents but their specific delivery to cancer cells in vivo without collateral damage to healthy cells remains challenging. We report here the application of RNA nanotechnology using the three-way junction motif (3WJ) from the phi29 DNA packaging motor packaging RNA for specific and efficient delivery of anti-miRNA seed-targeting sequence to block the growth of prostate cancer in mouse models. Utilizing the thermodynamically ultra-stable three-way junction of the pRNA, RNA nanoparticles were constructed by bottom-up self-assembly containing the anti-Prostate Specific Membrane Antigen aptamer as targeting ligand and anti-miR17 or anti-miR21 as therapeutic modules. The RNase resistant and thermodynamically stable RNA nanoparticles remained intact after systemic injection in mice and strongly bound to tumors with little or no accumulation in healthy organs 8 hr post-injection, and subsequently repressed tumor growth at low doses with high efficiency.

References:

Keywords: RNA nanotechnology, Prostate Cancer, Phi29
SnRK1 phosphorylates eIF4E/iso4E to regulate translation

Aaron N. Bruns (OSU), Sizhun Li (OSU), David M. Bisaro (OSU)

Abstract:
Plant SNF1-related kinase (SnRK1) belongs to a conserved family that includes animal AMP-activated protein kinase (AMPK) and yeast SNF1. These serine/threonine kinases sense the AMP:ATP ratio in cells and play key roles in maintaining energy homeostasis through a variety of pathways. SnRK1/SNF1/AMPK are also involved in both biotic and abiotic stress responses. Our lab previously identified SnRK1 as a target of suppression by geminivirus pathogenicity factors, and found that SnRK1 overexpression provides resistance to infection by the DNA containing geminiviruses as well as RNA viruses. However, the mechanism underlying this resistance remained unclear. In these studies, we demonstrate the presence of a previously unknown regulatory pathway in which SnRK1 interacts with and phosphorylates the mRNA cap-binding protein Eukaryotic Initiation Factor 4E (eIF4E) and its isoform (eIFiso4E). Additionally, we show that this inhibits translation in both yeast and plant (Nicotiana benthamiana) cells. These observations provide a direct link between a cellular energy sensing kinase and translation control, and likely also explain the virus resistance phenotype observed in SnRK1 overexpressing N. benthamiana. SnRK1 and eIF4E/iso4E are deeply conserved in plants, and SnRK1/SNF1/AMPK phosphorylation sites are also conserved in plant, fungal (e.g. Saccharomyces cerevisiae) and invertebrate animal (e.g. Drosophila melanogaster and Caenorhabditis elegans) eIF4E. Therefore, this novel regulatory mechanism may have broad importance in pathogen defense and stress response in many organisms, including valuable crop plants and potentially a wide variety of other non-vertebrate systems.

Keywords: Translation, eIF4E, AMPK SnRK1 SNF1
8) Crystal Structure of a Minimal Synaptosome Complex for DNA Repair by Single Strand Annealing

Brian J. Caldwell (OSBP), Chris E. Smith (OSBP), Charles E. Bell (Biological Chemistry and Pharmacology)

Abstract:
Single strand annealing (SSA) is one of three main pathways for repair of double stranded DNA breaks, a deleterious form of DNA damage that can lead to chromosome rearrangement. Bacteriophage lambda uses a simple two component “SynExo” (Synaptase-Exonuclease) recombination system for SSA that consists of a 5’-3’ exonuclease to resect DNA ends, and a protein called Red beta to anneal the resulting 3’ overhang to a complementary strand. Similar recombination systems exist in a wide variety of bacteriophage as well as in oncogenic dsDNA viruses such as HSV1. Due to the highly-evolved and simple nature of this recombination system, a mechanistic understanding of how it operates would provide a useful model for studying the more elaborate SSA reaction in mammalian cells. In addition, the SynExo proteins from bacteriophage lambda have been exploited in powerful new methods for genome engineering. Interestingly, the two components of the SynExo system in bacteriophage lambda form a complex called a “synaptosome”. The exact role of this complex is unknown but it may serve to integrate the two steps of the SSA reaction by physically loading the annealing protein onto the 3’ overhang as it is generated by the exonuclease. Knowledge of the overall architecture of the complex will shed light on how it operates. Here we have used x-ray crystallography to solve the structure of a “mini” synaptosome complex containing a lambda exonuclease trimer bound to three copies of the Red Beta C-terminal domain. The structure reveals the residues of each protein at the interface and thus provides a framework for design of mutational experiments to probe the role of the complex in promoting recombination in vivo.

Keywords: SynExo, Synaptosome
9) Inhibition of CDKs with PHA-848125 impairs estrogen receptor negative breast cancer progression and metastasis

Douglas Cheung (Molecular, Cellular, and Developmental Biology Graduate Program, The Ohio State University), Gianpiero di Leva, Matteo Fassan, Claudia Piovan (Department of Cancer Biology and Genetics, The Ohio State University), Krishna Patel, Arpan Kumar, Dorothee Wernicke, Stefano Volinia (Department of Cancer Biology and Genetics, The Ohio State University), Marina Ciomei (Business Unit Oncology, Nerviano Medical Sciences), Michela Garofalo (Transcriptional Networks in Lung Cancer, CRUK Manchester Institute), Carlo M. Croce (Department of Cancer Biology and Genetics, The Ohio State University)

Abstract:
The cell cycle is frequently deregulated in breast cancer, allowing rapid cell proliferation and tumor growth. The cell cycle is tightly regulated by cyclins and cyclin-dependent kinases (CDKs). We show here that estrogen receptor (ER) negative breast cancer cells are more sensitive to the multi-CDK inhibitor PHA-848125, which has the highest selectivity for CDK2, than ER positive breast cancer cells. PHA-848125 treatment of ER negative cells caused G1 cell cycle arrest and a reduction of phosphorylation of the retinoblastoma protein (RB), confirming the CDK2 inhibitory effect of the drug. Oral administration of PHA-848125 to mice bearing MDA-MB-231 xenograft TNBC tumors resulted in an inhibition of tumor growth and metastatic progression at tolerable doses. However, PHA-848125 had no effect in mice bearing MCF-7 ER-positive tumors. PHA-848125 treatment reduced both the sizes and the numbers of tumors in MMTV-PyMT mice. Our preclinical experiments therefore set the rationale for the clinical evaluation of PHA-848125 in the treatment of ER-negative breast cancer.

Keywords: cell cycle, CDK, breast cancer
10) LPR-3 as a cell non-autonomous regulator of activated LET-60/RAS in C. elegans

Marcos Corchado (Department of Molecular Genetics, Ohio State University, Columbus, Ohio 43210, USA), Komal Rambani (Department of Biomedical Sciences, Ohio State University, Columbus, Ohio 43210, USA), Gustavo Leone (Department of Molecular Genetics, Ohio State University, Columbus, Ohio 43210, USA), Helen M. Chamberlin (Department of Molecular Genetics, Ohio State University, Columbus, Ohio 43210, USA)

Abstract:
Extracellular signals from the stroma are required for the maintenance and progression of tumors. However, identifying such factors is particularly challenging due to the complexity of stromal cell types in mammals. In a recent collaboration, our lab co-developed a simple model for the tumor microenvironment based on C. elegans vulva development. Here, the mesoderm serves as a model for the stroma, while the epithelial vulva precursor cells (VPCs) model the tumor. Key to this system is a genetic modification restricting RNAi response to the mesoderm. Additionally, a gain-of-function mutation, let-60(gf), promoted increased cell division of VPCs, resulting in multi-vulva (MUV) animals with visible ventral protrusions. In humans, a hyperactive allele of Ras, the let-60 ortholog, leads to deregulation of the Ras-MAPK pathway, resulting in increased cell cycle progression and cancer. Using this system, a genome-wide RNAi screen was performed to identify genes that, upon knockdown, act as cell non-autonomous suppressors of let-60(gf). Surprisingly, only one gene encoded a secreted protein –lipocalin-related 3 (lpr-3). Most lipocalins transport hydrophobic ligands and initiate signaling cascades by binding to specific membrane receptors. The following work presents proposed research to elucidate the mechanism of LPR-3 and test the hypothesis that LPR-3 is secreted and binds to a receptor to promote activated let-60 signaling. In Aim 1 I will characterize the expression profile and conserved lipocalin functions of lpr-3. Aim 2 will test the hypothesis that LPR-3 binds to a receptor in the VPC membrane. Finally, Aim 3 focuses on identifying the endogenous LPR-3 ligand.

Keywords: lipocalins, Ras, C elegans
11) A programmable RNase for the targeted degradation of viral RNA

Daniel Dayeh (Chemistry & Biochemistry), William Cantara (Chemistry & Biochemistry), Karin Musier-Forsyth (Chemistry & Biochemistry), Kotaro Nakanishi (Chemistry & Biochemistry)

Abstract:
Since its discovery, RNA interference (RNAi) has enabled unprecedented advances to our understanding of cellular processes by employing small-interfering RNA (siRNA) to reduce endogenous protein levels for a gene product via degradation of messenger RNA (mRNA), yet its application towards other RNAs such as viral RNAs and long noncoding RNAs (lncRNAs) has been technically challenging. Here, we show that Argonaute (AGO), which plays a physiological role in RNAi in the budding yeast Kluyveromyces polysporus, can be programmed with a single-stranded guide DNA (gDNA) to site-specifically degrade the 5’ untranslated region of Human Immunodeficiency Virus - 1 (HIV-1 5’ UTR) by endonuclease activity. Site-specific degradation of viral RNAs will disrupt RNA:RNA and RNA:protein interactions by impairing or eliminating their binding sites, producing a map of the functional sites with nucleotide resolution. This approach is advantageous over existing methods due to the use of a DNA-induced slicing complex (DISC), which dramatically increases the stability of the DNA guides compared to the canonical RNA counterparts while reducing costs for largescale biotechnological applications. Overall, this strategy constitutes a high-throughput method for functional mapping of large, structured RNAs by identifying DISC-accessible sites, thus providing a platform for the investigation of physiologically and medically relevant RNAs.

Keywords: Argonaute
12) The required to maintain repression12 locus provides a novel mechanistic link between paramutation and developmental gene regulation in Zea mays

Natalie Deans (Department of Molecular Genetics, The Ohio State University, Columbus, OH, 43210), Brian Giacopelli (Department of Molecular Genetics, The Ohio State University, Columbus, OH, 43210), Daniel Hlavati, Emily McCormic (Department of Molecular Genetics, The Ohio State University, Columbus, OH, 43210), Charles Addo-Quaye (Department of Biochemistry, Purdue University, West Lafayette, IN, 47907), Brian Dilkes (Department of Biochemistry, Purdue University, West Lafayette, IN, 47907), Jay Hollick (Department of Molecular Genetics, and The Center for RNA Biology, OSU, Columbus, OH, 43210)

Abstract:

In Zea mays (maize), paramutations facilitate meiotically heritable changes in gene regulation for certain alleles of purple plant1, a gene encoding a transcription factor required for anthocyanin production1. A strongly expressed PI1-Rhoades allele is suppressed in trans when combined with a transcriptionally and post-transcriptionally repressed PI1-Rhoades allele, and both alleles are passed on in a repressed (denoted Pl’) state. At least sixteen loci whose functions are required to maintain repression (rmr) of Pl’ have been identified in an ethyl methanesulfonate mutagenesis screen. Known RMR proteins that mediate 24 nucleotide (24nt) RNA biogenesis in a pathway presumed to be orthologous to Arabidopsis RNA-directed DNA Methylation (RdDM) pathway which directs repressive chromatin modifications. The fourth protein is unique to 24nt RNA biogenesis in maize. Here we describe four recessive alleles which define the rmr12 locus and complement previously identified rmr factors. Unlike any other rmr-type mutations found to date2,3,4,5,6, rmr12 mutants display a unique combination of defects, including male gametophyte dysfunction, that indicate a novel mechanistic connection between paramutation and developmental gene control. Whole genome sequence analysis and molecular mapping place rmr12 in an interval on chromosome 9S, and individual rmr12-3 and rmr12-4 mutants have lesions in a gene encoding a chromodomain-helicase-DNA-binding protein 3 (chd3) within this region. CHD3 proteins are chromatin remodelers important for developmental gene regulation in both animals and plants, and defects in human CHD3 proteins have been associated with gastric and colorectal cancers7,8,9,10. Many poorly understood aspects of CHD3 biology, including its role in directing transgenerational changes in gene regulation, can now be addressed at a specific genomic target.

References:


Keywords: Development, Gene regulation, Chromatin
13) Characterization of the Function and Mechanism of an Orphan 3'-5' RNA Polymerase Involved in Noncoding RNA Processing.

Samantha Dodbele (OSBP), Blythe Moreland (Physics), Yicheng Long (OSBP), Spencer Gardner (Chemistry and Biochemistry), Ralf Bundschuh (Physics, RNA Center), Jane Jackman (Chemistry and Biochemistry, RNA Center)

Abstract:
Until the discovery of tRNAHis guanylyltransferase (Thg1) from Saccharomyces cerevisiae (Sc), nucleotide polymerization was believed to exclusively occur in the 5'-3' direction. Thg1 shifts this paradigm by catalyzing the non-templated addition of a required guanosine to the 5' end of tRNAHis in a 3'-5' direction. Enzymes exhibiting similarity to ScThg1, called Thg1-like proteins (TLPs) catalyze a Watson-Crick dependent 3'-5' polymerization. TLPs have been found in all three domains of life, including eukaryotic organisms such as Dictyostelium discoideum (Ddi). However, the roles and mechanisms of TLPs compared to their relatively more well-studied Thg1 counterparts are less understood.

Previous work by our group has demonstrated the functions of two TLPs in the model eukaryote Dictyostelium discoideum. These TLP enzymes, DdiTLP2, and DdiTLP3 catalyze a Watson-Crick dependent 3'-5' polymerization, and are responsible for mitochondrial tRNAHis maturation, and mitochondrial tRNA 5'-editing, respectively. However the biological function of a third TLP enzyme encoded in D. discoideum, DdiTLP4, remains unknown. In vitro studies suggest DdiTLP4 can act on several small, noncoding RNAs (ncRNA) in addition to tRNAs. Moreover, depletion of DdiTLP4 causes a severe growth defect in D. discoideum. We hypothesize that this essential function of DdiTLP4 is due to its role in small RNA processing and its activity on specific ncRNA substrates that remain yet to be positively identified. Depletion of DdiTLP4 followed by RNA-Seq is being used to identify in vivo substrates of DdiTLP4. This investigation will enable identification of any type of RNA whose 5'-end sequence is altered in the absence of DdiTLP4 activity, thus enabling the first comprehensive insight into 3'-5' polymerase substrate specificity, including into non-tRNA related activities associated with these enzymes. This investigation into the biological function of DdiTLP4 will provide greater understanding of the 5'-end maintenance machinery of eukaryotes and into diverse biological roles for 3'-5' polymerization.

Keywords: ncRNA, Thg1, 3 to 5 polymerase
14) Determining how pioneer factors access chromatin

**Ben Donovan** (biophysics)

**Abstract:**
Packaging the eukaryotic genome into nucleosomes greatly limits binding site accessibility for transcription factors (TFs) and other DNA binding proteins [1]. Because of this, nucleosomes are precisely positioned in regulatory regions of the genome [2], [3]. For example, transcription start sites (TSSs) are typically depleted of nucleosomes and contain specific patterns of post-translational modifications (PTMs) that recruit chromatin remodellers, histone chaperones, and other PTM-reading enzymes. It is well established that these epigenetic regulatory mechanisms control gene expression by modulating the accessibility of TF binding sites within nucleosomes. Recent studies indicate a new class of TFs, called pioneer factors, are the first TFs to target a gene before activation [4], [5]. Additionally, mis-regulation of these proteins are implicated in cancers [6], [7]. Despite links to disease, how pioneer factors access binding sites within condensed chromatin is not understood. These studies focus on Reb1, a pioneer factor from *S. cerevisiae* involved in establishing nucleosome depleted regions (NDRs) at TSSs and installing the histone variant H2A.Z at the 5’ ends of genes [8]. Together with collaborators, we show that insertion of a single Reb1 target site into the nucleosome dyad is sufficient to establish an NDR in vivo. Furthermore, electrophoretic mobility shift assays indicate Reb1 binds to sites within nucleosomes with similar affinities as to DNA. To probe how Reb1 effects nucleosome structure, we inserted binding sites in increments of 5 basepairs (bp) throughout the entry/exit region and monitored Reb1 induced ΔFRET between Cy3-DNA and Cy5-octamer. With this approach, we show that Reb1 binds to transiently exposed binding sites on unwrapped nucleosomal DNA without evicting histones. Additionally, we show that inserting a binding site into the dyad region increases Reb1 accessibility to entry/exit sites by 2-3 fold.

**References:**

**Keywords:** chromatin, biophysics, pioneer transcription factors
15) HIV-1 exploits dynamic multi-aminoacyl-tRNA synthetase complex to enhance viral reverse transcription and replication

Alice Duchon (Department of Chemistry and Biochemistry, Center for RNA Biology, Center for Retrovirus Research, The Ohio State University, Columbus, OH 43210), Nathan Titkemeier (Department of Chemistry and Biochemistry, Center for RNA Biology, Center for Retrovirus Research, The Ohio State University, Columbus, OH 43210), Corine St. Gelais (Center for RNA Biology, Center for Retrovirus Research, and Department of Veterinary Biosciences, The Ohio State University, Columbus, OH 43210), Li Wu (Center for RNA Biology, Center for Retrovirus Research, and Department of Veterinary Biosciences, The Ohio State University, Columbus, OH 43210), Karin Musier-Forsyth (Department of Chemistry and Biochemistry, Center for RNA Biology, Center for Retrovirus Research, The Ohio State University, Columbus, OH 43210)

Abstract:
A hallmark of retroviruses such as human immunodeficiency virus type-1 (HIV-1) is reverse transcription of genomic RNA into DNA, a reaction that is primed by host cell tRNAs. HIV-1 recruits human tRNA\textsuperscript{Lys}\textsuperscript{3} to serve as the reverse transcription primer, yet all three human tRNA\textsuperscript{Lys} isoacceptors are selectively packaged into virions via an interaction between lysyl-tRNA synthetase (LysRS) and the HIV-1 Gag polyprotein. LysRS is normally sequestered in a multi-aminoacyl-tRNA synthetase complex (MSC) together with 8 other tRNA-synthetases and three cellular factors. Previous studies have shown that components of the MSC can be mobilized in response to certain cellular stimuli. How LysRS is redirected from the MSC to viral particles for packaging is unknown. Here, we show that upon HIV-1 infection, LysRS is released from the MSC and partially relocalized to the nucleus. Heat inactivation of HIV-1 blocks nuclear localization but AZT treatment does not, suggesting LysRS release from the MSC occurs prior to reverse transcription. Knockdown of LysRS reduced progeny virion infectivity, release from the MSC and nuclear localization, as did treatment with a MEK inhibitor known to prevent phosphorylation of LysRS on Ser207. A phosphomimetic mutant (LysRSS207D) localized to the nucleus and rescued infectivity, whereas a phoshoablative mutant (LysRSS207A) remained cytosolic and failed to rescue infectivity. The ability of phoshoablative and phosphomimetic LysRS mutants to package tRNA\textsuperscript{Lys}\textsuperscript{3} is currently being assessed. Taken together, this work suggests that HIV-1 takes advantage of the dynamic nature of the MSC to redirect and co-opt host cellular factors and thereby enhance its own replication. As HIV-1 is less likely to develop resistance to drugs that target host factors, we anticipate that these findings will lead to development of new therapeutic approaches to treat HIV-positive individuals.

Keywords: HIV-1, Aminoacyl-tRNA synthetase
16) New tRNA contacts facilitate ligand binding in a Mycobacterium smegmatis T box riboswitch

Jane K. Frandsen (Ohio State Biochemistry Program, Cellular, Molecular & Biochemical Sciences Program, Center for RNA Biology), Anna V. Sherwood (Molecular Cellular and Developmental Biology, Center for RNA Biology), Frank J. Grundy (Department of Microbiology, Center for RNA Biology), Tina M. Henkin (Department of Microbiology, Center for RNA Biology)

Abstract:
T box riboswitches are RNA regulatory elements widely used by organisms in the phyla Firmicutes and Actinobacteria to regulate expression of amino acid-related genes. Expression of T box family genes is downregulated by transcription attenuation or inhibition of translation initiation in response to increased charging of the cognate tRNA. Three direct contacts with tRNA have been described; however, one of these contacts is absent in a subclass of T box RNAs, and the roles of several conserved structural domains are still unknown. In this study, structural elements of a Mycobacterium smegmatis ileS T box riboswitch variant with an Ultrashort Stem I were sequentially deleted, which resulted in a progressive decrease in binding affinity for the tRNA\textsuperscript{le} ligand. Selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE) showed structural changes in the conserved riboswitch domains upon interaction with the ligand tRNA. Cross-linking and mutational analyses identified two new interaction sites, one between the S-turn element in Stem II and the T arm of tRNA\textsuperscript{le} and the other between the Stem IIA/B pseudoknot and the D loop of tRNA\textsuperscript{le}. These newly identified RNA contacts add new features of tRNA recognition by the T box riboswitch and demonstrate a new role for the S-turn and pseudoknot elements that are common to many cellular RNAs.

References:

Keywords: Riboswitch, tRNA, S-turn, Pseudoknot, T arm, D loop
17) Delineating Rbfox-regulated splicing networks critical for vertebrate muscle development

Thomas L. Gallagher, Zachary T. Morrow (Dept. of Molecular Genetics, The Ohio State University), Swanny A. Lamboy Rodriguez (Dept. of Biology, University of Puerto Rico in Humacao), Marcus H. Stoiber, James B. Brown, Susan E. Celniker, John G. Conboy (Life Sciences Division, Lawrence Berkeley National Lab), Sharon L. Amacher (Dept. of Molecular Genetics, The Ohio State University)

Abstract:
Rbfox RNA binding proteins are regulators of phylogenetically-conserved alternative splicing events important for neuromuscular functions. To investigate rbfox gene function in muscle, we knocked down muscle-expressed rbfox1l and rbfox2 in zebrafish embryos using antisense morpholinos and showed that double morphant embryos have skeletal and cardiac muscle defects and exhibit changes in splicing of bioinformatically-predicted Rbfox target exons (Gallagher et al., 2011). Using CRISPR/Cas9 technology, we now have generated rbfox1l and rbfox2 single and compound null mutants. Whereas rbfox1l and rbfox2 single mutant embryos have normal myofibers and contractility, rbfox1l+/−; rbfox2+/− double homozygous mutant embryos have disorganized myofibrils coupled with complete paralysis, very much like double morphant embryos. Despite contractile defects, neuromuscular junctions appear normal in both compound and double mutants, as well as specification of slow- and fast-twitch muscle fiber types. Additionally, splicing analysis reveals that predicted Rbfox target exons are down-regulated in rbfox1l+/−; rbfox2+/− double homozygotes. Interestingly, compound mutant combinations have different effects: rbfox1l+/−; rbfox2+/− mutants have wavy myofibers and exhibit seizure/tremor-like behavior upon touch-evoked stimulation, whereas rbfox1l+/−; rbfox2+−/− mutants are indistinguishable from single mutants. Together, these results suggest that levels of Rbfox factors are critical for the coordinated regulation of an alternative splicing program essential for muscle function. In order to globally define this Rbfox-regulated splicing program, we have used RNA-Seq coupled with advanced computational strategies to identify Rbfox target exons that are misregulated in Rbfox-deficient muscle. Our goal is to understand how the network of Rbfox-regulated splicing events impacts muscle development and function that in the long term will allow us to rationally predict and functionally evaluate therapeutic approaches for splicing defects that lead to human muscle disease.

Keywords: Rbfox, alternative splicing, muscle
18) EJC acts via NMD to manipulate levels of key developmental regulators

**Pooja Gangras** (Department of Molecular Genetics), **Thomas L. Gallagher** (Department of Molecular Genetics), **Kiel T. Tietz** (Department of Molecular Genetics), **Natalie C. Deans** (Department of Molecular Genetics), **Sharon L. Amacher** (Department of Molecular Genetics), **Guramrit Singh** (Department of Molecular Genetics)

**Abstract:**

Developmental gene expression is regulated at transcriptional and post-transcriptional levels. One post-transcriptional regulatory mechanism is Nonsense Mediated mRNA Decay (NMD) which was originally described as a surveillance system to degrade aberrant mRNAs. It is now appreciated that NMD also degrades non-aberrant mRNAs containing ‘NMD-inducing features’ such as 3’ UTR introns. Post-splicing, an mRNA with a 3’ UTR intron has an Exon Junction Complex (EJC) bound downstream of the stop codon – if the distance between the two is ≥ 50 nts, the mRNA is recognized by the key NMD-regulator Upf1 and targeted for NMD. To study EJC function during development, we generated zebrafish mutants in EJC core proteins Rbm8a and Magoh. Homozygous rbm8a and magoh mutants are paralyzed and have disorganized myofibrils and stunted motoneuron axons. As expected, RNA profiling reveals that annotated NMD targets are significantly upregulated in EJC mutants; ten of these show the same trend in Upf1-deficient embryos and some contain known NMD-inducing features, suggesting that this subset may be regulated via NMD. Among the other upregulated transcripts, three contain a conserved 3’ UTR intron < 50 nts downstream of the stop codon. One of these encodes Foxo3b, a transcription factor that functions to inhibit Wnt signaling during development. We hypothesized that increased Foxo3b in EJC mutants might decrease Wnt signaling below a critical threshold. To test this, we treated EJC mutants with a Wnt agonist BIO, and preliminary results show dramatic rescue of mutant morphological defects. We hypothesize that a distal EJC (≥ 50 nts downstream of the stop codon) directs rapid mRNA turnover while a more proximal EJC (< 50 nts) creates a sub-optimal NMD target that decays more gradually, allowing finer tuning of expression levels. To test this hypothesis, I will conduct foxo3b mini-gene experiments and probe our datasets to discover additional NMD targets and atypical NMD-inducing features.

**Keywords:** Exon Junction Complex, NMD, development
Conserved GTPase LepA (Elongation Factor 4) functions in biogenesis of the 30S subunit of the 70S ribosome

Michelle R. Gibbs (Department of Microbiology, Center for RNA Biology, The Ohio State University), Kyung-Mee Moon (Department of Biochemistry and Molecular Biology, University of British Columbia), Menglin Chen (Department of Microbiology, Center for RNA Biology, Ohio State Biochemistry Program, The Ohio State University), Rohan Balakrishnan (Department of Microbiology, Center for RNA Biology, Ohio State Biochemistry Program, The Ohio State University), Leonard J. Foster (Department of Biochemistry and Molecular Biology, University of British Columbia), Kurt Fredrick (Department of Microbiology, Center for RNA Biology, Ohio State Biochemistry Program, The Ohio State University)

Abstract:
The physiological role of LepA, a paralog of EF-G found in all bacteria, has been a mystery for decades. Here, we show that LepA functions in ribosome biogenesis. In cells lacking LepA, immature 30S particles accumulate. Four proteins are specifically underrepresented in these particles—S3, S10, S14, and S21—all of which bind late in the assembly process and contribute to the folding of the 3′ domain of 16S rRNA. Processing of 16S rRNA is also delayed in the mutant strain, as indicated by increased levels of precursor 17S rRNA in assembly intermediates. Mutation ΔlepA confers a synthetic phenotype in absence of RsgA, another GTPase, well known to act in 30S subunit assembly. Analysis of the ΔrsgA strain reveals accumulation of intermediates that resemble those seen in the absence of LepA. These data suggest that RsgA and LepA play partially redundant roles to ensure efficient 30S assembly.

Keywords: protein synthesis, translation, RsgA
Identifying novel protein components of the cytoplasmic capping complex using proximity-dependent biotinylation

Andrew J. Giltmier (Biological Chemistry and Pharmacology), Jackson B. Trotman (Biological Chemistry and Pharmacology)

Abstract:
mRNAs that appear without a 5’ cap in the cytoplasm are readily identified and degraded by cytoplasmic enzymes. However, a smaller set of uncapped mRNAs can be recapped in the cytoplasm, protecting them from degradation by exonucleases, allowing them to return to a translationally active state [1]. The cytoplasmic capping complex consists of cytoplasmic capping enzyme (cCE), RNA-guanine-7methyltransferase (RNMT), and a 5’ RNA kinase that assemble on adapter protein Nck1. This complex is the set of proteins responsible for replacing the 5’ cap that provides a unique form of mRNA regulation [2]. Although the basic structure of the cytoplasmic capping complex is established, many details remain unknown. Our goal is to complete our understanding of the structure of this complex using a new in vivo approach for proximity-dependent labeling of proteins called BioID (proximity-dependent biotin identification). Using a promiscuous biotin ligase bound to cCE, we identify known and unknown candidates of the cytoplasmic capping complex by biotin tagging proteins as they interact with the complex. These tagged candidate proteins are isolated via streptavidin affinity chromatography and identified with mass spectrometry analysis. Identification of unknown interactors has the potential to better understand the biological relevance of a unique mechanism of post-transcriptional regulation.

References:


Keywords: Capping, BioID
21) Regulation of geminivirus late gene expression by PRC2

Elizabeth Regedanz, Department of Molecular Genetics, The Ohio State University, Dr. Garry Sunter, Department of Biology, The University of Texas at San Antonio, Dr. David Bisaro, Department of Molecular Genetics, The Ohio State University

Geminiviruses are small ssDNA viruses that infect many agriculturally important crops and cause significant loss in yield. Geminiviruses also serve as excellent models for de novo epigenetic processes. Upon entry into the nucleus, the virus replicates its genome through host enzymes to generate a dsDNA replicative form (RF). The RF associates with histones to form non-integrating episomes that both facilitate virus transcription and serve as targets of host defense pathways. Polycomb Repressive Complex 2 (PRC2) is an important repressive regulator of developmental processes via its ability to deposit histone 3 lysine 27 trimethylation (H3K27me3), but it is unclear how PRC2 is recruited to target genes. We have preliminary data suggesting viral late gene expression is regulated by PRC2. We have found that H3K27me3 is localized to a late viral gene. Additionally, we have data suggesting that PRC2 is recruited to viral chromatin through interaction with the transcription factor TCP24 (TEOSINTE BRANCHED 1, CYCLOIDEA, and PCF Family 24). TCP24 is regulated post-transcriptionally by an endogenous miRNA (miR319) through mRNA degradation. Interestingly, TCP24 mRNA levels are decreased in geminivirus infected plants.

We hypothesize that TCP24 recruits PRC2 to establish H3K27me3 on viral chromatin. Early in infection, this H3K27me3 deposition inhibits viral late gene expression. Once a threshold of viral genomes is produced, TCP24 expression is decreased, late genes begin to be expressed, and the genomes are encapsidated. We are testing this hypothesis by directly measuring PRC2 association with viral chromatin via chromatin immunoprecipitation (ChIP). We are also investigating possible mechanisms of TCP24 regulation by monitoring both miR319 expression and methylation of the TCP24 promoter over the course of infection. These experiments will provide insight into how cells target PRC2 to specific loci, and will also illuminate a key facet of geminivirus gene regulation.
22) Characterization of HIV-1 Gag and Gag-RNA interactions by native mass spectrometry

Samantha H. Hinckley (Chemistry and Biochemistry, The Ohio State University), Erik D. Olson (Chemistry and Biochemistry, The Ohio State University), Karin Musier-Forsyth (Chemistry and Biochemistry, The Ohio State University), Vicki H. Wysocki (Chemistry and Biochemistry, The Ohio State University)

Abstract:
Most HIV-1 therapies are focused on targeting viral enzymes. Due to the common drug resistance which occurs when HIV-1 therapies based on targeting viral enzymes are employed new strategies for treatment must be developed. The HIV-1 Gag polyprotein and its mature protein products are critical for the lifecycle of this retrovirus. It is established that the presence of both the membrane and nucleic acids lead to linear Gag conformations associated with infectious viral particle formation a defined mechanism has not been established. To better understand the lifecycle of the HIV-1 retrovirus the oligomeric states of Gag and the binding stoichiometry of Gag in the presence of the trans activating response (TAR) element and the packaging signal sequence (PSI) have been determined with native mass spectrometry. A systematic approach has been used to dissect the interactions and of Gag with TAR-polyA and PSI.

In vitro transcribed TAR-polyA (A34U) RNA (81 µM) was obtained from the Musier Forsyth lab and folded in ammonium acetate (50 mM). The solution was heated up to 80°C for 2 minutes, subsequently incubated at 60°C for 2 minutes and then magnesium acetate (10mM) was added before chilling on ice for 30 minutes. The folded TAR-polyA was then incubated with GagΔp6, GagΔsp1Δp6, nucleocapsid, or matrix (7 µM each) in 500 mM ammonium acetate for 10 minutes at 25°C. The same protocol was followed for forming the Gag-Psi complex. The complex was then analyzed on an in house modified Thermo Q-Exactive plus EMR with an installed quadrupole and S1D device.

Upon analysis, the complex was seen with a mass of 86 kDa, indicating a one to one stoichiometry in these conditions. The Psi-Gag mixture was observed to yield a 200 kDa complex indicating that either higher oligomeric states of Gag or a three to one stoichiometry in these conditions. Apo-gag polyprotein was observed to have multiple oligomeric states ranging from monomer to hexamer.

This is the first high resolution mass spectrum of the intact Gag: TAR-polyA and Gag: Psi RNA-protein complex and observation of the various oligomeric states of Apo-gag.

References:

Keywords: HIV-1 Packaging, Native Mass Spectrometry, RNA Protein Complexes
23) Probing the mechanism of homotropic allostery in TRAP using native mass spectrometry

Melody Pepsi Holmquist (Ohio State Biochemistry Program, The Ohio State University), Elihu C. Ihms (Department of Chemistry and Biochemistry, The Ohio State University), Paul Gollnick (Department of Biological Sciences, State University of New York at Buffalo), Vicki Wysocki (Department of Chemistry and Biochemistry, The Ohio State University), Mark P. Foster (Department of Chemistry and Biochemistry, The Ohio State University)

Abstract:
Homotropic allostery describes the cooperative binding of ligands to a macromolecule with multiple binding sites for the same ligand. In the case of positive cooperativity, binding of one ligand increases the affinity of the macromolecule for additional ligands; oxygen binding by hemoglobin is a classic example. In order to understand the mechanism of such allostery, one must first quantify the thermodynamic coupling between ligand binding sites. This is generally difficult because experimental measurements of affinity yield an apparent macroscopic parameter that is composed of many microscopic parameters, including the site-site coupling terms of interest. We are developing and testing an approach to provide access to those microscopic interaction terms by studying allostery in trp RNA binding attenuation protein (TRAP). TRAP is an oligomeric protein with 11 identical binding sites for the ligand tryptophan, Trp. TRAP becomes activated upon Trp binding and binds to the 5' leader region of the trp mRNA, resulting in both transcription attenuation and translation inhibition, presenting a regulated feedback loop that controls tryptophan biosynthesis in Bacillus cells. A key difficulty with accurately obtaining microscopic parameters describing cooperativity is that in typical binding measurements, where binding isotherms are constructed from ligand-dependent changes in fluorescence, NMR chemical shift, or enthalpy, allostery may distort the proportion between the measurable and the number of the bound ligands. However, the mass of a protein with \( n \) ligands bound is not biased by allosteric effects and reports directly the number of bound ligands. As long as populations can be accurately obtained from the ions counted by MS detectors, MS can be used to measure populations of bound species, thereby increasing the accuracy with which microscopic parameters can be obtained. By using native and ion mobility mass spectrometry, we measured concentration dependent binding of Trp to TRAP and use a nearest-neighbor thermodynamic model to quantify the microscopic thermodynamics of the cooperativity in TRAP. In addition, we used solution measurements (ITC) in MS-compatible buffers to test and validate native MS as a tool to measure microscopic thermodynamic parameters.

Keywords: microscopic thermodynamics, native mass spectrometry, isothermal titration calorimetry
24) A family expands: Characterization of human TRMT10B, a multifunctional tRNA methyltransferase

Nathan Howell (Ohio State University), Jane Jackman (Ohio State University)

Abstract:
By linking genetic information with protein synthesis, tRNA performs a critical role in biology. To satisfy the cell's need for a high quality pool of tRNA, substantial resources are invested into producing functional tRNA molecules, including an extensive system of post-transcriptional nucleotide modifications. Through this universal process, chemical functional groups are changed, rearranged, and added to individual nucleotide residues in a pattern that is specific for each target tRNA. One such modification is the addition of a methyl group to the N-1 atom of ninth-position purines (m1N9), which occurs in archaea and eukarya and is catalyzed by the Trm10 family of enzymes. Initially discovered in S. cerevisiae, this SPOUT methyltransferase family was believed to comprise members that only exhibit m1G9 activity. Recently however, several archaeal and mitochondrial Trm10 homologs were unexpectedly demonstrated to form m1A9, either in addition to or instead of m1G9; this activity is not well understood, particularly in light of different pKa values exhibited by the methylated atoms. Intriguingly, higher eukaryotes encode multiple Trm10 paralogs, including two cytoplasmic enzymes in humans: TRMT10A and TRMT10B. TRMT10A is suggested to be responsible for formation of all m1G9 in human cytosolic tRNA, whereas TRMT10B has been resistant to biochemical characterization. However, yeast complementation experiments and human disease models suggest that TRMT10B is not functionally redundant with TRMT10A. Using in vitro biochemical assays, primer extension, and HPLC analysis, we report that TRMT10B is indeed a tRNA methyltransferase, and is responsible for the newly reported m1A9 modification in human tRNA-Asp. Furthermore, our data support the role of TRMT10B as a multifunctional methyltransferase capable of both A and G methylation, forming distinct patterns of previously unknown methylation products in a substrate-dependent manner on human tRNA. Human TRMT10B is the first eukaryotic cytosolic enzyme to display such unique functionality in terms of its target nucleotide identity and substrate discrimination. Our results not only illuminate the enzyme responsible for a newly identified m1A9 in human tRNA, but shed further light on the unusually complex biochemical repertoire of the Trm10 enzyme family.

Keywords: tRNA modifications, enzymology, Trm10
The Effect of Size and Shape of RNA Nanoparticle on Biodistribution

Daniel L. Jasinski (College of Pharmacy and College of Medicine, The Ohio State University), Hui Li (College of Pharmacy and College of Medicine, The Ohio State University), Peixuan Guo (College of Pharmacy and College of Medicine, The Ohio State University)

Abstract:
In vivo liver or other organ accumulation of drugs or nanoparticles is a hurdle in therapeutics due to the consequence of toxicity and side effects. The mechanism of organ accumulation has been extensively investigated, and it has been reported that the accumulation is size-dependent and in large part due to engulfing of nanoparticles by macrophages such as the Kupffer cell in livers. However, findings in literature about the effect of size on organ accumulation are inconsistent due to the complication by the effect of shape, which varies from nanoparticle to nanoparticles. RNA nanoparticles are tunable in size and shape, making them an ideal system to study how these factors affect in vivo delivery properties such as circulation time and biodistribution. Unique to RNA nanoparticles, size could be studied without change in shape, resulting in a true comparison of size effect on pharmacokinetics and biodistribution. In this study, we constructed RNA triangles, squares, and pentagons that display different size. The effect of particles size and shape in relation to biodistribution is investigated using the RNA polygons with variable size but identical shape, and with variable shape but identical size. It was found that increasing the size of the nanoparticles increased circulation time, while still avoiding significant entrapment in the liver and kidneys. Difference in shape appeared to have no effect on final biodistribution, however, evidence points towards diverse routes of clearance among different RNA polygons. Despite increasing size, we still see little accumulation in the liver, kidneys, and spleen after 24 h of circulation with particle with 5-25 nanometers.

References:

Keywords: RNA Nanotechnology, biodistribution, size and shape effects
26) Interaction between human Glutamyl-Prolyl tRNA synthetase Linker domain and HIV-1
Matrix: implications for viral infectivity

Danni Jin, Nathan Titkemeier, Alice Duchon (Department of Chemistry and Biochemistry, Center for RNA Biology, Center for Retrovirus Research, The Ohio State University, Columbus, OH, 43210), Yiping Zhu, Stephen P. Goff (Department of Biochemistry and Molecular Biophysics, Howard Hughes Medical Institute, Columbia University, New York, NY, 10032), Corine St. Gelais, Li Wu (Center for RNA Biology, Center for Retrovirus Research, Department of Veterinary Biosciences, The Ohio State University, Columbus, OH 43210), Karin Musier-Forsyth (Department of Chemistry and Biochemistry, Center for RNA Biology, Center for Retrovirus Research, The Ohio State University, Columbus, OH, 43210)

Abstract:
Aminoacyl-tRNA synthetases (AARSs) are part of the cellular translational machinery and primarily function to ligate specific amino acids to cognate tRNAs. Mammalian Glutamyl-Prolyl tRNA Synthetase (EPRS) is a bifunctional AARS with a unique Linker domain between two synthetase domains, which has been shown to be involved in several non-canonical functions. EPRS is a component of the multi-aminoacyl-tRNA synthetase complex (MSC) together with seven other AARSs and three scaffold proteins. The genome of Human Immunodeficiency Virus Type 1 (HIV-1) encodes 15 viral proteins, which interact with host factors that affect viral replication. Based on published studies, many MSC components are included in the interactome of HIV-1 Matrix (MA) protein (Jäger, S., et al. (2012) Nature 481, 365-370). We hypothesize that this virus-host interaction contributes to efficient viral infection. Our new data suggest that the expression of EPRS is downregulated in HIV-1 infected cells, and HIV-1 infectivity decreases in 293T cells stably overexpressing the Linker. Preliminary data also show that MA interacts with the MSC through the Linker domain in an RNA-dependent manner, but the specific RNA mediating the interaction is unknown. We are unable to detect an interaction between MA and the purified EPRS Linker in vitro in the absence of RNA, and both proteins interact with many tRNA species with similar affinity. MA mutants that fail to interact with the MSC in cells were identified by alanine-scanning mutagenesis, and viruses expressing these MA mutants are less infectious. Based on these observations, we propose that the Linker domain of EPRS restricts HIV-1 infection, and that the virus has evolved to downregulate this host cell protein.

References:

Keywords: EPRS, HIV-1
27) Tumor suppressor genes in vulval development reveal cryptic genetic variation between C. elegans and C. briggsae

Leanne H. Kelley (Department of Molecular Genetics, Ohio State University), Marcos Corchado (Department of Molecular Genetics, Ohio State University), Abdulrahman M. Jama (Department of Molecular Genetics, Ohio State University), Helen M. Chamberlin (Department of Molecular Genetics, Ohio State University)

Abstract:
The EGFR/Ras/MAPK pathway, which is conserved across metazoans, is an important regulator of cell proliferation. Defects in this pathway are hallmarks of some cancers, such as melanoma. Our research uses Caenorhabditis vulval development as a model to enhance our understanding of the mechanisms that govern regulation in the EGFR/Ras/MAPK pathway. A recent study has identified four prospective tumor suppressor genes in C. briggsae that inhibit vulval cell hyperproliferation, indicated by the presence of the multivulva (Muv) phenotype when these genes are altered (Sharanya et al. 2015). Whole-genome sequencing has identified the C. briggsae Muv genes as Cbr-spr-4, Cbr-gon-14, Cbr-htz-1, and CBG03376. Given that the Muv genes encode nuclear proteins, it is hypothesized that these genes regulate vulval development by altering the transcription of other genes. Rescue experiments suggest that Cbr-htz-1 functions cell non-autonomously to regulate vulval cell fate, leading us to hypothesize that these genes may increase the transcription of the ligand gene lin-3/EGF. However, qRT-PCR data does not show an increase in lin-3/EGF transcript abundance for the C. briggsae Muv mutants, while RNA-seq data for two of the C. briggsae Muv mutants show an increase in lin-3/EGF transcription abundance. lin-3 RNAi knockdown experiments may further clarify whether these C. briggsae Muv mutants regulate vulval development through lin-3/EGF. In efforts to determine if these genes similarly impact the EGFR/Ras/MAPK pathway in C. elegans, mutant C. elegans lines have been established for three of the four Muv genes. Two of these mutant lines (Cel-gon-14 and Cel-htz-1) confer a Muv phenotype, while the mutant Cel-spr-4 line does not. Uncovering cryptic genetic variations within vulval development between C. briggsae and C. elegans will ultimately broaden our understanding of nematode vulval development and the role of tumor suppressors in EGFR/Ras/MAPK pathway regulation.

References:

Keywords: Caenorhabditis, tumor suppressor
28) Identification of Aminoacyl-tRNA Synthetase Inhibitors against Eukaryotic Pathogens

Paul Kelly (Molecular, Cellular, and Developmental Biology Program, Ohio State University), Tammy Bullwinkle (Department of Microbiology, Ohio State University), David Ardell (Program in Quantitative Systems Biology, University of California, Merced), Roger Linington (Department of Chemistry, Simon Fraser University), Abhay Satoskar (Department of Microbiology and Department of Pathology, Ohio State University), Michael Ibba (Department of Microbiology, Ohio State University)

Abstract:
While antibiotics have been developed to specifically target prokaryotic cellular machinery, developing therapies against eukaryotic pathogens has proven to be more challenging due to conservation between potential pathogen drug targets and their host counterparts. Previous studies have identified antifungal therapies that target pathogen aminoacyl-tRNA synthetases (aaRS) with some degree of specificity, indicating that these essential enzymes could be developed as drug targets for a variety of infections. AaRS transfer amino acids on to their corresponding tRNA in a two-step process: 1) binding to the free cognate amino acid in an ATP-dependent manner (activation) and 2) aminoacylation of the amino acid on to the cognate tRNA (transfer). Using bioinformatic and in vitro biochemical analysis, we have identified alanyl-tRNA synthetase (AlaRS) in the eukaryotic parasite, Leishmania major as a potential target for antileishmanial therapies. Furthermore, preliminary identification of potential inhibitors prevented amino acid activation of the L. major AlaRS but had no effect on the human counterpart enzyme. Future studies look to determine the mechanism of action of these inhibitors and determine the broad spectrum potential of these inhibitors against other eukaryotic pathogens.

Keywords: Aminoacyl-tRNA synthetase, tRNA, Drug Discovery
Investigating the Conformational Changes in the HIV-1 5'UTR and Their Impact on gRNA Selective Packaging

Jonathan Kitzrow (The Ohio State University), Erik Olson (The Ohio State University), Karin Musier-Forsyth (The Ohio State University)

Abstract:
HIV-1 packages its genetic material into budding virions as a dimer of full length-genomic RNA (gRNA). HIV-1 gRNA packaging is facilitated through interactions between the NC domain of the HIV-1 Gag protein and specific structural elements in the 5'-untranslated region (5'UTR) of gRNA. HIV-1 gRNA also serves as the template for Gag/Gag-Pol translation, essential structural and enzymatic polyproteins of the HIV-1 virion. A conformational change in the 5'UTR has previously been proposed to govern a switch from translation competent or packaging competent gRNA. NMR studies have shown that the U5 region of the 5'UTR can interact with both the dimer initiation signal (DIS) and Gag start codon (AUG), located in the 5'UTR. These interactions selectively expose either the DIS (packaging competent form) or AUG (translation competent form) elements in a mutually exclusive manner. Gag, Gag-NC, and tRNALys3 may all effect this conformational equilibria. To test these interactions we have designed a FRET based assay to probe the HIV-1 5’UTR conformational state in dimeric and monomeric conditions.

Keywords: HIV-1 gRNA, FRET
Mechanistic features of the bifunctional tRNA methyltransferase, Trm10

Aiswarya Krishnamohan (Ohio State Biochemistry Program, Dept. of Chemistry and Biochemistry), Jane Jackman (Ohio State Biochemistry Program, Dept. of Chemistry and Biochemistry)

Abstract:
The tRNA m1G9 methyltransferase (Trm10) family enzymes methylate the 9th nucleotide position of multiple tRNAs using S-adenosyl methionine (SAM) as the methyl group donor. Although the founding member of the Trm10 family, Saccharomyces cerevisiae Trm10 (ScTrm10), is an obligate m1G9 methyltransferase, some homologs of Trm10 have been shown to also methylate adenosine (A9) to form m1A9, an expansion of substrate specificity not observed in other characterized methyltransferases. Moreover, although Trm10 has been classified as a member of the SpoU-TrmD (SPOUT) family of methyltransferases, it does not share key mechanistic features associated with members of this enzyme family, including the characteristic dimeric structure that is essential for forming the active site in most SPOUT enzymes. These observations suggest that Trm10 enzymes exhibit a distinct active site and therefore a novel mechanism of catalysis compared to other SPOUT members. To address these questions about the mechanism and the unique substrate specificity of Trm10 enzymes, a detailed biochemical and kinetic analysis of the m1G9 catalyzing ScTrm10 and a m1A9/m1G9 catalyzing bifunctional Thermococcus kodakarenensis Trm10 (TkTrm10) was performed, providing evidence for an atypical tRNA methyltransferase mechanism that characterizes this enzyme family.

Using a structure of Trm10 solved in the absence of tRNA as a guide, we investigated the catalytic roles of residues proposed to interact with the SAM methyl donor, a putative general base, and a proposed tRNA binding surface. To study the monomeric form of Trm10 binding to tRNA we used chemical and nuclease footprinting to identify elements on the tRNA that is recognized by Trm10. While our data support the crystallographically observed SAM binding site, we provide direct evidence contradicting the identification of the proposed general base and tRNA binding surface. These new and unexpected findings will enable future efforts to fully understand the mechanism of catalysis and substrate recognition by Trm10 so that its complex effects on biology can be understood.

Keywords: tRNA modification, tRNA methylation, Enzyme mechanism
Quality control by trans-editing factor prevents global mistranslation of non-protein amino acid alpha-aminobutyrate

Lexie Kuzmishin (The Ohio State Biochemistry Program, Department of Chemistry and Biochemistry, and Center for RNA Biology, The Ohio State University), William Cantara (Department of Chemistry and Biochemistry, and Center for RNA Biology, The Ohio State University), Jo Marie Bacusmo (Department of Chemistry and Biochemistry, and Center for RNA Biology, The Ohio State University), Karin Musier-Forsyth (The Ohio State Biochemistry Program, Department of Chemistry and Biochemistry, and Center for RNA Biology, The Ohio State University)

Abstract:
Aminoacyl-tRNA synthetases attach cognate amino acids to tRNAs, and are a critical checkpoint in protein synthesis. ARSs misactivate amino acids that are similar to their cognate substrates; thus, aminoacyl-tRNA editing mechanisms are needed to prevent widespread mistranslation. In addition to Ala-tRNAPro editing mediated by the insertion (INS) domain present in most bacterial prolyl-tRNA synthetases (ProRS), single-domain trans-editing factors structurally homologous to INS are present in some organisms. To date, INS-like trans-editing proteins have been shown to act on tRNAs mischarged with proteogenic amino acids. Here, we show that Rhodopseudomonas palustris ProXp-x, a previously uncharacterized INS homolog, displays robust editing of tRNAPro and tRNAVal mischarged with the non-protein amino acid α-aminobutyrate (2-Abu) in vitro. 2-Abu is the product of the transamination of oxobutyrate, a metabolite in Ile biosynthesis, and is a precursor metabolite in Thr biosynthesis. 2-Abu is mischarged by *E. coli* ValRS and ProRS in vitro. In vivo experiments showed that 2-Abu is toxic to an *E. coli* strain encoding an editing-defective ValRS. However, expression of *R. palustris* ProXp-x rescues cell growth, presumably by removing 2-Abu that has been mischarged onto tRNAVal by the editing-deficient ValRS. We hypothesize that ProXp-x serves as a general 2-Abu-tRNA deacylase to prevent accumulation of this species under growth conditions wherein cellular 2-Abu concentrations are high. A *R. palustris* ProXp-x deletion strain has been constructed, and experiments are underway to identify the environmental stresses that impart a growth defect in the null strain.

Keywords: trans-editing, ami, Rhodopseudomonas palustris
Engineering Polyvalent DLL1 Ligands for Cancer Immunotherapy

Nicholas E. Long (Ohio State Biochemistry Program), Ming Poi (The Ohio State University College of Pharmacy), Mikhail M. Dikov (The Ohio State University College of Medicine), Thomas J. Magliery (The Ohio State University Department of Chemistry and Biochemistry)

Abstract:
Currently, immunotherapy is one of the most promising forms of cancer therapy. This treatment method relies on the host immune system’s natural ability to fight aberrant cell development and growth (i.e. cancer). However, in many cases a tumor finds ways to evade immune cell detection. Undeterred, these cancers can continue to grow and metastasize, leading to progression of the disease and poor patient prognosis. Previous proof-of-concept experiments performed in the Dikov lab have shown promise in “re-energizing” immune T-cell function even after cancer has previously evaded detection. Through activation of the Notch signaling pathway in T-cells using clustered DLL1 ligands, mouse model studies have shown overall decreased tumor growth and longer survival time. Unfortunately, this DLL1 cluster does not satisfy requirements for FDA approval, cannot be patent protected, and is economically unfeasible for large-scaled industrial production. For these reasons, an alternative protein therapeutic is needed which has the same potency (and mode of Notch activation) as the DLL1 cluster, but without its restrictions.

In a collaborative effort directed by The Ohio State Drug Development Institute, the Dikov, Poi, and Magliery Labs have successfully engineered and characterized a new multivalent DLL1 ligand based Notch activator. This was accomplished by truncation studies of DLL1 which indicated the minimum domains required for function and various genetic and chemical methods to make multivalent conjugates thereof. This protein therapeutic has been shown in vitro to activate the Notch signaling pathway in mouse EL4 T Cells, is novel intellectual property, and can be reliably and cheaply produced in E. coli. It is our hope that with the addition of in vivo tumor studies, we will have created a novel immunotherapy cancer drug with broad applications.

Keywords: Immunotherapy, Protein Engineering, Cancer
N6-methyladenosine of HIV-1 RNA regulates viral replication and gene expression

Wuxun Lu (Center for Retrovirus Research; Department of Veterinary Biosciences, The Ohio State University), Nagaraja Tirumuru (Center for Retrovirus Research; Department of Veterinary Biosciences, The Ohio State University), Pratibha Chowdary Koneru (Center for Retrovirus Research; College of Pharmacy, The Ohio State University), Chang Liu (Department of Chemistry, Department of Biochemistry and Molecular Biology, Institute for Biophysical Dynamics, The University of Chicago), Mamuka Kvaratskhelia (Center for Retrovirus Research; College of Pharmacy, The Ohio State University), Chuan He (Department of Chemistry, Department of Biochemistry and Molecular Biology, Institute for Biophysical Dynamics, The University of Chicago)

Abstract:
The internal N6-methyladenosine (m6A) modification of cellular RNA controls post-transcriptional gene expression. The dynamic addition, removal, and recognition of m6A in cellular RNAs are coordinately regulated by three groups of host proteins, including methyltransferases (writers), demethylases (erasers), and m6A-binding proteins (readers). The YTH domain family proteins (YTHDF1-3, readers) bind to m6A-modified cellular RNA and modulate RNA metabolism and processing. Three recent studies highlighted the importance of m6A modification of HIV-1 RNA in regulating viral replication and gene expression (Lichinchi, Nat. Microbiol. 2016; Kennedy, Cell Host Microbe. 2016; Tirumuru, eLife, 2016). We reported that overexpression of YTHDF1-3 proteins in cells inhibits HIV-1 infection, mainly by decreasing HIV-1 reverse transcription, while knockdown of endogenous YTHDF1-3 in primary CD4+ T-cells increases HIV-1 infection (Tirumuru, eLife, 2016). Here, we aim to better understand the mechanisms by which YTHDF1-3 inhibit HIV-1 infection. We hypothesize that the m6A modification of HIV-1 RNA may affect its structure and/or binding to YTHDF1-3 proteins, thereby regulating HIV-1 reverse transcription and viral replication. Using an AlphaScreen-based binding assay, we determined that recombinant YTHDF1-3 proteins strongly bound to an m6A-modified HIV-1 RNA fragment compared to an unmodified RNA counterpart. We generated three HIV-1 mutants that could impair m6A modification in the 5’ leader sequence of HIV-1 RNA. We investigated the effect of these mutations on HIV-1 replication and viral protein expression in cells. Our results indicate an important role of m6A modification of HIV-1 RNA in viral replication and gene expression.

Keywords: m6A modification, HIV-1 replication
35) Utilization of an RNase P-based assay for accurate measurement of 5'-modification in tRNAs

Seth Lyon (Chemistry and Biochemistry), Edward J. Behrman (Chemistry and Biochemistry), Venkat Gopalan (Chemistry and Biochemistry)

Abstract:
RNAs perform an array of functions in all life (e.g., catalysis, chromatin remodeling, structural scaffolds for large assemblies). Understanding this versatility of RNAs requires knowledge of their structure-function relationships. Probing RNA structure often requires spectroscopic methods, which in turn necessitates strategies for post-synthetic, site-specific incorporation of chemical probes into target RNAs. One method to achieve this goal is through in vitro transcription (IVT) of RNAs by T7 RNA polymerase and a GTP-initiating class III Φ6.5 promoter. In addition to GTP, T7 RNA polymerase can incorporate 5'-modified guanosine analogs during transcriptional priming. Because the nucleoside/nucleotide monophosphate guanosine analog cannot be used in elongation, it can only serve as the initiator. By using a 4-fold excess of the 5'-modified guanosine analog:GTP in the IVT, others have successfully biased the polymerase to initiate with the analog. We have now rigorously examined the extent of this bias with different RNAs and with 5'-deoxy-5'-azidoguanosine (Az-G) as the modifier. For small RNAs (5 nts), T7 RNA polymerase indeed generates mostly 5'-Az-G-modified RNAs (~75%). With tRNAs (150 nts), we determined that there is an unexpected maximum threshold (<50%) for the incorporation of Az-G into full-length transcripts even across a range of [Az-G]:[GTP] ratios. The use of RNase P, which catalyzes the cleavage of the 5'-leader in pre-tRNAs, was essential to obtain this accurate measurement. The ceiling that we observed for initiation of pre-tRNAs with 5'-modified guanosine analogs likely reflects a preponderance of 5'-Az-G-containing abortive transcripts, which are less likely when GTP is the initiator. Our work suggests that spectrophotometric (or other) methods previously used to determine the extent of incorporation overestimate the extent of 5'-modification by including both aborted and full-length RNAs. Our results should motivate use of T7 RNA polymerase mutants that have a decreased tendency for premature termination upon initiation with guanosine analogs.

Keywords: RNA-labeling
36) SELECTIVITY mechanisms of aminoacyl-tRNA trans-editing factors

Xiao Ma (Department of Chemistry and Biochemistry, Center for RNA Biology, The Ohio State University), Eric M. Danhart, Marina Bakhtina, Will Cantara (Department of Chemistry and Biochemistry, Center for RNA Biology, The Ohio State University), Lexie Kuzminshin, Brianne Sanford (Department of Chemistry and Biochemistry, Center for RNA Biology, The Ohio State University), Marija Kouti, Ronald Micura (Institute of Organic Chemistry and Center for Molecular Biosciences, Innsbruck CMBI Leopold Franzens University), Karin Musier-Forsyth, Kotaro Nakanishi, Mark P. Foster (Department of Chemistry and Biochemistry, Center for RNA Biology, The Ohio State University)

Abstract:
Aminoacyl-tRNA synthetases (aaRSs) are responsible for charging cognate amino acids onto their corresponding tRNAs, which are then delivered to the ribosome by EF-Tu. The structural features of the aaRS catalytic domain provide a high degree of selectivity for attachment of the correct amino acid; however, given the similar stereochemical properties of many of the amino acids, errors in tRNA charging can occur leading to mistranslation, especially for the smaller and isometric amino acids. In addition to the selectivity and proofreading capabilities provided by the synthetases, to maintain high-fidelity translation many organisms possess free-standing editing enzymes that function in trans to hydrolyze mischarged tRNAs. Caulobacter crescentus ProXp-ala, a structural homolog of the bacterial prolyl-tRNA synthetase editing domain, can deacylate mischarged Ala-tRNA^{Pro}. To determine how ProXp-ala selectively deacylates Ala-tRNA^{Pro} over the cognate Pro-tRNA^{Pro}, we performed NMR-mapping studies, site-directed mutagenesis experiments, and MD simulations of ProXp-ala bound to an uncharged microhelix^{Pro} and a non-hydrolyzable, amide-linked Ala-microhelix^{Pro} substrate analog. These studies show that helix α2, which exhibits dynamics on the ps-ns timescale, is less mobile when bound to Ala-microhelix^{Pro}, but remains dynamic when bound to the uncharged microhelix^{Pro}. Flexibility of helix α2 is also supported by comparison of a new crystal structure of the protein we obtained to a previously published structure. The structures are virtually identical but differ only in the helix α2 orientation. Correspondence between stereochemical properties of mischarged amino acids and the deacylation activity of ProXp-ala shed light on both the role of size and aminoacyl functional groups in the discrimination mechanism. Taken together, these data suggest that size exclusion, chemical and conformational selection contribute to specific deacylation of Ala-tRNA^{Pro} by ProXp-ala.

References:
3. Danhart E. et. al (In revision)

Keywords: trans-editing, NMR, RNA-protein interaction
37) Alternate Exon Junction Complexes potentiate branched nonsense-mediated mRNA decay pathways

Justin Mabin (Molecular Genetics, The Ohio State University), Lauren Woodward (Molecular Genetics, The Ohio State University), Robert Patton (Department of Physics, The Ohio State University), Mengxuan Jia, Vicki Wysocki (Department of Chemistry and Biochemistry, The Ohio State University), Ralf Bundschuh (Department of Physics, Division of Hematology, College of Medicine, Center for RNA Biology, The Ohio State University), Guramrit Singh (Department of Molecular Genetics, Center for RNA Biology, The Ohio State University)

Abstract:
Pre-mRNA splicing deposits the exon junction complex (EJC) 24 nt upstream of most exon-exon junctions in a sequence-independent manner. The stable EJC core consists of eIF4AIII, Y14 and Magoh, which serves as an interaction platform for more dynamic peripheral EJC proteins that direct mRNA export, localization, translation and nonsense-mediated mRNA decay (NMD). To date, the specificity of the peripheral EJC proteins in target RNA selection and the resulting impact on mRNA fate remains largely unknown. We recently reported that MLN51, a protein widely presumed to be an EJC core factor, and several peripheral EJC proteins are sub-stoichiometric in EJCs purified from human cells. We have now discovered that MLN51 and RNPS1, two EJC proteins previously implicated in NMD, exist in two mutually exclusive EJCs in human and mouse cells. Surprisingly, the alternate EJCs have similar occupancy on endogenous mRNAs including those targeted for NMD. However, as compared to MLN51-EJCs, RNPS1-EJCs are more enriched in non-canonical sites, which we previously showed to overlap with SR protein binding sites. Indeed, biochemical and proteomic analysis of purified MLN51 and RNPS1 complexes from HEK293 cells shows that while the two alternate EJCs share the three EJC core proteins, only RNPS1-EJCs associate with SR proteins. Intriguingly, RNPS1 depletion causes >2-fold upregulation of most of the endogenous NMD targets tested while MLN51 depletion leads to modest or no increase in their levels. Overall, our data suggests that endogenous EJCs exist in at least two mutually exclusive complexes defined by either RNPS1 or MLN51. While both complexes are likely to be active in NMD, they may represent two distinct NMD branches in which RNPS1-EJCs, in cooperation with SR proteins, promote more efficient RNA turnover as compared to SR protein devoid MLN51-EJCs. We are currently testing this model to understand how EJC composition and its cooperativity with SR protein impacts mRNA fate.

Keywords: Exon-Junction Complex, Nonsense-mediated mRNA decay, Post-transcriptional gene regulation
38) Isoacceptor specific characterization of tRNA aminoacylation and misacylation in vivo

Kyle Mohler (Department of Microbiology, Center for RNA Biology), Rebecca Mann (Ohio State Biochemistry Program), Michael Ibba (Department of Microbiology, Center for RNA Biology, Ohio State Biochemistry Program)

Abstract:
Amino acid misincorporation during protein synthesis occurs due to misacylation of tRNAs or defects in decoding at the ribosome. While misincorporation of amino acids has been observed in a variety of contexts, less work has been done to directly assess the extent to which specific tRNAs are misacylated in vivo, and the identity of the misacylated amino acid moiety. Here we describe tRNA isoacceptor specific aminoacylation profiling (ISAP), a method to identify and quantify the amino acids attached to a tRNA species in vivo. ISAP allows compilation of aminoacylation profiles for specific isoacceptors tRNAs. To demonstrate the efficacy and broad applicability of ISAP, tRNA$^{\text{Phe}}$ and tRNA$^{\text{Tyr}}$ species were isolated from total aminoacyl-tRNA extracted from both yeast and Escherichia coli. Isolated aminoacyl-tRNAs were washed until free of detectable unbound amino acid and subsequently deacylated. Free amino acids from the deacylated fraction were then identified and quantified by mass spectrometry. Using ISAP allowed quantification of the effects of quality control on the accumulation of misacylated tRNA species under different growth conditions.

References:

Keywords: tRNA, Misacylation, Quality Control
Dissecting structural and functional cooperation in RNase P, a multi-subunit catalytic ribonucleoprotein

Ila Marathe (Department of Chemistry and Biochemistry), Lien Lai (Department of Chemistry and Biochemistry), Akiko Tanimoto (Department of Chemistry and Biochemistry), Yi Luo (Department of Physics), Vicki Wysocki (Department of Chemistry and Biochemistry), Michael Poirier (Department of Physics)

Abstract:
RNase P is an essential ribozyme that catalyzes Mg$^{2+}$-aided maturation of tRNAs in all life forms. An RNase P RNA (RPR) and five RNase P proteins (RPPs) make up archaeal RNase P. In addition to serving as a model to uncover the cooperation between a catalytic RNA and multiple protein subunits, archaeal RNase P is an excellent proxy for the intractable and homologous eukaryotic cousin whose dysregulation leads to disease. Towards our goal of understanding how proteins modulate RNA catalysis, we focus here on archaeal L7Ae, a ribosomal protein that also acts as an RPP. L7Ae facilitates axial bending of RNAs by binding kink-turns, a widespread structural motif implicated in various aspects of RNA function. To test the hypothesis that L7Ae might aid in recruiting other RPPs to the RPR at physiological [Mg$^{2+}$], we seek to uncover its roles in assembly of and catalysis by archaeal RNase P. By employing native mass spectrometry, single-molecule fluorescence, and activity assays, we expect to gain insights into: (i) stoichiometry of L7Ae, (ii) influence of L7Ae on the RNase P assembly landscape, and (iii) the ability of L7Ae to modulate the Mg$^{2+}$ dependence of RNase P activity. Our results support the use of these methods to map the hierarchy and cooperation among RPPs in assembly and function of archaeal RNase P, and more broadly help elucidate the basis of human diseases where RNA-protein cooperation is defective.

Keywords: RNase P, RNA-protein cooperation, L7Ae
40) Role of Chromatin Insulator CTCF in HTLV-1 Retroviral Pathogenesis

**Michael P. Martinez** (Department of Veterinary Biosciences, The Ohio State University, Columbus, OH), **Jacob J. Al-Saleem** (Department of Veterinary Biosciences, The Ohio State University, Columbus, OH), **Amanda R. Panfil** (Department of Veterinary Biosciences, The Ohio State University, Columbus, OH), **Lee Ratner** (Department of Medicine, Washington University, St. Louis, MO), **Patrick L. Green** (Department of Veterinary Biosciences, The Ohio State University, Columbus, OH)

**Abstract:**
Human T-cell leukemia virus (HTLV-1) is a deltaretrovirus endemic to the Caribbean and Japan. HTLV-1 is the etiologic agent of adult T-cell leukemia and the neurological disorder HAM/TSP. Approximately 5-10% of infected individuals will develop disease after a prolonged latency. The exact mechanisms through which this variable latency is regulated remains nebulous. CTCF-binding factor (CTCF) is an 11-zinc finger transcriptional repressor that acts through the induction of conformational changes in chromatin structure and subsequent enhancer-blocking activity. A CTCF-binding site was recently identified within the HTLV-1 provirus and shown to affect the anti-sense derived hbz transcript. HBZ provides tumor maintenance function and supports viral persistence. Therefore, we propose to study the epigenetic effects of CTCF on HTLV-1-induced in vitro immortalization using short-term viral co-culture assays. Using an HTLV-1 proviral molecular clone, a ∆CTCF mutant was generated using site-directed mutagenesis. The ∆CTCF mutation prevents CTCF-binding via EMSA, but does not disrupt overlapping reading frames or splice sites. The ∆CTCF mutant is transcriptionally similar to WT, as demonstrated by LTR-based reporter gene assays and viral gag release. Since efficient in vitro infection of naïve T-cells by HTLV-1 requires co-cultivation with infected cells, we next used stable transfection of the WT and ∆CTCF proviral clones into a susceptible human B-cell line to produce consistent and quantifiable amounts of virus. Briefly, irradiated viral producer cells are co-cultured with freshly isolated peripheral blood lymphocytes (PBL). The initiation of transformation is apparent within 5-6 weeks following co-culture as detected by expansion of cells from the PBL cell population. Preliminary results indicate the ∆CTCF mutant has similar immortalization potential compared to WT. Future experiments will examine the effect of the CTCF mutation on viral persistence using a rabbit model of infection. Ultimately, understanding epigenetic regulation of HTLV-1 latency could provide meaningful insights into mechanisms of immune evasion.

**Keywords:** CTCF, HTLV-1, ATL
41) Investigating the biological roles for thg1-like proteins in Myxococcus xanthus

Ashanti Matlock (Chemistry and Biochemistry), Jane Jackman (Chemistry and Biochemistry)

Abstract:
The \( \text{tRNA}^{\text{His}} \) guanylyltransferase (Thg1) enzyme family is a unique group of proteins that catalyze 3' to 5' nucleotide addition. The biological function of 3' to 5' nucleotide addition catalyzed by Thg1 in eukaryotes is to add a single essential guanine to the 5' end of \( \text{tRNA}^{\text{His}} \). By modifying \( \text{tRNA}^{\text{His}} \) with an additional guanine at the -1 position, Thg1 ensures translational fidelity by guaranteeing proper charging by histidyl amino acyl synthetase. Conversely, the biological significance of TLPs in bacteria and archaea is less clear. It is likely that TLPs are not used for \( \text{tRNA}^{\text{His}} \) maturation because \( G_{-1} \) is predicted to be retained in the mature transcript after 5' end processing by RNase P. Possible functions for TLPs include 5' end repair of truncated mitochondrial tRNAs, as recently demonstrated inDictyostelium discoideum. However, biological roles for bacterial TLPs remain elusive due to the difficulty of obtaining viable knockout in organisms that encode TLPs. To determine the function of TLPs in vivo we have generated a viable deletion of a TLP in \textit{Myxococcus xanthus} and are characterizing the in vitro activity of MxTLP to obtain insight into potential biological substrates.

Keywords: Thg1, new function, RNA
42) Driving protein conformational changes with light: Evidence for photoinduced structural rearrangement in a heterobimetallic Mn/Fe oxidase

Pearson T. Maugeri (Biophysics Graduate Program), Effie K. Miller (Ohio State Biochemistry Program), Zach R. Smith (Department of Chemistry and Biochemistry)

Abstract:
Metalloenzymes play a critical role in many of the life-giving reactions on the planet, such as photosynthesis and respiration. One group of metalloproteins, the ferritin-like superfamily, is involved in a diverse group of functions ranging from Fe and O₂ storage and transport to C-H bond activation and deoxyribonucleotide biosynthesis. Recently, a new subgroup has been identified within the ferritin-like superfamily that can bind both Mn(II) and Fe(II) ions, counter to the well-established Irving-Williams series. This new group has been named the R2-like ligand-binding oxidases, or R2lox, due to its similarity in sequence and metal binding properties to the class 1c R2 subunit of the ribonucleotide reductases. R2lox possesses many characteristics that distinguish it from members of the canonical di-iron ferritin-like proteins, such as the ability to bind either Fe/Fe or Mn/Fe cofactors, the presence of an exogenous fatty acid lipid bound to the metal centers, and the capability to perform two-electron oxidation reactions, as evidenced by formation of a Tyr-Val crosslink upon oxygen activation. Recently, we have observed that R2lox undergoes a structural rearrangement in the active site upon photoirradiation. Using a variety of spectroscopic and photochemical methods, including a state-of-the-art resonance Raman system, a structure and mechanism for formation of this new state has been proposed. Future experiments relating this state to a possible biological function will be discussed.

References:

Keywords: metalloproteins, resonance Raman, photochemistry
Cell-autonomous action of RNA polymerase IV maintains the epigenetic repression of a paramutant pl1 allele

Allison McClish (Department of Molecular Genetics, The Ohio State University), Brian Giacopelli (Molecular, Cellular and Developmental Biology Graduate Program, The Ohio State University), Jay B. Hollick (Department of Molecular Genetics, The Ohio State University; Center for RNA Biology, The Ohio State University)

Abstract:
Paramutation describes a heritable epigenetic change in gene regulation initiated by trans-homolog interactions and maintained by components, including the largest subunit of RNA polymerase IV (RPD1), required for the biogenesis of small RNA (sRNA)1. Because sRNAs in eudicots can move from cell to cell, exerting non-cell-autonomous effects on gene expression, we tested the cell-autonomous action of presumed sRNAs in maintaining repressed expression of a paramutant purple plant1 (pl1) allele (Pl1-Rh) by evaluating genetic mosaics for anthocyanin production related to RPD1 function. RPD1 dysfunction is associated with increased pigmentation in plants that are homozygous for repressed Pl1-Rh alleles (designated Pl'). By comparing pigmentation in hemizygous rpd1 mutant sectors to that of adjacent non-mutant tissues we could evaluate the potential cell-autonomy of normal RPD1 function in repressing Pl'. If RPD1 function acts in a cell-autonomous manner, increased anthocyanin production would be expected throughout the sector, while non-cell-autonomous function would maintain pigment repression, at least at sector boundaries. One thousand nine plants (half heterozygous for the rpd1-9 mutant allele) from irradiated seeds were evaluated in summer 2016. Of the 72 sectors greater than 0.5cm in width that were examined, 60 had sharp boundaries of dark pigment coincident with the cell-autonomous albino phenotype marking RPD1 loss. This result indicates that RPD1 actions - including the resulting sRNAs - are cell autonomous in their ability to induce and maintain Pl' states. This interpretation stands in contrast to results from grafting experiments in eudicots in which mobile sRNAs direct systemic epigenetic modifications3.

References:
1. Brink 1958 CSH Symp Quant Biol 23, 379
2. Erhard 2009 Science 27, 323
3. Lewsey 2016 PNAS 9, 113

Keywords: gene-regulation, mobility, maize
44) Elucidating the mechanisms for MDM2 alternative splicing regulation: The role of SRSF2 and miR29-b

Matias Montes (Molecular, Cellular and Developmental Biology Graduate Program, The Ohio State University), Daniel Comiskey (Molecular, Cellular and Developmental Biology Graduate Program, The Ohio State University), Dawn Chandler (Center for Childhood Cancer and Blood Diseases, Nationwide Childrens Hospital)

Abstract:
MDM2, a well-known negative regulator of the tumor suppressor protein P53, goes through alternative splicing and one of its spliced isoforms, MDM2-ALT1 comprised of exons 3 and 12, is highly expressed in several cancers such as lung carcinoma, liposarcoma or rhabdomyosarcoma, correlating its expression with a poor disease prognosis. MDM2-ALT1 expression is also upregulated under conditions of cellular genotoxic stress. Nevertheless, the way that MDM2 splicing is controlled is not entirely understood.

The micro RNA 29-b is part of the miR-29 family, and has been reported as one of the miRNAs that localize inside of the nucleus, but its function there still it is not well understood. Additionally, this miRNA has been shown to act as a tumor suppressor in certain cancer and also being downregulated in rhabdomyosarcoma. Recent studies have shown that some of the splicing factors can be regulated through miRNA-mediated gene silencing, nevertheless, to this day there is no decisive evidence showing direct regulation of alternative splicing by miRNAs.

In this study by using our minigene system and the CRISPR-Cas9 technology, we explore the role of the splicing factor SRSF2 in the alternative splicing of MDM2, hypothesizing that the splicing factor SRSF2 is involved in the splicing of MDM2. On the other hand, we investigate the idea that the miR29-b also plays a role in the alternative splicing of MDM2, by transfecting either a miR-29b or a non-specific miRNA precursor and measuring its effects on the MDM2-ALT1 expression after genotoxic stress. Our results show that SRSF2 acts as a positive regulator of the MDM2 alternative splicing, which is conserved in both mice and humans. Additionally, we show that the miR-29b is implicated in the expression of MDM2-ALT1, which could implicate for the very first time that a miRNA would be acting as a splicing factor, a novel function for these non-coding RNAs.

Keywords: miR-29b, SRSF2, MDM2-Alt1
45) Development of binding and stability assays of the anti-tumor Ab fragment 3E8 for high throughput cancer screening

Callie L. Moore (College of Arts and Sciences), Nicholas E. Long (Ohio State Biochemistry Program), Thomas J. Magliery (Ohio State Department of Chemistry and Biochemistry)

Abstract:
Tragedy falls upon more than 1,500 people a day when they lose their battle with cancer. Surgical resection of the cancerous tumor continues to be one of the most successful methods of cancer treatment. In order to remove cancerous tumors, the surgeon needs to have the ability to differentiate healthy from diseased tissue. Doctors use cancer imaging for this specific reason. In order to see the cancer, a tumor antigen, which is an antigenic substance produced within tumor cells, can be used as a marker. One of these tumor antigens is Tag72 which contains a unique sugar, Sialyl-Tn. The Magliery lab has engineered an antibody fragment, 3E8.scFv, that is able to specifically bind to this disaccharide. Using the diagnostics developed in this lab, it is our hope that surgeons will soon have a more proactive way to screen for cancer. There are still many tests needed to be performed in order to make sure this method is as safe and effective for the patients as possible. Our research is working towards learning more about the binding and stability properties of the antibody 3E8 in order to provide an effective way for surgeons to screen for cancer and safely remove the cancerous tumor. Specifically, my research involves the performance of immunohistochemistry on cancerous tissues positive for Tag72 and developing a high throughput screen to more effectively study 3E8.scFv.

Keywords: Immunohistochemistry, Cancer, High Throughput
Identification and characterization of Medicago truncatula LINC complex components with potential functions in root symbioses

Anna Newman-Griffis (Department of Molecular Genetics and Center for RNA Biology, The Ohio State University), Katherine Beigel (Department of Molecular Genetics, The Ohio State University), Iris Meier (Department of Molecular Genetics and Center for RNA Biology, The Ohio State University)

Abstract:
Symbiotic interactions with soil-dwelling microbes are vital for the fitness of many plant species, including leguminous crops such as soybean. Legumes can form symbioses with both rhizobia and arbuscular mycorrhizal fungi (AMF). Observations since the 1950s have implied, but never definitively established, that nuclear movement is involved in the initiation of both these symbioses. Recently, our lab has shown that nuclear envelope (NE)-spanning complexes comprised of outer nuclear membrane KASH proteins and inner nuclear membrane SUN proteins (known as LINC complexes) are involved in nuclear movement and positioning in Arabidopsis thaliana. These proteins interact in the nuclear envelope lumen through the C-terminal tail of KASH proteins and the SUN domain of SUN proteins. Arabidopsis is susceptible neither to rhizobia nor to AMF, so to translate our work on LINC complexes to the study of symbiosis, we must identify analogous proteins in a new model system.
To determine whether plant LINC complexes are involved in nuclear movement in root-microbe symbioses, we have adopted a reverse genetic approach in the model legume Medicago truncatula. Using the bioinformatic tools developed to identify KASH proteins in Arabidopsis, we have identified Medicago genes that encode 9 putative KASH proteins and one putative SUN protein. A bona fide KASH protein must localize to the NE, interact with SUN proteins in a KASH tail- and SUN domain-dependent manner, and require SUN interaction for NE localization. To validate the putative KASH proteins, we have performed localization and co-immunoprecipitation experiments designed to establish whether they satisfy these criteria.
Thus far we have shown that 6 KASH proteins and the SUN protein localize to the NE in Nicotiana benthamiana leaf epidermal cells. All 6 KASH proteins interact with Arabidopsis SUN in a SUN domain-dependent manner in co-immunoprecipitation experiments. Furthermore, two of the six have been shown to depend upon their KASH tails to localize properly and bind Arabidopsis SUN. We are continuing our analysis on the SUN protein and remaining KASH proteins. Our data suggest that the Medicago genome encodes bona fide KASH and SUN proteins, and that these proteins can function as part of a LINC complex in planta.

Keywords: Plant Biology, Symbiosis, Cell Biology
47) Reversible Aptamer Inhibition of Von Willebrand Factor is a Potent Thrombolytic and Ameliorates Stroke Burden Following Vascular Injury Compared to Recombinant Tissue Plasminogen Activator

David Dornbos III, Debra G. Wheeler, Allyson Huttinger, (Department of Neurological Surgery), Hallie Harris, Surya Gnyawali, (Department of Surgery), Cameron Rink, Chandan K. Sen (Department of Surgery), Nicholas Venetos, Spencer Talantino, Nicholas Musgrave (Department of Neurological Surgery), Cheyenne Jones (Department of Neurological Surgery), Jay Zweier (Department of Medicine)

Abstract:
Introduction: While recombinant tissue plasminogen activator (rTPA) is the mainstay of ischemic stroke treatment, few patients are eligible for treatment, and recanalization is only seen in 25-50%. Von Willebrand Factor (VWF) inhibition may play a role in thrombolysis.

Hypothesis: VWF inhibition with an RNA aptamer lysed arterial thrombus and decreases ischemic injury. Furthermore, aptamer reversal with an antidote oligonucleotide ameliorates intracranial hemorrhage (ICH).

Methods: Adult wild-type (C57BL/6J) mice were anesthetized, and the right carotid artery was exposed. Baseline carotid flow was obtained using a Doppler flow probe, and thrombotic occlusion was induced with a ferric chloride patch. After clot stabilization, mice were administered vehicle (platelet binding buffer, n=11), no infusion (n=8), rTPA (n=5) or VWF aptamer (n=5). Carotid flow was monitored for an additional 100 minutes. In a second cohort of mice, a 6-0 nylon suture was advanced within the carotid artery to generate vascular injury and ICH. Mice were given vehicle (n=16), rTPA (n=11), VWF aptamer (n=9) or aptamer/antidote (n=8). An MRI was obtained after 90 minutes to assess stroke and ICH volumes.

Results: VWF aptamer successfully restored carotid blood flow 45 minutes following carotid occlusion (Figure 1) compared to controls (p<0.01*) and rTPA (p<0.05+). Stroke volume was significantly decreased in mice treated with VWF aptamer (23.03 ± 6.81 mm3) and aptamer/antidote (12.48 ± 5.68 mm3) compared to vehicle (45.25 ± 4.14 mm3, p<0.01). ICH volumes in mice treated with rTPA (2.64 ± 0.84 mm3) were trending higher than vehicle (1.51 ± 0.17 mm3), VWF aptamer (1.92 ± 0.22 mm3) or aptamer/antidote (1.31 ± 0.35 mm3).

Conclusions: Aptamer inhibition of VWF is a potent thrombolytic agent with greater efficacy compared to rTPA. VWF inhibition appears safe with a trend toward lower ICH volumes in animals treated with aptamer and aptamer/antidote compared to rTPA.

Keywords: thrombosis, aptamer, antidote oligonucleotide
48) Biogenesis and functional consequences of the intronic Drosophila RNase P RNA

Geeta Palsule (The Ohio state University, Department of Molecular Genetics), Lien B. Lai (The Ohio state University, Department of Chemistry and Biochemistry ), Venkat Gopalan (The Ohio state University, Department of Chemistry and Biochemistry ), Amanda Simcox (The Ohio state University, Department of Molecular Genetics)

Abstract:
RNase P, an essential and conserved ribonucleoprotein complex that catalyzes removal of the 5' leader from pre-tRNAs, is comprised of a catalytic RNA (RNase P RNA, RPR) and a variable number of proteins (1). In eukaryotes, RPR was considered a canonical RNA Pol III-regulated transcript, until we discovered that in insects and crustaceans it is embedded in an intron of a Pol II recipient gene and lacks signals for Pol III transcription. In Drosophila, we showed that RPR is transcribed as part of a Pol II-regulated recipient gene transcript (2). To enable this transcriptional switch, that occurred ~500 mya, we hypothesize that nucleases involved in processing other ncRNAs were coopted to trim the 5' and 3' ends of the RPR’s recipient intron and generate the mature RPR. To identify these nucleases, I used RNAi knockdown of candidates in Drosophila cells and examined the impact on RPR biogenesis. My results suggest that XRN2 and the exosome are involved in maturation of RPR. Interestingly, XRN2, a 5' to 3' exonuclease, affects both 5' and 3' processing. I am currently investigating how 5' and 3' end processing are coupled by determining if XRN2 recruits factors to the 3' end and/or resolves a secondary structure in the RNA to reverse a block on 3' processing. To determine whether the mode of transcription has functional consequence(s), I am engineering transgenes regulated by an ancestral-type, Pol III promoter. Purification of RNase P from cells expressing a transgene shows that Pol III-derived RPR co-purifies with the holoenzyme. Preliminary results from pre-tRNA cleavage assays suggest that the Pol III-derived RPR may be active. I am using CRISPR/Cas9 genome editing to generate a fly solely expressing a Pol III-regulated RPR, as this will enable a phenotypic analysis at the molecular, cellular and organismal levels. Collectively, these results will determine whether there is a link between transcriptional control, biogenesis, and function in Drosophila RPR.

References:

Keywords: RNase P, RNA processing, Transcriptional regulation
49) Structural and Functional study of human Argonaute3

Mi Seul Park (Department of Chemistry and Biochemistry, The Ohio State University), Hong-Duc Phan, Florian M. Busch, Kimberly Nguyen (Department of Chemistry and Biochemistry, The Ohio State University), Junan Li, Ming Poi (Division of Pharmacy Practice and Science, College of Pharmacy, The Ohio State University), Vicki H. Wysocki (Department of Chemistry and Biochemistry, The Ohio State University), Kotaro Nakanishi (Department of Chemistry and Biochemistry, The Ohio State University)

Abstract:
microRNAs cooperates with Argonaute proteins in the RNA-induced silencing complexes that post-transcriptionally regulate gene expression by degrading target mRNAs. In humans, there are four Argonaute paralogs: Argonaute1, Argonaute2, Argonaute3, and Argonaute4. To date, Argonaute2 has been reported to function as the only RNA slicer, but Argonaute3 retains the same set of the catalytic tetrad, Asp-Glu-Asp-His, that Argonaute2 possesses. Therefore, why Argonaute3 has retained the catalytic tetrad throughout its molecular evolution remains a long-standing question. Here we report that Argonaute3 indeed cleaves RNAs. To revisit this question, we purified recombinant Argonaute3 from insect cells and immunopurified FLAG-tagged Argonaute3 from HEK293T cells. Both proteins showed cleavage activities though the immunopurified protein showed much higher slicer activity than the recombinant protein. Intriguingly, our biochemical data revealed that the slicer efficiencies of AGO3 heavily depended on the guide sequence and the length of target RNAs. To understand the molecular basis for the target cleavage, we also determined the first crystal structure of Argonaute3 in complex with guide RNA. Comparison with the structure of Argonaute2 elucidated the molecular mechanism that the intrinsic slicer activity of Argonaute3 is restrained by the incompletion of the nucleic acid binding channel due to the flexible N domain and its unique insertion. Given the discrepancy in the observed activities between the recombinant and immunopurified samples, we surmise that unidentified binding factor(s) in the cells stabilizes the channel, thereby activating Argonaute3 for RNA cleavage.

References:
2. Schirle, N. T., & MacRae, I. J. (2012). The crystal structure of human Argonaute2. Science (New York, N.Y.), 336(6084), 1037–1040. doi:10.1126/science.1221551

Keywords: microRNA, Argonaute
50) Fundamental Study on Nucleic Acid Three-way Junction Inspired by phi29 Bacteriophage Packaging RNA

Xijun Piao (College of Pharmacy), Hongran Yin (College of Pharmacy), Zhefeng Li (College of Pharmacy), Congcong Xu (College of Pharmacy), Hongzhi Wang, Daniel Binzel, Daniel Jasinski, (College of Pharmacy), Peixuan Guo (Center for RNA Nanobiotechnology and Nanomedicine; College of Pharmacy, Division of Pharmaceutics and Pharmaceutical Chemistry; College of Medicine, Department of Physiology & Cell Biology)

Abstract:
RNA three-way junction is common in nature and the three-way junction in phi29 bacteriophage packaging RNA has recently been used as the core in RNA nanotechnology for treatments of various cancers for the reasons listed below: 1) anionic charge disallows nonspecific passage across negatively charged cell membrane; 2) RNA nanoparticles can be readily functionalized with RNA aptamer to enhance the homing of RNA nanoparticles to cancer cells; 3) nanoscale size and shape avoids rapid renal clearance and engulfment by lung macrophages and liver Kupffer cells; 4) RNA nanoparticles show favorable biodistribution profiles with little accumulation in healthy organs, minimizing nonspecific side effects. DNA, RNA and 2′-F 3WJs have previously been studied in our group. To fully characterize phi29 3WJ and promote RNA nanotechnology, this study incorporates two well-known nuclease-resistant oligonucleotides, phosphorothioate DNA (PS DNA) and locked nucleic acid (LNA). In excellent agreement with duplex system, the thermostability trend of these five oligonucleotides within phi29 3WJ is PS DNA < DNA < RNA < 2′-F < LNA. LNA 3WJ shows significant resistance to heat or 8 M urea denaturation, as well as serum degradation. LNA 3WJ inspired by phi29 bacteriophage pRNA shows potential as a novel drug delivery platform.

References:

Keywords: phi29 three-way junction, RNA nanotechnology, thermodynamic stability
51) Using Proximity-Dependent Biotinylation to Identify Proteins Proximal to Histone Acetyltransferase 1 in vivo

Liudmila Popova (MCDB), Mark Parthun (Biological Chemistry and Pharmaclogy), Miranda Gardner (OSBP), Michael Freitas (Cancer Biology and Genetics)

Abstract:
Histone Acetyltransferase 1 (Hat1) is an evolutionarily conserved enzyme known to acetylate lysines 5 and 12 in the tail of the newly synthesized histone H4. Besides playing this role in replication-coupled chromatin assembly, Hat1 has been shown to contribute to DNA damage repair in a variety of organisms. However, much remains unknown about Hat1 functions in vivo. In order to gain detailed insight into cellular roles of Hat1 in mammalian cells we utilized a proximity-dependent biotinylation approach (BioID), which relies on fusing a mutant biotin ligase BirA (R118G) to the protein of interest (Hat1) in order to allow for biotinylation of proteins vicinal to the protein of interest in vivo. For this experiment, a triplicate of HEK 293 cells was transfected i with Hat1-BirA (R118G) construct and treated with exogenous biotin for 24 hours. Biotinylated proteins were isolated, and the pull-downs were analyzed by mass spectrometry. Statistical analysis yielded a list of ~60 proteins enriched in the Hat1-BirA (R118G) transfected-exogenous biotin treated sample compared to the control. The list of enriched proteins included actin-related proteins, transcriptional regulator KAISO, RNA-splicing-related protein GEMIN5 and other proteins. Further experiments are necessary to uncover the nature of Hat1’s relationship to the aforementioned proteins.

References:

Keywords: Hat1, BioID
Mechanistic studies of alternate functions exhibited by tRNA-His guanylyltransferase (Thg1)

Tracy M. Roach (OSBP), Jane Jackman, PhD (Department of Chemistry and Biochemistry, The Ohio State University)

Abstract:
tRNA-His guanylyltransferase (Thg1) is responsible for adding a G-1 to tRNAHis across from an A73 discriminator nucleotide. This step is essential for histidyl tRNA synthetase to recognize and aminoacylate tRNAHis. Depletion of Thg1 in several different eukaryotes results in phenotypes, ranging from cell cycle defects to temperature sensitivity, which are not readily explained by the lack of mature tRNAHises. Therefore, we hypothesize that Thg1 may exhibit other functions beyond its role in tRNAHises metabolism. To understand these possible functions, it is important to establish the molecular basis for substrate recognition by Thg1 enzymes. To provide a uniform platform for comparing activities of different Thg1 family enzymes, Thg1 activity will be tested with a set of model RNAs. The substrates will be 5-18 base pair stem-loops that mimic the coaxial stacking of the acceptor stem and T stem of tRNAHises. In order to provide a broad framework for understanding diverse enzyme recognition mechanisms, I will collectively and systematically characterize Thg1 family proteins from three eukaryotic organisms, S. cerevisiae, A. thaliana, and D. discoideum, for which distinct biochemical features of these enzymes have already been demonstrated. Among these enzymes, preferences for different substrates (tRNA vs. non-tRNA) and distinct gene structures have been observed, suggesting that these enzymes will exhibit distinct biochemical behavior with the tested RNAs that will help to provide insight into their biological role(s).

Keywords: 3-5 polymerization, RNA editing, tRNAHis guanylyltransferase
53) Modulating insulin receptor splicing as a potential therapeutic approach for rhabdomyosarcoma

Brianne Sanford (The Ohio State University Comprehensive Cancer Center, Columbus, OH 4321; Center for Childhood Cancer and Blood Diseases, The Research Institute at Nationwide Childrens Hospital, Columbus, OH, 43205), Chelsea Brown, Hemant Bid, Thomas Bebee, Daniel Comiskey Jr, (Center for Childhood Cancer and Blood Diseases, The Research Institute at Nationwide Childrens Hospital, Columbus, Ohio, 43205), Frank Rigo (Ionis Pharmaceuticals, Carlsbad, California, 92105), Peter Houghton (Greehey Childrens Cancer Research Institute, University of Texas Health Science Center, San Antonio, Texas, 78229), Dawn Chandler (1Center for Childhood Cancer and Blood Diseases, The Research Institute at Nationwide Childrens Hospital, Columbus, OH, 43205; Department of Pediatrics, The Ohio State University, Columbus, OH, 43210)

Abstract:
The insulin receptor (IR) is subject to alternative splicing to produce two isoforms: full-length IR-B and an isoform lacking exon 11 known as IR-A, the predominant isoform expressed in sarcomas. This exon encodes for 12 amino acids and results in a receptor with high affinity for a growth hormone called insulin-like growth factor 2 (IGF2), which responds to autocrine and paracrine signaling. Increased IR-A levels observed in rhabdomyosarcoma (RMS) coupled with increased expression of IGF2, programs the tumor cell for enhanced growth and angiogenesis. Furthermore, IGF2 can maintain angiogenesis through IR-A when the cognate receptor for IGF2, the IGF-1 receptor, is blocked. Interestingly we have shown hypoxia increases alternative splicing to produce more IR-A. Adaptation to the hypoxic environment is a hallmark of the neoplastic phenotype. Therefore, we hypothesize that expression of splicing factors is altered under hypoxic conditions which leads to increased alternative splicing and generation of IR-A, allowing the cancer cells a growth advantage which contributes to metastasis beyond the micrometastatic phase.

To characterize sequence elements and splicing factors involved in the regulation of INSR alternative splicing, we have developed a hypoxia-inducible splicing system, which recapitulates the splicing patterns observed in tumors. We have shown that sequence elements preceding exon 11 are critical to the increased alternative splicing we see under hypoxic conditions. As such, we have targeted these regions for antisense oligonucleotide (ASO) development to increase IR-B, with the goal of restricting proliferative signaling by IGF2 activation of IR-A. We performed an ASO walk to target regions important for exon inclusion or exclusion and have successfully targeted a binding site for the splicing factor CUGBP1. RMS-derived cell lines almost exclusively express IR-A but when treated with our lead ASO compound we see a dramatic decrease in alternative splicing resulting in increased levels of IR-B. We have also shown that RMS-derived cells treated with our splice modulating ASO exhibit reduced cell proliferation and migratory properties. We postulate that modulation of INSR splicing can be used in conjunction with already established anti-IGF-1 receptor therapies to treat RMS.

Keywords: Splicing
Regulation of myoblast migration by Eph-Ephrin signaling

Melissa Siebert (OSBP), Denis Guttridge (Molecular Virology, Immunology and Medical Genetics)

Abstract:
After birth, the largest increase in neonatal mass comes from skeletal muscle growth and this growth is necessary to gain motility and posture. Although, neonatal growth depends on myoblast precursor cells fusion to the ends of muscle fibers, our laboratory was the first to give a mechanism explaining this phenomenon. We found NG2 interstitial cells promotes the migration of myoblasts to the ends of muscle fibers through the production of a newly discovered NF-kappaB target gene, EphrinA5. The aim in this project will be to identify the Ephrin receptor (Eph) on myoblasts that interacts with the EphrinA5. I have found that of the six known receptors that can bind EphrinA5; only EphA3, EphA4 and EphB2 RNA is expressed in C2C12 cells and primary myoblasts. EphA3 and EphB2 protein is also expressed on myoblasts although EphA4 protein is barely detectable in primary myoblasts. Similarly, in P7 EDL muscle, Pax7 positive interstitial cells co-stain with EphA3 and EphB2 but not EphA4. Based on these preliminary results, I hypothesize EphA3 and/or EphB2 are binding to EphrinA5 to promote myoblast migration. To test this hypothesis I will measure whether EphrinA5 can promote the migration of EphA3 and EphB2 knockout C2C12 myoblasts. Currently I have knocked out EphB2 from C2C12 cells using CRISPR-Cas9. After I knockout EphA3 from C2C12 cells, I will measure the relative migration of the knockout C2C12 cells through a boyden chamber compared to wild type C2C12 cells in the presence of EphrinA5. This work will give us a more complete understanding of how EphrinA5 promotes myoblast migration.

References:

Keywords: Ephrin-Eph Signaling, Muscle Development
56) The transposable element’s Achilles’ heel: do double-strand breaks trigger recognition and epigenetic silencing?

Meredith J. Sigman (Molecular Genetics, OSU), R. Keith Slotkin (Molecular Genetics, OSU)

Abstract:
Transposable elements (TEs) are mobile fragments of DNA that cause double-stranded DNA breaks upon transposition and are thus inherently mutagenic. To prevent DNA damage, multicellular organisms such as the model plant Arabidopsis thaliana transcriptionally silence TEs via heterochromatin formation enacted through epigenetic modifications such as DNA and histone methylation. Cytosine methylation is added to a TE locus through a small RNA-guided pathway termed RNA-directed DNA Methylation (RdDM). It has been previously demonstrated that RNA Polymerase V, a plant specific polymerase derived from Pol II, plays a role in DNA double-stranded break repair (1), but thus far, it is unknown whether this involvement can trigger methylation around break site. The dual role of POL V as well as other canonical RdDM proteins in double-stranded break repair begs the question of whether this pathway can also serve as a preliminary surveillance mechanism capable of targeting TE and exogenous DNA at the genome integration step. I hypothesize that the cell uses the double-strand break that the TE creates upon transposition to target DNA methylation (and hence epigenetic silencing) to a TE locus as soon as it duplicates. This study utilizes CRISPR-Cas9 mediated double-stranded breaks to assay whether a double-stranded break is sufficient to recruit proteins involved RdDM and trigger cytosine methylation. Preliminary evidence shows low level CHH methylation at a previously unmethylated region near the break site in wildtype plants. In addition to furthering the understanding of RdDM and DNA silencing, this project has broader impacts: If a double stranded break alone is sufficient to initiate DNA methylation, many researchers employing CRISPR may be drawing less that accurate conclusions due to regional epigenetic alterations around a targeted break.

References:

Keywords: DNA Methylation, Double-stranded break, Epigenetic
Overcoming a “molecular ruler” mechanism: the unusual heterotrimeric tRNA splicing endonuclease of Trypanosoma brucei

Gabriel Silveira dAlmeida (Department of Microbiology and OSU Center for RNA Biology, The Ohio State University), Mary Anne Rubio (Department of Microbiology and OSU Center for RNA Biology, The Ohio State University), Christopher Trotta (PTC Therapeutics Inc), Arthur Gnzl (School of Medicine, University of Connecticut), Juan Alfonzo (Department of Microbiology and OSU Center for RNA Biology, The Ohio State University)

Abstract:
Introns interrupt tRNA sequences in all major lines of descent (Bacteria, Archaea and Eukarya), rendering them nonfunctional for protein synthesis. Intron removal is therefore, essential. In all known eukaryotes, intron cleavage, the first step in the tRNA splicing pathway, is catalyzed by a conserved heterotetrameric tRNA splicing endonuclease (Sen) composed of four subunits: Sen54, 34, 15 and 2. Bioinformatic analysis using previously published eukaryotic Sen sequences led us to the identification of only one homolog of the tRNA splicing endonuclease in Trypanosoma brucei (Sen34), suggesting that either the other subunits are missing or, as a whole, the enzyme is highly divergent in these organisms. In this work, we present evidence for a divergent and unique enzyme composed of three subunits: homologs of Sen34, 15 and 2. By performing tandem affinity chromatography followed by mass spectrometry analysis we purified and identified TbSen subunits from a T. brucei S100 fraction. Gel filtration chromatography and in vitro activity assays revealed that the active enzyme had a size within the range of 58 to 72 kDa, consistent with that of a heterotrimer. Furthermore, immunofluorescence localization assays showed that the enzyme was cytoplasmic, in stark contrast to the nuclear localization of Sen in most eukaryotes. The results presented here demonstrate that TbSen greatly diverges from previously described eukaryotic enzymes in both structure and localization. Interestingly, in most eukaryotes, Sen54 serves as a “molecular ruler” that carefully measures the distance between the splice sites and the backbone of the folded tRNA, aiding in substrate identification and catalytic site positioning. Our finding of a heterotrimeric endonuclease then obviates the need for a Sen54 subunit and may remove the substrate recognition restriction set forth by the “molecular ruler” mechanism. These observations have direct implications for both the evolution of the enzyme in trypanosomes and its potential for targeting of additional substrates while not just being limited to tRNAs.

Keywords: tRNA, splicing, trypanosoma
58) Elucidating how the LptB motor drives Lipopolysaccharide transport

Brent W. Simpson (Department of Microbiology), Rebecca M. Davis (Department of Microbiology), Alex Mestre (Department of Microbiology), Natividad Ruiz (Department of Microbiology)

Abstract:
Bacterial interactions with the environment are primarily mediated by the cell surface. To tightly control what enters the cell, many bacteria, the Gram-negatives, coat the cell surface with a highly impermeable lipid called lipopolysaccharide (LPS). This layer of LPS comprises the outer leaflet of the outer membrane and prevents toxic compounds, like antibiotics, from entering. After it is synthesized at the cytoplasmic membrane, LPS needs to be transported across the periplasm and the outer membrane to be assembled at the cell surface. This process is best studied in Escherichia coli which requires seven essential lpt (LPS transport) genes to encode the machinery. Here we explore the function of an unusual ATP-binding cassette (ABC) transporter, LptB2FG, that provides energy for LPS transport. The dimer of LptB utilizes the conserved features of ABC motor domains to hydrolyze ATP and cause conformational movements. These conformational changes are transmitted to transmembrane proteins LptFG to drive LPS transport. Utilizing structure-function and suppressor analyses, we have identified unique features of the ATPase LptB required for LPS transport. While LptB shares motifs with other members of the ABC transporter family for ATP binding and hydrolysis, it has a unique motif at its C-terminus that is essential for function. We demonstrate that the C-terminus of LptB functions with ATP-binding motifs in LptB and transmembrane regions of LptFG to coordinate ATP hydrolysis with LPS transport.

Keywords: Cell envelope, Lipopolysaccharide, Antibiotic resistance
59) Understanding the role of FACT during early C. elegans development

Brittany Suggs (Molecular Genetics)

Abstract:
The FACT (Facilitates Chromatin Transcription) complex is known to be involved in the chromatin remodeling processes during gene transcription, DNA repair and replication. Two proteins comprise this complex: SSRP1 and SPT16. We are focusing on the functions of this complex in influencing gene transcription that are well characterized in yeast, but less well understood in the development of multicellular organisms. We have found that a double RNAi knockdown of HMG-3 and HMG-4, the orthologs for SSRP1, as well as RNAi against F55A3.3, the SPT16 ortholog, causes high levels of embryonic lethality in C. elegans. Surprisingly, as FACT is believed to be widely expressed and knockdown is cell-lethal in mouse embryos, the phenotype shows a very specific requirement for the complex during early embryogenesis: when either component is absent, the anterior pharynx fails to form. We aim to determine when in pharyngeal development FACT is needed and which of its functions is necessary to ensure proper development of the anterior pharynx.

Keywords: embryogenesis, FACT
Pnrc2 regulates 3’UTR-mediated decay of cyclic transcripts during somitogenesis

Kiel T. Tietz (Department of Molecular Genetics, The Ohio State University), Thomas L. Gallagher (Department of Molecular Genetics, The Ohio State University), Zachary T. Morrow (Department of Molecular Genetics, The Ohio State University), Nicolas L. Derr (Department of Molecular Genetics, The Ohio State University), Sharon L. Amacher (Department of Molecular Genetics, The Ohio State University)

Abstract:
Vertebrate segmentation is regulated by the segmentation clock, a biological oscillator that controls periodic formation of embryonic segments. This molecular oscillator generates cyclic gene expression in the tissue that generates somites and has the same periodicity as somite formation. Molecular components of the clock include the her/Hes family of transcriptional repressors, but additional transcripts also cycle. Maintenance of clock oscillation requires that transcriptional activation and repression, RNA turnover, translation, and protein degradation are rapid (one cycle is 30 minutes in the zebrafish). We previously isolated a zebrafish segmentation clock mutant, tortuga, that has elevated levels of cyclic transcripts. We show that loss of proline-rich nuclear receptor coactivator protein Pnrc2 is responsible for cyclic transcript accumulation in tortuga deletion mutants and that a new pnrc2 loss-of-function mutant displays an identical phenotype. pnrc2 mRNA is maternally provided and zygotically expressed, and in maternal-zygotic pnrc2 mutants, cyclic transcripts perdure even longer. We show that the her1 3’UTR confers instability to otherwise stable transcripts in a Pnrc2-dependent manner indicating that the 3’UTR of cyclic transcripts is critical for Pnrc2-mediated decay. Preliminary data suggests the last half of the 725 nucleotide (nt) her1 3’UTR is sufficient to convey rapid instability and we are currently examining features that reside in the last 363 nts of the 3’UTR that may confer Pnrc2-mediated decay. Interestingly, cyclic protein levels do not accumulate in pnrc2 mutants, suggesting that stabilized cyclic transcripts are not efficiently translated and that translation may be controlled by an additional post-transcriptional mechanism. Our work explores mechanisms regulating oscillation dynamics during somitogenesis and will further our understanding of pathways controlling post-transcriptional gene regulation.

Keywords: mRNA decay, her1, genetic oscillations
61) Functional significance of the demethylase ALKBH1 in HIV-1 infection and gene expression

Nagaraja Tirumuru (Center for Retrovirus Research; Department of Veterinary Biosciences; Center for RNA Biology, The Ohio State University, Columbus, Ohio 43221, USA), Chuan He (Department of Chemistry, Department of Biochemistry and Molecular Biology, Institute for Biophysical Dynamics; Howard Hughes Medical Institute, The University of Chicago, Chicago, Illinois 60637, USA), Li Wu (Center for Retrovirus Research; Department of Veterinary Biosciences; Center for RNA Biology, The Ohio State University, Columbus, Ohio 43221, USA)

Abstract:
Modification of cellular RNA controls the expression of different genes in organisms, ranging from humans to bacteria. Such modifications are regulated by a diverse group of demethylases including the ALKBH (AlkB homolog) family proteins, which has 9 members including ALKBH1-8 and fat mass and obesity-associated protein (FTO). ALKBH1 has six distinct enzymatic activities, including demethylation of m^5C in DNA, demethylation of methylated lysine in histone H2A, demethylation of m^6A in DNA, double hydroxylation of m^5C in mitochondrial tRNA, demethylation of m^1A in tRNA, and lyase cleavage of apurinic/apyrimidinic sites in DNA. We have reported that the HIV-1 genome RNA contains m^5A modification and that ALKBH5 and FTO negatively regulate HIV-1 protein expression in virus-producing cells (Tirumuru et al, eLife, 2016). However, the role of ALKBH1 in HIV-1 infection is unknown. Since HIV-1 replication requires human tRNAs in its lifecycle, we investigated whether ALKBH1 plays a role in HIV-1 infection and viral gene expression. We hypothesize that ALKBH1 may affect HIV-1 infection by regulating proviral DNA transcription, viral protein synthesis and virus production. We knocked-down ALKBH1 expression in target cells with specific siRNA, and infected the cells with single-cycle reporter HIV-1. We found that knockdown of ALKBH1 reduced HIV-1 Gag protein synthesis, while overexpression of ALKBH1 increased HIV-1 Gag protein synthesis in virus-producing cells. Moreover, compared to control cells, ALKBH1 knockdown or overexpression either reduced or increased cell proliferation, respectively. To examine the role of ALKBH1 in HIV-1 production, we transfected an HIV-1 proviral DNA plasmid into ALKBH1-overexpressing cells and observed reduced HIV-1 protein synthesis compared to control cells. Our results suggest that ALKBH1 plays an important role in cell proliferation and translation of HIV-1 proteins.

Keywords: ALKBH1, HIV-1, Demethylase
62) Promoter Mutation in Ccna2 Reveals Novel Functions of the Protein in Spermatogenesis

Manuel Torres (Department of Molecular Genetics, The Ohio State University), Lindsey Kent (Medical University of South Carolina, Hollings Cancer Center)

Abstract:
Spermatogenesis is the process by which germ cells called Spermatogonia (SSCs) undergo subsequent differentiation events until spermatozoa are produced (1). As they cycle, SSCs express Cyclin A2 (CCNA2/Ccna2), which regulates the phosphorylation of proteins that drive the S phase and G2/M transition phase of the cell cycle (2). CCNA2 mediates cellular processes like double-stranded break (DSB) repair and DNA replication termination and is expressed exclusively in proliferating spermatogonia and preleptotene spermatocytes (3). These stem cells regulate the expression of cell cycle genes (such as Ccna2) via the E2F family of transcription factors, which can bind to a specific DNA sequence and alter gene expression (4). The relevance of E2F-driven Ccna2 expression has not been thoroughly studied in vivo, so to evaluate the importance of E2F-mediated regulation of Ccna2, we generated mice with a null E2F binding site in its promoter. Although mice are viable and appear healthy, we observed infertility and early testicular atrophy in males homozygous for the promoter mutation. Mouse testes were collected at 1, 2, 3, 4, 6, and 12 weeks of age and were fixed in either bouins or formalin for further experiments. Histological analysis revealed a cellular arrest at the preleptotene stage, an expanded SSC population, and lack of mature sperm in the mutants. Using RT-qPCR, Ccna2 levels were found to be upregulated at two weeks and through adulthood, while anti-yH2AX stains revealed elevated DNA damage in the mutants, suggesting unresolved DSBs. Although the molecular mechanism is still not clear, the data strongly suggests that E2F regulation of Ccna2 is vital for fertility and a successful first wave of spermatogenesis.

References:

Keywords: Spermatogenesis, Cell Cycle, Transcriptional Regulation
EGL-38/PAX protein coordination of cell fates in the Caenorhabditis elegans egg-laying system

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

Abstract: The Paired-box family of transcription factors is an essential regulator of coordinated development throughout Metazoans, necessary for cell growth, differentiation, and specification. We seek to understand how PAX functions in unrelated but neighboring cells to synchronize cell fates by utilizing the PAX2/5/8 Caenorhabditis elegans ortholog EGL-38. EGL-38 is required in both epithelial and mesodermal cells of the egg-laying system for creation of the vulval-uterine connection. In the epithelial vulF cell, EGL-38 is known to be required for expression of lin-3/egf, which initiates the EGF pathway to specify the uv1 cell identity. In the mesodermal uv1 cell, we have discovered that egl-38 uv1 expression is dependent on EGL-38 in vulF as well as on the EGF pathway signal. Conversely, we have shown that EGL-38 activation of the uv1 neuropeptide nlp-2 is EGF pathway independent, and are currently investigating EGL-38 activation of the related nlp-7 in uv1. We are also examining the role of LIN-1, a potential EGL-38 co-factor, for involvement with lin-3/egf, nlp-2, and nlp-7 expression; a co-factor would provide a mechanism of restricting EGL-38 activities to specific cells. This dichotomous EGL-38 activity- triggering a signaling pathway in one cell type and responding to that signal in a different cell type- is an unusual behavioral motif that may be found in other Pax proteins to explain how they coordinate the fates of disparate cells.

Keywords: Pax proteins, Signaling pathway, Transcription
Multi-step 5-aminopentanol assembly on Elongation factor P influences antibiotic resistance in Bacillus subtilis

Anne Witzky (Department of Molecular Genetics, Ohio State University, Columbus, Ohio), Rodney Tollerson II (Department of Microbiology, Ohio State University, Columbus, Ohio), Katherine R. Hummels (Department of Biology, Indiana University, Bloomington, Indiana), Andrei Rajkovic (Molecular Cellular Developmental Biology Program, Ohio State University, Columbus Ohio), Daniel B. Kearns (Department if Biology, Indiana University, Bloomington, Indiana), Michael Ibba (Department of Microbiology, Ohio State University, Columbus, Ohio)

Abstract:
Elongation Factor P (EF-P) is a universally conserved translation factor that facilitates translation of polyproline motifs. In order to perform this function, EF-P requires a post-translational modification (PTM) on a conserved residue. Although the position of the modification is highly conserved, the structure can vary widely between organisms. In Bacillus subtilis, EF-P is modified at Lysine 32 with a 5-aminopentanol moiety. However, the modification pathway for this PTM is not known. Here, we use a forward genetic screen to identify genes required for 5-aminopentanolylation. When each gene is deleted, EF-P retains a unique incomplete modification, indicating that 5-aminopentanol is likely directly assembled on EF-P in a multi-step manner. In the presence of each incomplete modification, EF-P activity is impaired to varying degrees. In further phenotypic characterization of these mutants, we found that a Δefp strain was resistant to sulfonamide drugs and puromycin. The modification mutants had varying degrees of intermediate phenotypes. These findings not only establish a novel EF-P PTM pathway, but they also highlight the importance of EF-P in antibiotic resistance.

References:

Keywords: Elongation Factor P, Translation, Post Translational Modification
Translating ribosomes displace Exon Junction Complexes to the 3’UTR before their disassembly

Lauren Woodward (Department of Molecular Genetics), Justin Mabin (Department of Molecular Genetics), Guramrit Singh (Department of Molecular Genetics, Center for RNA Biology)

Abstract:
The EJC is a multiprotein complex that is deposited 24nt upstream of exon junctions during pre-mRNA splicing, and couples splicing to RNA export, localization, translation, and decay. Current models state that the EJC remains bound at the -24nt position until its disassembly during the pioneer round of translation by an elongating ribosome. Following translation termination, if an EJC remains bound to mRNA sufficiently downstream of a stop codon, such mRNAs can be rapidly degraded by nonsense-mediated mRNA decay (NMD). Here, we provide evidence that EJCs are not removed immediately upon contact with the pioneer ribosome but are first displaced before their disassembly. Interestingly, in vivo EJC footprints are not restricted to the -24 position. Indeed, ~8% of EJC footprints map to the last exon, i.e. downstream of the last expected EJC site. We find that translation inhibition leads to a decrease in the number of reads mapping downstream of stop codons. We also observe a direct relationship between the number of EJC deposition sites in the coding sequence and the amount of EJC signal at the stop codon. Further, we observe a higher 3’UTR accumulation of EJCs that contain a disassembly-deficient EJC core protein Magoh, suggesting that at least some EJC disassembly occurs after its displacement to the 3’UTR. Our preliminary analysis shows that on some messages, EJCs displaced to 3’UTRs may be active in NMD, as depletion of EJC core proteins increases the abundance of mRNAs that have a high 3’UTR EJC signal. To assess how much of the total EJC footprint signal in 3’UTRs is comprised of displaced EJCs, I am currently comparing the EJC occupancy landscape in newly synthesized versus actively translating mRNPs. I am also investigating the role of 3’UTR EJCs in NMD. Altogether, this study reveals a new step in the EJC lifecycle that may expand its role in influencing mRNA fate.

Keywords: EJC, translation, 3UTR
Kinetic characterization of the human DNA polymerase ε holoenzyme

**Walter Zahurancik** (Ohio State Biochemistry Program, Department of Chemistry and Biochemistry)

**Abstract:**
Numerous genetic studies have provided compelling evidence to establish DNA polymerase ε (Pol ε) as the primary DNA polymerase responsible for leading strand synthesis during eukaryotic nuclear genome replication. Pol ε is a heterotetramer consisting of a large catalytic subunit that contains the conserved polymerase core domain as well as a 3'→5' exonuclease domain common to many replicative polymerases. In addition, Pol ε possesses three small subunits with no known catalytic activity that associate with components involved in a variety of DNA replication and maintenance processes. Previous enzymatic characterization of Pol ε from budding yeast suggested that the small subunits slightly enhance DNA synthesis by Pol ε in vitro. However, similar studies of human Pol ε (hPol ε) have been limited by the difficulty of obtaining hPol ε in quantities suitable for thorough investigation of its catalytic activity. Utilization of a baculovirus expression system for overexpression and purification of hPol ε from insect host cells has allowed for isolation of greater amounts of active hPol ε, thus enabling a more detailed kinetic comparison between hPol ε and an active N-terminal fragment of the hPol ε catalytic subunit (p261N), which is readily overexpressed in Escherichia coli. Here, we report the first pre-steady-state studies of fully-assembled hPol ε. We observe that the small subunits increase DNA binding by hPol ε relative to p261N, but do not significantly affect the nucleotide incorporation rate constant or nucleotide binding affinity. Furthermore, the small subunits do not appear to affect the rate-limiting step of correct nucleotide incorporation. Together, these data suggest that the role of the small subunits in vivo is primarily limited to mediating protein-DNA and protein-protein interactions. Importantly, this study provides the framework for future kinetic investigation of the impact of the other proteins known to interact with Pol ε at the replication fork.

**Keywords:** human DNA polymerase epsilon, DNA replication, pre-steady-state kinetics