ADDENDUM to the PROGRAM – RNA 2015

May 26 – 31, 2015
University of Wisconsin - Madison

DRAFT

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

Friday, May 29: 9:00 a.m. – 12:30 p.m.
Plenary session 5: Short non-coding RNAs **Shannon Hall**

Withdrawn from session, will be presented as a POSTER:

59 ADAR1 is required for differentiation and neural induction by regulating microRNA processing in a catalytically independent manner

This abstract will be presented as poster number 106 on Wednesday evening.

Friday, May 29: 2:00 – 3:30 p.m.
Concurrent session 6C: RNA modification and editing **Play Circle Theater**

WITHDRAWN:

80 5' phospho-methylation regulates fate of processed RNAs

REPLACED with:

251 Dynamic regulation of RNA modifications and its role in translational control and disease

Mary McMahon, Adrian Contreras, Dayle Juliano, Cristian Bellodi, Davide Ruggero

POSTER CHANGES

ADDED in topic area Emerging & High-throughput Techniques:

106 ADAR1 is required for differentiation and neural induction by regulating microRNA processing in a catalytically independent manner

Refer to abstract 59.

ADDED in topic area Emerging & High-throughput Techniques, placement after poster number 123:

ADD1 Revisiting a Classic Reaction: High-Throughput Fluorescence for T7 RNA Polymerase

Zachary J. Kartje, Eman Ageely, Kushal Rohilla, Keith T. Gagnon
Southern Illinois University, Carbondale, IL USA

For over 40 years the minimal viral RNA polymerases and their mechanisms, especially from the T7 bacteriophage, have been a paradigm in transcription and RNA research. These systems have also emerged as work-horses for nucleic acid biochemists and structural biologists. *In vitro* transcription by viral RNA polymerases like T7 are well-established and have been in use for decades. Despite major improvements in biotechnology, several crystal structures, and substrate specificity and enzymatic studies, these systems have not been completely characterized or optimized.

In vitro transcription by T7 RNA polymerase is quite efficient, making the need for further optimization seem unapparent. However, assays for evaluating activity of T7 RNA polymerase preparations still rely on quantifying incorporation of radioactive nucleotides or simple trial-and-error in the laboratory. While recently preparing large *in vitro* transcription reactions for NMR of a fluorescent RNA aptamer, we were frustrated by conflicting buffer recipes, claims of improved

transcription with certain additives, proprietary buffer systems in commercial kits, the occasional lack of reproducibility, and the cost of large reaction volumes despite using our own purified T7 RNA polymerase.

We discovered that the fluorescent RNA aptamer we were studying, called "broccoli," could readily be combined with small transcription volumes and high-throughput fluorescence plate reading. We have now used this simplified assay to begin systematically characterizing optimal *in vitro* transcription conditions and kinetics for T7 RNA polymerase in real-time, including various buffer component concentrations, site-directed mutagenesis of promoter sequences, and template lengths.

This system provides a rapid, cost-effective and safe method for nearly any laboratory with a fluorescent plate reader or qPCR machine to screen and optimize transcription conditions for many polymerases, including T7, T3 and SP6. This fluorescent high-throughput method should shed new light on a classic system and ultimately improve the efficiency of *in vitro* transcriptions, reduce the cost of these reactions, and decrease reliance on expensive commercial kits when performing large-scale transcriptions.

WITHDRAWN:

193 Novel computational metrics and approaches for evaluating population-wide differences in alternative splicing at the single-cell level

ADDED under RNA Bioinformatics, placement after poster number 211:

ADD2 The Mix^2 Model Leads To Significantly Improved Accuracy In Transcript Concentration Estimates And Detection Of Differential Expression In RNA-Seq Data

Andreas Tuerk, Gregor Wiktorin, Serhat Güler
Lexogen GmbH, Vienna, Austria

Quantification of gene isoforms with RNA-Seq is inaccurate due to varying positional coverage bias. Here we propose a statistical model for RNA-Seq data, the Mix^2 model (rd. "mixquare"), which represents this bias by mixtures of probability distributions. The parameters of the Mix^2 model can be efficiently trained with the EM algorithm yielding simultaneous estimates for the relative abundance of gene isoforms and the positional coverage bias.

We evaluated the Mix^2 model on the Universal Human Reference (UHR) and Brain (HBR) sample of the Microarray Quality Control (MAQC) data. Comparing the correlation between qPCR and FPKM values to that of Cufflinks and PennSeq we obtain an increase from 0.44 to 0.6 and from 0.34 to 0.54 in R2 value for UHR and HBR, respectively. The detection of differential expression between UHR and HBR, based on the FPKM fold change, yields for the Mix^2 model an increase in true positive rate from 0.44 to 0.71 at a false positive rate of 0.1. Clustering the positional coverage bias learned by the Mix^2 model exhibits 6 dominant bias types in the MAQC data, which cannot be detected by other methods. Due to the fast convergence of the Mix^2 model we obtain a reduction in run-time by up to a factor of 60 in comparison to Cufflinks with bias correction.

We further used the Association of Biomolecular Resources Facility (ABRF) RNA-Seq data to evaluate the correlation of concentration estimates by the Mix^2 Model between different sequencing facilities, library preparations and RNA degradations. These experiments show that the concentration estimates of the Mix^2 model across different conditions are much better comparable than those by Cufflinks. In particular, for all 51 tested comparisons we achieve an increase in R2 value of between 15% and 30%.

Our results suggest that in comparison to state-of-the-art methods the Mix^2 model yields substantially improved concentration estimates of gene isoforms from RNA-Seq data and leads therefore to higher accuracy in the detection of differential expression. Furthermore, concentration estimation with the Mix^2 model is substantially faster than that of other methods and leads to better comparability between different sequencing facilities and library preparations.

ADDED under RNA Bioinformatics, placement before poster number 212:

ADD3 NPDock – a web server for protein-nucleic acid docking

Marcin Magnus[1], Irina Tuszynska[1], Katarzyna Jonak[1], Wayne Dawson[1], Janusz M. Bujnicki[1,2]
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Protein-RNA and protein-DNA interactions play fundamental roles in many biological processes. Detailed understanding of these interactions requires the knowledge of protein-nucleic acid complex structures. However, experimental determination of

macromolecular structures is time-consuming and difficult. Computational docking methods have been developed for predicting complex structures, starting from the separate structures of molecules expected to bind to each other. Docking methods have been particularly widely used to study protein-protein interactions, however, only a few methods have been made available to model protein-nucleic acid complexes. Here, we present NPDock (Nucleic acid Protein Docking), a novel web server for predicting protein-nucleic acid complex structures, which implements a computational workflow that includes docking, scoring of poses, clustering of best-scored models, and refinement of most promising solutions. The NPDock server provides a user-friendly interface and 3D visualization of the results. The smallest set of input data consists of a protein structure and a DNA or RNA structure in the pdb format. Advanced options are available to control details of the docking process and obtain intermediate results. The web server is available at http://genesilico.pl/NPDock.

CORRECTION of authors:

271 The role of the mammalian methyltransferase Tgs1 in RNA processing

Li Chen[1,2], Allie Burns[1], Rutendo Sigauke[1], Peter Baumann[1,2,3]
[1]Stowers Institute for Medical Research, Kansas City, MO, USA, [2]Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS, USA, [3]Howard Hughes Medical Institute, Kansas City, MO, USA

WITHDRAWN:

323 Towards computing the effective concentrations that define RNA tertiary structure

ADDED under RNA Structure and Folding, placement after poster number 331:

ADD4 Hepatitis C virus 3'X terminal RNA domain analysis by NMR

Ángel Cantero-Camacho and José Gallego
Universidad Católica de Valencia, Valencia, Spain

The hepatitis C virus (HCV) is a single-stranded RNA virus belonging to the Flaviviridae family. The HCV genome comprises a single open reading frame (ORF) flanked at either end by untranslated regions (UTRs). The 5'-UTR contains an internal ribosome entry site (IRES) that mediates initiation of protein synthesis, whereas the 3'-UTR is involved in viral replication. The 3'-UTR region consists of a highly variable region immediately downstream of the ORF stop codon, a polypyrimidine tract of variable length, and a highly conserved 98-nt-long domain designated 3'X.

While the three-dimensional structure of the HCV IRES has been extensively studied by nuclear magnetic resonance (NMR), X-ray crystallography and cryo-electron microscopy methods, the structure of the 3'X region has only been studied by enzymatic and chemical footprinting experiments. These experiments have established that the last 46 nucleotides of the 3'X domain form a stable stem-loop, SL1, but the secondary structure of the remaining 3'X 52 nucleotides is not well defined. Although most reports have concluded that they form two additional stem-loops (SL2 and SL3), alternative folds comprising one or three hairpins have also been proposed. This 3'X 52-nt subdomain comprises sequences involved in a distal loop-loop interaction with a nearby cis-acting sequence located in the ORF (termed CRE), and in an intermolecular kissing loop interaction leading to genomic RNA dimerization in vitro. Since the alternative 3'X structures expose to different degrees these nucleotides, several authors have proposed that this domain may act as a dynamic switch signaling the transition between the replication, translation and possibly packaging processes of the virus.

We are studying the solution structure of the HCV RNA 3'X domain using NMR spectroscopy. To facilitate this study, separate SL1, Sl2, Sl3 and SL2+SL3 subdomains and the complete 3'X domain are currently being analyzed using natural and 15N-labeled samples. Utilizing the information supplied by these experiments, HCV replicon mutants modulating the structure of 3'X will be designed, and their activity will be evaluated with cellular assays.

ADDED under RNA Structure and Folding, placement before poster number 332:

ADD5 Spinach RNA aptamer: Applications for detection of lead (II) and miRNA with high selectivity

Sandip A. Shelke[1], Saurja DasGupta[2], Nan-Sheng Li[1] and Joseph A. Piccirilli [1,2]
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Nucleic acid molecular beacons or aptamers are emerging tools for imaging RNA oligonucleotides as well as detection of small molecules and metal ions. Along these lines, we demonstrate applications of Spinach, an RNA mimic of GFP, for selective detection of Pb^{2+} and micro-RNA (miRNA). The Spinach RNA aptamer has a G-quadruplex motif that acts as a binding platform for fluorophore DFHBI and activation of its fluorescence. We show that Pb^{2+} induced stabilization of Spinach's G-quadruplex allows DFHBI binding and gives a strong fluorescence signal, thereby allowing its detection with high selectivity and sensitivity. Practical applicability of this sensor has been demonstrated by detection of Pb^{2+} in tap water. This is the first example of an RNA based sensor that provides a simple and inexpensive tool for Pb^{2+} detection. We also present a rationally designed Spinach based molecular beacon for highly specific detection of miRNA. In the Spinach molecular beacon, G-quadruplex formation is inhibited a by a nucleotide sequence which complements the target miRNA. The presence of a cognate miRNA triggers the folding of Spinach to its active conformation and results in fluorescence signal. Together, these sensors expand the applicability of the Spinach RNA aptamer.

ADDED under RNA Transport and Localization:

353 *Drosophila* germ granules are structured and contain homotypic mRNA clusters

Refer to abstract 20.

ADDED under RNA-protein Interactions:

390 IGF2BP3 controls a malignant RNA regulon by modulating RISC function

Hanane Ennajdaoui[1], Jonathan R Howard[1], Tim Sterne-Weiler[2], Doyle J Coyne[1], Sol Katzman[1], Phil J Uren[4], Luiz O Penalva[3], Andrew D Smith[4], Jeremy R Sanford[1]
[1]University of California Santa Cruz, Santa Cruz, CA, USA, [2]University of Toronto, Toronto, ON, Canada, [3]University Texas San Antonio, San Antonio,TX, USA, [4]University Southern California, Los Angeles, CA, USA

The Insulin-like growth factor 2 mRNA binding protein 3 (IGF2BP3) is overexpressed in a diverse array of cancers but its regulatory targets and role in pathogenesis are poorly understood. We applied a combination of high throughput approaches including individual-nucleotide resolution crosslinking immunoprecipitation (iCLIP), RNA immunoprecipitation (RIP) and gene expression profiling to determine the regulatory landscape of IGF2BP3 in pancreatic ductal adenocarcinoma (PDAC) cell models. These experiments defined a coherent set of target transcripts with roles in cancer cell biology as well as a strong enrichment for binding sites within 3' untranslated regions (3'UTRs). The RNA map also revealed significant sequence similarity and positional overlap of IGF2BP3 binding sites and cancer related microRNA target sites. Depletion of IGF2BP3 from PDAC cells alters the steady state levels of target transcripts, association with the RNA induced silencing complex (RISC) and their sedimentation properties in sucrose gradients. These results suggest that IGF2BP3 promotes a malignant gene expression program by modulating microRNA-mediated gene regulation.

WITHDRAWN:

406 Biological functions associated with potential interactions between human proteins and their own transcripts

ADDED under RNA-protein Interactions, placement after poster number 419:

ADD6 iSRIM (In Vitro specificity based RNA regulatory protein identification method)

Shungo Adachi and Tohru Natsume
Molecular Profiling Research Center for Drug Discovery (molprof), National Institute of Advanced Industrial Science and Technology (AIST), Tokyo, Japan

It is very important to identify the critical RNA-binding proteins (RBPs) that interact with and regulate a particular RNA; however, It is still difficult, because hundreds of different RBPs interact with a single RNA molecule *In Vitro*, making it difficult to determine which RBP is the critical regulator. It is also still under debate whether *In Vitro* identified proteins interact functionally with the RNA *In Vivo.* Here we developed the *In Vitro* proteomics based important-RNA-regulating-protein identification method, named iSRIM. This method is based on the hypothesis that important RNA regulating proteins specifically interact with their target RNA even *In Vitro*. And experimental key points in our method are our RNA purification

system using Flag-peptide-tagged RNA and our highly reproducible mass spectrometry (MS) system. iSRIM consists of following 6 steps: 1, Select bait-RNAs including a target RNA and several other unrelated RNAs as controls. 2, Synthesize these RNAs *In Vitro* and a Flag-peptide was conjugated to their 3'-ends. 3, Perform an immunoprecipitation experiment using these bait RNAs and cell lysate. 4, Identify the whole proteins that interact to each bait-RNA with MS system. 5, Select target RNA specific binding proteins from the identified proteins as regulator candidates. 6, Validate the function of the candidate proteins on a target RNA. To show the usefulness of iSRIM, we used LDLR mRNA as a model. We tried to identify the critical regulator that controls the stability of LDLR-mRNA. We successfully identified ZFP36L1 and ZFP36L2 as LDLR mRNA specific binding proteins and we found that these proteins are critical regulators of LDLR mRNA stability. Further, we found that many well-known important RNA-RBP interactions, such as TNF-α mRNA-Roquin, Histon mRNA-SLBP, 7SK RNA-pTEF and so on are bait-RNA specific. These results indicate that our method is very useful and can be used to analyze the regulation of a wide variety of RNAs.

WITHDRAWN:

435 Probing the Accessibility of Repeat Expansion RNA with Molecular Beacons

WITHDRAWN:

440 Long non-coding RNA genes are direct disease causal candidates in human metabolic disorders: insights from the GWAS-transcriptome interface

WITHDRAWN:

479 Multiplex STTMs for functional interaction of microRNAs: A comparison between poly-cis STTMs and poly-ribozyne-cleaved STTMs

ADDED under Small RNAs, placement after poster number 482:

ADD7 Temperature-dependent sRNA transcriptome of the Lyme disease spirochete

Lybecker M[1,2], Popitsch N[3,4], Rescheneder P[4], Bilusic I[2] and Schroeder R[2]

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Hfq is an RNA chaperone that is often required for small regulatory RNAs (sRNAs) to function. The atypical Hfq ortholog in *Borrelia burgdorferi*, the causative agent of Lyme disease, is required for mouse infection via needle inoculation and has a pleiotropic phenotype *in vitro*. We hypothesized that Hfq_{Bb} plays a global role in post-transcriptional gene regulation via an extensive sRNA network that contributes to the pathogenesis of *B. burgdorferi*. We took a genome-wide high-throughput approach to identify temperature and Hfq-dependent sRNAs using RNA-seq technology. We specifically sequenced only the sRNA transcriptome by size selecting for sRNAs prior to cDNA library construction. The sRNA transcriptome was characterized in wild-type and *hfq* mutant strains at both 23°C and 37°C. Our preliminary bioinformatic analyses identified over 100 sRNAs in *B. burgdorferi* with subsets being differentially expressed between the wild type and *hfq* mutant and/or the two temperatures. The sRNAs are found in intergenic regions, within open reading frames, and antisense to open reading frames. Twenty sRNAs have been confirmed via Northern blot analyses. Our study demonstrates that sRNAs are abundant and differentially expressed under relevant environmental conditions suggesting that gene regulation via sRNAs is a common mechanism utilized in *B. burgdorferi*.