



Banff International Research Station

for Mathematical Innovation and Discovery

Advances and challenges in Protein-RNA: recognition, regulation and prediction

Jun 07 - Jun 12, 2015

GENERAL INFORMATION

*All lectures will be held in the lecture theater in the TransCanada Pipelines Pavilion (TCPL).

* Breakfast, Lunch and Dinner are served in the Sally Borden Building

Breakfast (Buffet): 7:00 – 9:30

Lunch (Buffet): 11:30– 13:30

Dinner (Buffet): 17:30 – 19:30

* Beverages and small assortment of snacks are available on a cash honor system in 2nd floor lounge, Corbett Hall

PROGRAM

Sunday

16:00 Check-in begins (Front Desk – Professional Development Centre - open 24 hours)

17:30-19:30 Buffet Dinner

20:00-20:30 **Opening and Introduction (TCPL)**

20:30- **Informal gathering in 2nd floor lounge, Corbett Hall**

Monday

7:00-8:45 **BREAKFAST**

8:45-9:00 Introduction and Welcome by BIRS Station Manager, TCPL

9:00-12:30 Session 1-

High throughput approaches to study protein-RNA interactions

Chair: Neelangan Mukherjee

9:00-9:30 Gene Yeo, University of California San Diego, CA, USA

RNA binding proteins: large-scale mapping of binding sites and identifying functions of these proteins

9:30-10:00 Jernej Ule, UCL Institute of Neurology, London, UK

Experimental and computational tools for improved assignment of protein-RNA binding sites through iCLIP

10:00-10:30 **COFFEE BREAK**

10:30-11:00 Markus Landthaler, Max-Delbrück Center for Molecular Medicine, Berlin, Germany
RC3H1 posttranscriptionally regulates A20 mRNA and modulates the activity of the IKK/NF- κ B pathway

11:00-11:30 Jack D. Keene, Duke University, Durham, NC, USA,
Quantifying RNA-Binding and RNP Codes that Coordinate mRNAs

11:30-13:00 **LUNCH**

13:00-14:00 Guided Tour of The Banff Centre; meet in the 2nd floor lounge, Corbett Hall

14:00 Group Photo; meet in foyer of TCPL

14:30-17:00 Session 2
Exploring the RNA binding proteome
Chair: Shlomi Dvir

14:30-15:00 Matt Friedersdorf, Duke University, Durham, NC, USA
Identifying cooperative binding mechanisms of RNA recognition in cells with a novel technique, DO-RIP-seq

15:00-15:30 **COFFEE BREAK**

15:30-16:00 André Gerber, University of Surrey, Guildford, UK
Conserved mRNA-binding proteomes in eukaryotic organisms

16:00-16:30 Benedikt Beckmann, Humboldt-Universität zu Berlin, Germany
The RNA-binding proteomes from yeast to man harbor conserved enigmatic RBPs

16:30-17:00 Miha Milek, Max-Delbrück Center for Molecular Medicine, Berlin, Germany
Functional characterization of proteins differentially bound to mRNA upon genotoxic stress

17:30-19:30 **DINNER**

19:30-20:30 Session 3
Non-coding RNPs
Chair: Iris Dror

19:30-20:00 Uwe Ohler, Max-Delbrück Center for Molecular Medicine, Berlin, Germany
A principled computational approach to define open reading frames from ribosome footprinting data

20:00-20:30 Neelanjan Mukherjee, Max-Delbrück Center for Molecular Medicine, Berlin, Germany
Differences in RNA metabolism between human coding and non-coding RNA

20:30- Informal gathering in 2nd floor lounge, Corbett Hall

Tuesday

7:00-9:00 **BREAKFAST**

9:00-12:30 **Session 4**

Experimental and computational approaches for mapping binding motifs

Chair: Alona Rabner

9:00-9:30 Hanah Margalit, The Hebrew University of Jerusalem, Jerusalem, Israel
Transcriptome-wide mapping of small RNA-target interactions in bacteria

9:30-10:00 Tim Hughes, University of Toronto, Toronto, Canada
Mapping RNA binding motifs

10:00-10:30 **COFFEE BREAK**

10:30-11:00 Quaid Morris, University of Toronto, Toronto, Canada
Motif models for RBPs with complex binding preferences

11:00-11:30 Rolf Backofen, University of Freiburg, Freiburg, Germany
How to make Sense out of CLIP-seq data

11:30-12:00 Iris Dror, Technion, Israel Institute of Technology, Haifa, Israel
Beyond the consensus: The role of the motif environment on transcription factor binding

12:00-13:30 **LUNCH**

14:30-17:00 **Session 5**

Structural, biochemical and computational approaches to study protein RNA recognition (I)

Chair : Irina Tuszynska

14:30-15:00 Frederic Allain, Institute of Molecular Biology and Biophysics, ETH Zurich, Switzerland
NMR investigation of splicing factors: how single and tandem RRM's recognized their targets

15:00-15:30 **COFFEE BREAK**

15:30-16:00 Andres Ramos, Mill Hill Laboratory, London, UK
KH-RNA interactions regulating RNA metabolism

16:00-16:30 Traci M.T. Hall, NIEHS,NIH, NC, USA
Long lost relatives: The expanding PUF protein family

16:30-17:00 Arnon Henn, Technion, Israel Institute of Technology, Haifa, Israel,
RNA recognition and helix destabilization by human Pumilio 2

17:30-19:30 **DINNER**

19:30-20:30 Session 6

RNA-ligand and RNA-peptide interactions

Chair: Jernej Murn

19:30-20:00 Paolo Carloni, University of Aachen (RWTH), Aachen, Germany.
Investigating and designing ligands interfering with RNA/protein interaction using *in silico* methods

20:00-20:30 Gabriele Varani, University of Washington, Seattle WA, USA
Design of peptides and proteins to target specific RNA sequences

20:30- Informal gathering in 2nd floor lounge, Corbett Hall

Wednesday

7:00-9:00 **BREAKFAST**

9:00-12:30 Session 7

Functional analysis of RBPs

Chair: Miha Milek

9:00-9:30 Lynne E. Maquat University of Rochester, Rochester, NY, USA
SINES of Anarchy: How Alu Elements Alter mRNA Metabolism

9:30-10:00 Eduardo Eryas, Pompeu Fabra University, Barcelona, Spain
RNA processing alterations as drivers of cancer

10:00-10:30 **COFFEE BREAK**

10:30-11:00 Sam Fagg, University of California, Santa Cruz, CA, USA
Functional analysis of Quaking RNA binding protein isoforms reveals unique auto-regulatory interactions

11:00-11:30 Jernej Murn, Children hospital , Harvard Medical School, Boston, MA, USA
Recognition of distinct RNA motifs by clustered CCCH zinc fingers of the neuronal protein Unkempt

11:30-13:30 **LUNCH**

Free afternoon and evening

Thursday

7:00-9:00 **BREAKFAST**

9:00-12:30 **Session 8**

Structural, biochemical and computational approaches to study protein RNA recognition (II)

Chair: Sam Fagg

9:00-9:30 Michael Sattler, Helmholtz Zentrum München, Neuherberg, Germany
Molecular recognition and dynamics of protein-RNA interactions in posttranscriptional regulation of gene expression

9:30-10:00 Thomas Leeper, The University of Akron, OH, USA
Integrated approaches to structure determination of SRA lncRNA RNP complexes

10:00-10:30 **COFFEE BREAK**

10:30-11:00 Blanton Tolbert, CASE university Cleveland, OH, USA
Structural Studies of a UP1-RNA Complex Reveals a New Look for an Old RNA Binding Protein

11:00-11:30 Eckhard Jankowsky, Case Western Reserve University, Cleveland, OH, USA
Hidden specificity in an apparently nonspecific RNA-binding protein

11:30-12:00 Yael Mandel-Gutfreund, Technion, Israel Institute of Technology, Haifa, Israel,
The role of RNA conformation in RNA-protein recognition

12:00-13:30 **LUNCH**

14:30-17:00 **Session 9**

Predicting and characterizing RNA binding interfaces

Chair: Matt Friedersdorf

14:30-15:00 Eric Westhof, Université de Strasbourg, Strasbourg France
The Predictions of RNA binding probabilities in nucleic acids binding proteins.

15:00-15:30 **COFFEE BREAK**

15:30-16:00 Drena Dobbs, Iowa State University, IA, USA
Predicting RNA-Protein Interfaces and Interaction Networks

16:00-16:30 Janusz Bujnicki, International Institute of Molecular and Cell Biology, Warsaw, Poland
3D Modeling of protein-RNA complex structures

16:30-17:00 Irinia Tuszynska, International Institute of Molecular and Cell Biology, Warsaw, Poland
NPdock – a web server for protein-nucleic acid docking

17:30-19:00 **DINNER**

19:30-20:30 **CLOSING DISCUSSION**
Lead by Manny Ares

20:30- **Informal gathering in 2nd floor lounge, Corbett Hall**
Friday

7:00-9:30 **BREAKFAST**

9:30-12:00 **Checkout****

** All participants are welcome to use BIRS facilities (lunch, BIRS Coffee Lounge, TCPL and Reading Room) until 3 pm on Friday.

Notice ! all are required to checkout of the guest rooms by 12 noon.



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Advances and challenges in Protein-RNA: recognition, regulation and prediction

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ABSTRACTS

Speaker: Allain Frederic (Institute of molecular biology and biophysics ETH Zurich, Switzerland)

Title: NMR investigation of splicing factors: how single and tandem RRM recognized their targets

Abstract: Splicing regulation is still primarily determined by the binding of a few dozens of the trans-acting factors that for a great majority contain one to several copies of a small RNA recognition motif referred to as RRM. Over the years, we have characterized the structures in solution using NMR of many RRM-containing alternative-splicing factors in complex with RNA. We will present here how our recent structures of TDP43 *(NSMB, 2013), CPEB1 (Gene & Dev, 2014), hnRNP C (JACS, 2014), hnRNP L and SRSF1 bound to RNA shed light on their mechanism of action and on the extraordinary diversity in RNA binding modes proposed by RRMs and in particular tandem RRMs.

Speaker: Backofen Rolf (University of Freiburg, Freiburg, Germany)

Title: How to make Sense out of CLIP-seq data

Abstract: It is becoming increasingly clear that a RNA-binding proteins are key elements in regulating the cell's transcriptome. Thus, unraveling the interaction network of the RNA-binding proteins by determining their binding sites is becoming an increasingly important topic. There are several high-throughput methods available to detect binding sites such as CLIP-seq. Since not all possible binding sites are covered due to differential expression in tissues and developmental states, the main problem is to come up with good motif descriptions to find missing binding sites and to evaluate the binding strength. Our new approach GraphProt uses an advanced machine learning approach based on our graph-kernel, and is able to use both structural profiles as well as detailed 2D-structures, and predicts missing binding sites with an high accuracy.

Speaker: Beckmann Benedikt (Humboldt-Universität zu Berlin, Germany)

Title: The RNA-binding proteomes from yeast to man harbor conserved enigmatic RBP

Abstract: RNA-binding proteins (RBPs) play a pivotal role in posttranscriptional regulation of gene expression. We determined the RNA-binding proteome of yeast and human hepatocytic cells and identified hundreds of RBPs with unknown function in RNA biology. Among these enigmatic RBPs are many enzymes, particularly of central carbon metabolism. Comparison of human and yeast RNA interactomes allowed us to describe the first conserved "core" of eukaryotic RBPs and revealed remarkable differences in short unstructured regions of those RNA-binders.

Finally, we are investigating novel approaches to study crosslinked RNP complexes and its utilization in infection models.

Speaker: Bujnicki Janusz (International Institute of Molecular and Cell Biology, Warsaw, Poland)

Title: 3D Modeling of protein-RNA complex structures

Abstract: Protein-RNA interactions play fundamental roles in many biological processes, such as regulation of gene expression, RNA splicing, and protein synthesis. The understanding of these processes improves as new structures of protein-RNA complexes are solved and the molecular details of interactions analyzed. However, experimental determination of protein-RNA complex structures by high-resolution methods is tedious and difficult. Therefore, studies on protein-RNA recognition and complex formation present major technical challenges for macromolecular structural biology. Alternatively, protein-RNA interactions can be predicted by computational methods. Although less accurate than experimental measurements, theoretical models of macromolecular structures can be sufficiently accurate to prompt functional hypotheses and guide e.g. identification of important amino acid or nucleotide residues. I will present an overview of strategies and methods for computational modeling of proteins, RNAs and their complexes with emphasis on software developed in our laboratory (available at <http://genesilico.pl>), and I will illustrate it with practical examples of structural predictions.

Speaker: Carloni Paolo (University of Aachen RWTH, Aachen, Germany)

Title: Investigating and designing ligands interfering with RNA/protein interactions using in silica methods

Abstract: We will review our recent attempts at investigating computationally molecules interfering with RNA/protein complexes which are considered promising targets for anti-HIV-1 and anti-Huntington's therapies. We will show that the use of advanced simulation methods, such as well-tempered metadynamics, may significantly help predict poses and potency of ligands targeting RNA's, taking into account the full flexibility of the biomolecules. A perspective for drug design of these methods will be also provided

Speaker: Dobb Drena (Low State University, Ames, IA , USA)

Title: Predicting RNA-Protein Interfaces and Interaction Networks

Abstract: RNA molecules play spectacularly diverse functional and structural roles in living cells, many of which depend on precisely orchestrated interactions between RNA and proteins. To begin to identify sequence and structural signals that specify and regulate these interactions, we have developed machine learning and homology-based methods for predicting: i) whether a given RNA-protein pair will interact; and ii) which amino acids in a given protein directly contact RNA. Using a new dataset of RNA-protein interaction motifs, we have obtained substantial improvements in both partner and interface prediction. Application of these approaches to infer RNA-protein interaction networks will be presented.

Speaker: Dror Iris (Technion-Israel Institute of Technology, Haifa, Israel)

Title: Beyond the consensus: The role of the motif environment on transcription factor binding

Abstract: Transcription factors (TFs) bind to short DNA motifs. However, only a small fraction of all potential binding sites containing motifs actually bind the protein. We tested whether the surroundings of cognate binding sites have unique characteristics, which distinguish them from unbound sequences, by conducting a comprehensive analysis of different features surrounding the motifs of hundreds of TFs extracted from in-vitro and in-vivo assays. Comparing the regions around TF-bound sites to the counterpart unbound regions containing the same motifs revealed significant differences in sequence compositions, DNA shape, and similarity to the consensus motif, which extended far beyond the core binding site.

Speaker: Eyras Eduadro (Pompeu Fabra University, Barcelona, Spain)

Title: RNA processing alterations as drivers of cancer

Abstract: Alterations in RNA processing are emerging as important signatures to understand tumor formation and to develop new therapeutic strategies. However, it is not yet known the extent to which these alterations can be considered drivers or whether specific patterns of RNA processing can be predictive of prognosis. We describe our efforts to determine the functional impact and relevance in cancer of RNA processing alterations measured in 11 cancer types. We describe multiple alterations in RNA binding proteins, including mutation, copy-number alterations and expression changes, and identify their target genes. We further describe the functional impact of these alterations and to which extent they can be considered as drivers of cancer. These novel signatures expand the catalogue of candidate actionable alterations in tumors and potentially complement current strategies in precision cancer medicine.

Speaker: Fagg Sam (University of California Santa Cruz, CA, USA)

Title: Functional analysis of Quaking RNA binding protein isoforms reveals unique auto-regulatory interactions

Abstract: Mammalian RNA binding protein families have members with indistinguishable RNA sequence recognition properties, but different functions, suggesting structural elements that define distinct functions reside outside the RNA binding domains. The Quaking proteins (Qk5 and Qk6) arise from a single gene through alternative splicing and differ only at the C-terminus. To define isoform-specific functions of Qk proteins we have undertaken various approaches that demonstrate Qk5 isoform is required for not only known splicing functions, but also expression of other Qk isoforms, highlighting a unique autoregulatory pathway. We are currently exploring the mechanisms of these regulatory constraints on this unique RBP family.

Speaker: Friedersdorf Matt (Duke University, Durham, CA, USA)

Title: Identifying cooperative binding mechanisms of RNA recognition in cells with a novel technique, DO-RIP-seq

Abstract: To address limitations in current global RNA-protein interaction techniques we developed a native immunoprecipitation protocol called DO-RIP-seq. Do-RIP-seq quantifies relative binding strength of an RBP to sites in their native cellular context. Using this approach we identified global binding sites for RBM38. We find there are at least three distinct and substantial RBM38 RNA recognition mechanisms within cells. Two of the mechanisms appear to be distinct cooperative binding events with HuR and Tra2B and the third mechanism is similar to RBM38's RNA recognition in reconstitution assays. These insights enhance our understanding of the cellular RNP code and its mechanisms.

Speaker: Gerber Andre (University of Surrey, Guildford, UK)

Title: Conserved mRNA-binding proteomes in eukaryotic organisms

Abstract: Recent high-throughput screens have dramatically increased the number of experimentally identified RNA-binding proteins; however, comprehensive *in vivo* identification of RBPs within living organisms, as opposed to cell cultures, has been elusive. Here, we describe the repertoire of 765 and 594 proteins that reproducibly interact with polyadenylated mRNAs in the yeast *Saccharomyces cerevisiae* and in the nematode *Caenorhabditis elegans*, respectively. Strikingly, most proteins comprising these mRNA-binding proteomes (mRBPomes) are evolutionarily conserved, including components of early metabolic pathways such as glycolysis or protein complexes such as the proteasome, which possibly represents 'memory' towards RNA as a relict from an ancient RNA world.

Speaker: Hall Tracy (NIEHS, NIH, USA)

Title: Long lost relatives: The expanding PUF protein family

Abstract: RNA regulation occurs at many levels including processing to mature forms, subcellular localization, and translation. RNA-binding proteins direct and regulate these processes. PUF proteins bind sequence specifically to mRNA targets using their RNA-binding domains comprising eight α -helical repeats (PUM repeats). Our previous structural studies revealed curved structures and sequence specificity unique to these classical PUF proteins. In fact, the sequence specificity of classical PUF proteins can be designed by site-directed mutagenesis. PUM repeats have now been identified in proteins, including human Puf-A and yeast Puf6, that function in pre-rRNA processing, a new role for PUF proteins. Our crystal structures of these novel proteins illustrate how PUM repeats have evolved to form different shapes. Moreover, these new PUM repeat proteins exhibit RNA-binding properties that have diverged from the sequence-specific RNA recognition of classical PUF proteins.

Speaker: Henn Arnon (Technion-Israel Institute of Technology, Haifa, Israel)

Title: RNA recognition and helix destabilization by human Pumilio 2

Abstract: The human PUM2 is an RNA binding protein, which regulates mRNA expression post-transcriptionally. We utilized biophysical approaches to investigate PUM2 binding to its cognate RNA. Our substrate design strategy is based on previous computational analysis that revealed a consensus sequence/structural motif. We employed fluorescence anisotropy to investigate the binding of PUM2 to structured and non-structured consensus binding motifs. Interestingly, we found that PUM2 binds both targets in a similar mode. Furthermore, we utilized time resolved kinetics to show RNA remodeling by PUM2. We propose that PUM2 may contribute to miRNA regulation by binding to structural regions at the 3'UTR regions of mRNAs.

Speaker: Jankowsky, Eckhard (Case Western Reserve University, Cleveland, OH, USA)

Title: Hidden specificity in an apparently nonspecific RNA-binding protein

Abstract: RNA-binding proteins (RBPs) are generally classified as specific or nonspecific. We have examined substrate binding by the apparently nonspecific RBP C5 from *E. coli*, which binds precursor tRNAs at a site without sequence or structure signatures. We simultaneously measure processing of thousands of RNA species and find distributions of affinities of C5 for all substrate variants that resemble distributions for highly specific RBPs. Unlike specific RBPs, C5 does not bind its physiological RNA targets with the highest affinity, but with affinities near the median of the distribution. Our findings suggest that apparently nonspecific and specific RBPs may not differ fundamentally, but represent distinct parts of common affinity distributions.

Speaker: Keene Jack (Duke University, Durham, CA, USA)

Title: Quantifying RNA-Binding and RNP Codes that Coordinate mRNAs

Abstract: RNA regulons are probabilistic and directed by RNP codes. We will describe the DO-RIP-Seq procedure that can identify all RNA binding sites of RBPs and validate non-binding sites. This approach uses LOD scores of saturated binding sites as a measure of quantitative RBP binding strength. DO-RIP-Seq reveals on a genome-wide scale overlapping binding sites of RBPs that cooperate or possibly compete. Our goal is to derive metrics of RNA targeting that indicate regulatory RNP codes (USERS) and dynamic RNA regulons as a quantitative gateway to exploring functional mechanisms within RNP complexes under varying biological conditions.

Speaker: Landthaler Markus (Berlin Institute of Medical Systems Biology at the MDC Berlin, Germany)

Title: RC3H1 posttranscriptionally regulates A20 mRNA and modulates the activity of the IKK/NF- κ B pathway

Abstract: The RNA-binding protein RC3H1 promotes TNF α mRNA decay via a 3'UTR constitutive decay element (CDE). Here, we applied PAR-CLIP to human RC3H1 to identify about 3800 mRNA targets with more than 16000 binding sites. A large number of sites are distinct from the consensus CDE and revealed a structure-sequence motif with U-rich sequences embedded in hairpins. RC3H1 binds preferentially short-lived and DNA damage induced mRNAs, indicating a role of this RNA-binding protein in the posttranscriptional regulation of the DNA damage response. Intriguingly, RC3H1 affects expression of NF- κ B pathway regulators such as I κ B α and A20. Knockdown of RC3H1 resulted in increased A20 protein expression, thereby interfering with I κ B kinase and NF- κ B activities, demonstrating that RC3H1 can modulate the activity of the IKK/NF- κ B pathway.

Speaker: Leeper Thomas (The University of Akron, OH, USA)

Title: Integrated approaches to structure determination of SRA lncRNA RNP complexes.

Abstract: Steroid Receptor Activator RNA (SRA) is a long non-coding RNA transcript that has been found to act as both epigenetic regulatory molecule and to encode a protein (SRA1p). Most lncRNAs do not participate in translation to make proteins, but instead regulate gene expression by interacting with RNA-binding proteins, becoming coactivators for transcription factors, and repressing promoters. SRA, in particular, has been proposed to form in multiple complexes that regulate the transcription of nuclear steroid receptors. Previous work had suggested that the SRA1p protein directly bound and regulated SRA action. Our recent NMR structure, along with a crystal structure from Tom Cech's group published at the same time, refutes this notion and suggests that other RNA binding proteins must be involved. One protein that has been linked to SRA action, SMRT/HDAC1 Associated Repressor Protein (SHARP), is a multi-domain protein that is involved in multiple transcription regulatory pathways. Of particular interest are the four RNA Recognition Motifs (RRMs) that are found near the N-terminus of the protein. Previous data has suggested that SHARP is involved in interacting and regulating the action of the SRA through the use of these RRM. Direct RNA binding of SHARP via these RRM causes repression of the RNA's activity. Chemical shift perturbation studies via ¹⁵N heteronuclear single quantum correlation (HSQC) of SHARP RRM domains and large portions of the SRA RNA suggest that there are multiple modes of binding employed during this interaction. NMR structures of the domains plausibly support RNA recognition via canonical RRM-RNA interactions but also suggest that at least one of these domains may utilize novel features for binding. Structural remodeling of the SRA RNA upon SHARP RRM binding is also indicated by chemical footprinting methods. Because the sizes of these RNP complexes are larger than what is typically studied by NMR, we are combining PRE-monitored NMR data with SAXS to provide new views of these complexes. These integrated SAXS-NMR structural studies on SRA lncRNA complexes with SHARP RRM and SRAs demonstrate that single methods of structural analysis are generally inadequate for illuminating the dynamic rearrangements and complex structures associated with lncRNA function. It is our conclusion that such integrated structure determination strategies will be required for these complicated, dynamic, weakly stabilized, and large RNP systems.

Speaker: Mandel-Gutfreund Yael (Technion-Israel Institute of Technology, Haifa, Israel)

Title: The role of RNA conformation in RNA-protein recognition.

Abstract: Here we studied protein-RNA complexes from different RNA binding domain families solved by NMR and x-ray crystallography. Characterizing the structural properties of the RNA at the binding interfaces revealed an unexpected number of nucleotides with unusual RNA conformations, specifically found in RNA-RRM complexes. Moreover, we observed that the RNA nucleotides that are directly involved in interactions with the RRM domains, via hydrogen bonds and hydrophobic contacts, are significantly enriched with unique RNA conformations. Overall, this study suggests an additional way by which the RRM domain recognizes its RNA target, involving a conformational readout.

Speaker: Maquat Lynne (University of Rochester, Rochester, NY, USA)

Title: SINES of Anarchy: How Alu Elements Alter mRNA Metabolism.

Abstract: Staufen1-mediated mRNA decay (SMD), which occurs when translation terminates sufficiently upstream of a STAU-binding site (SBS), is important to developmental and homeostatic pathways. An SBS can be created by intramolecular base-pairing within an mRNA 3'UTR or by intermolecular base-pairing between a 3'UTR and one or more lncRNAs. Intermolecular base-pairing in humans involves Alu elements, which are a type of small interspersed repetitive element (SINE), whereas intermolecular base-pairing in rodents involves B and identifier SINEs⁵. Roles of STAU1 dimerization and the STAU1 paralog STAU2 in SMD will be discussed. A mechanism by which mRNAs crosstalk in a way that involves direct mRNA–mRNA interactions between 3'UTR Alu elements in each mRNA will be described, uncovering a new role for mammalian-cell mRNAs. This unexpected function, together with our discovering how STAU1 binding to inverted repeated 3'UTR Alu elements (*IRAlus*) competes with nuclear retention mediated by p54nrb binding to 3'UTR *IRAlus* and also the repression of cytoplasmic translation mediated by PKR binding to 3'UTR *IRAlus*, adds new layers of complexity to the network of post-transcriptional interactions that regulate gene expression and involve ncRNA.

Speaker: Milek Miha (Berlin Institute of Medical Systems Biology at the MDC Berlin, Germany)

Title: Functional characterization of proteins differentially bound to mRNA upon genotoxic stress

Abstract: The contribution of post-transcriptional regulatory networks to DNA damage response (DDR) has not been extensively studied. We present a quantitative approach to systematically identify the differentially bound proteins to polyadenylated transcripts upon ionizing irradiation of MCF-7 cells. The majority of the differentially bound proteins showed increased binding to mRNA (N=184) upon irradiation and contained known RNA-binding proteins with predominant nuclear localization. We are further investigating the involvement of an HNRNP protein and an RNA helicase in the DDR, by assaying their impact on the early DDR-induced transcriptional response and cell survival.

Speaker: Mukherjee Neelanjan (Berlin Institute of Medical Systems Biology at the MDC Berlin, Germany)

Title: Differences in RNA metabolism between human coding and non-coding RNA

Abstract: The metabolism of protein-coding RNA is a highly regulated and dynamic process coordinated by numerous ribonucleoprotein complexes. In order to understand the function of ribonucleoprotein complexes it is important to have quantitative data on various steps in RNA metabolism, such as processing, stability, localization and translation. We integrate these data as quantitative phenotypes descriptive of the effect of the RBP-RNA interactions. Specifically, compared differences between protein-coding and long non-coding RNAs. We identify distinct classes of lncRNAs expressed in a human cell line based on their metabolism. Generally, lncRNA are less stable, more likely to be post-transcriptionally spliced, and have different intrinsic splicing-relevant distance and sequence features. The majority of the stable cytoplasmic lncRNAs contain short open reading frames. The challenge moving forward will be to determine which RBP-RNA interactions determine these differences.

Speaker: Murn Jernej (Children hospital, Harvard Medical School, Boston, MA, USA)

Title: Recognition of distinct RNA motifs by clustered CCCH zinc fingers of the neuronal protein Unkempt

Abstract: Unkempt is an evolutionarily deeply conserved RNA-binding protein that regulates translation of its target genes required for the establishment of the early neuronal morphology. How the predicted CCCH zinc fingers (ZFs) of Unkempt recognize a bipartite RNA recognition element (RRE) consisting of UAG and a poorly defined U/A-rich motif is unknown. Here we present X-ray structures of all six CCCH ZFs bound to a complete RRE. The six ZFs arrange to form two compact clusters of three ZFs, with each cluster recognizing a different trinucleotide RNA motif; cluster ZF1-3 recognizes the U/A-rich motif and cluster ZF4-6 binds with high affinity to the UAG motif. Both ZF clusters recognize specific RNA sequences predominantly through hydrogen bonding with the peptide backbone, and exhibit similar overall topologies that are unique in the annotated metazoan proteomes. Structure-guided point mutations reduced the RNA-binding affinity of Unkempt and ablated its translational control, as well as the capacity to induce a bipolar cellular morphology. Our studies unravel a novel mode of RNA sequence recognition by clusters of CCCH ZFs that is critical for post-transcriptional control of neuronal morphology by Unkempt. These findings have implications for several unstudied tandem CCCH ZF proteins found in diverse organisms, including humans.

Speaker: Ohler Uwe (Berlin Institute of Medical Systems Biology at the MDC Berlin, Germany)

Title: Differences in RNA metabolism between human coding and non-coding RNA

Abstract: Several studies have used the deep sequencing protocol of Ribosome Profiling (RP) to provide global insights into the extent of pervasive translation and the coding potential of putative long non-coding RNAs. However, with the high resolution and depth obtained in recent experiments, ribosome profiling lets us pinpoint the exact codons that are processed by the translation machinery. More precisely, the profiles exhibit a three-nucleotide periodicity pattern of footprints along the reading frame. This sub-codon resolution enables bottom-up, data-driven analyses of the coding potential of small regions down to a size of 50 nucleotides or less.

To take full advantage of this information, we have developed a new computational strategy based on spectral analysis methods. This principled approach allows us to quantify the statistical significance of periodic patterns individually for each candidate locus, and unravels the presence of high-confidence coding loci outside of canonical coding sequences. Based on newly generated, deep data for the human cell line HEK293, we define hundreds of translated small Open Reading Frames, located mostly in the 5'UTR of coding transcripts, but also in annotated lincRNA and other non-coding genes. Comparison with shotgun mass-spectrometry data demonstrates this strategy to be at least on par with very deep proteomics datasets.

Speaker: Ramos Andres (UCL and The Francis Crick Institute Mill Hill Laboratory, London, UK)

Title: KH-RNA interactions regulating RNA metabolism

Abstract: The hnRNP K-homology (KH) domain is a single stranded, sequence specific, nucleic acid binding domain present in many proteins that regulate gene expression. Eukaryotic KH domains recognize RNA nucleobases using a conserved hydrophobic groove and share many interaction features. Further, most eukaryotic KH domains cooperate with other KH domains of the same protein to recognize RNA targets. However, our recent analysis shows a remarkable diversity in the role of individual KH domains in RNA recognition and in their degree of sequence specificity. We discuss here how we manipulate protein-RNA interactions to relate this diversity to protein function.

Speaker: Sattler Michael (Institute of Structural Biology, Helmholtz Zentrum München, Neuherberg, Germany)

Title: Molecular recognition and dynamics of protein-RNA interactions in posttranscriptional regulation of gene expression

Abstract: RNA plays essential roles in virtually all aspects of gene regulation. These processes involve the recognition of *cis* elements in the RNA, which provide binding sites for RNA binding proteins (RBPs). Most eukaryotic RBPs are multi-domain proteins that comprise multiple structural domains for protein-RNA but also protein-protein interactions. We employ integrated structural biology combining solution techniques such as NMR-spectroscopy and SAXS/SANS with X-ray crystallography to study the structure and dynamics of such multi-domain RNA binding proteins. I will present examples that highlight the role of conformational dynamics in the recognition of the 3' splice site RNA during eukaryotic pre-mRNA splicing, translational regulation and RNA stability.

Speaker: Blanton Tolbert (CASE Western Reserve University Cleveland, OH, USA)

Title: Structural Studies of a UP1-RNA Complex Reveals a New Look for an Old RNA Binding Protein

Abstract: Human hnRNP A1 is a multifunctional protein involved in many RNA processing events. Structural insights into hnRNP A1-RNA function derive from crystal structures of its UP1 domain bound to ssDNA; yet, chemical footprinting often show discrete protection patterns in stable RNA secondary structure. We solved the crystal structure (1.92 Å) of a UP1-(AGU) complex. The structure reveals single-site RNA binding through the RRM1 domain and inter-RRM linker only. SAXS reconstructions reveal UP1 binds the HIV ESS3 stem loop using a similar mechanism. Our structural/biophysical studies open a very intriguing question: can binding of RNA through RRM1 and the inter-RRM linker allosterically regulate hnRNP A1?

Speaker: Tuszynska Irinia (International Institute of Molecular and Cell Biology, Warsaw, Poland)

Title: NPDock – a web server for protein-nucleic acid docking

Abstract: A detailed understanding of Protein-RNA and protein-DNA interactions requires knowledge about protein-nucleic acid complex structures. Docking methods are widely employed to study protein-protein interactions; however, only a few methods have been made available to model protein-nucleic acid complexes. We developed NPDock (Nucleic acid – Protein Docking); a novel web server for predicting complexes of protein-nucleic acid structures that implements a computational workflow that includes docking, scoring of poses, clustering of the best-scored models, and refinement of the most promising solutions. The NPDock server provides a user-friendly interface and 3D visualization of the results. The web server is available at <http://genesilico.pl/NPDock>.

Speaker: Ule Jernej (UCL Institute of Neurology, London, UK)

Title: Experimental and computational tools for improved assignment of protein-RNA binding sites through iCLIP

Abstract: Individual-nucleotide resolution crosslinking and immunoprecipitation (iCLIP) provides transcriptome-wide maps of protein crosslink sites on endogenous transcripts. We developed a modified iCLIP protocol that allows to separately identify cDNAs that truncate at crosslink sites and those that read through them. We applied this method to PTBP1, which showed that >96% of PTBP1 iCLIP cDNAs truncate at crosslink sites. This showed that cDNAs of different lengths identify different crosslink sites because of a sequence bias at read ends. I will present experimental and computational solutions that can avoid this bias to enable comprehensive assignment of protein-RNA binding sites with iCLIP.

Speaker: Varani Gabriele (University of Washington, Seattle WA, USA)

Title: Design of peptides and proteins to target specific RNA sequences

Abstract: Mis-regulation of regulatory RNA-protein interactions is common in chronic and infectious disease. The ability to engineer proteins and peptides that target RNA sequence specifically would provide protein mimics to develop into pharmaceuticals, but also provide a stringent test of our understanding of the molecular basis of specificity. We have mimicked RNA-binding proteins by using designed conformationally rigid cyclic peptides, constrained to form tight β -hairpin structures and cyclized to provide proteolytic stability and structural rigidity. Using a structure-driven approach, we have explored optimal chemistry at the turns, and investigated natural and un-natural side chain identity to optimize RNA binding activity. Several rounds of design and testing yielded molecules that bind to RNA with low picomolar activity and discriminate very effectively even against closely related RNAs. The best molecules inhibit HIV replication with activity comparable to FDA-approved drugs. The same chemistry is applied to discover new inhibitors of microRNA maturation that down regulate the expression of oncogenic microRNAs. At the same time we are using protein design to engineer RNA-binding proteins that bind to microRNA precursors sequence specifically. These proteins provide tools to investigate whether the inhibition of the biogenesis of specific microRNAs can have pharmaceutical benefits, to validate them as target for intervention in cancer and inflammation, and might be developed into therapeutic agents as well.

Speaker: Westhof Eric (Université de Strasbourg, CNRS, Strasbourg France)

Title: The Predictions of RNA binding probabilities in nucleic acids binding proteins

Abstract: The understanding of the recognition principles of RNA binding to proteins is necessary to predict the binding interfaces. In the past decade, tens of computational prediction algorithms have been developed to predict RNA binding sites based on either protein sequences or protein structures. Most of the state-of-art methods depend on machine learning approach based on PSSM and other residue propensities, ranging from SVM, neural network to random forest and Naive Bayes. However, in order to discriminate RNA binding sites from non-binding sites, the existing programs are all-or-none classifications. Here, we propose a simple score to predict RNA binding probabilities based on a combination of protein sequences and structures. The prediction score is based on physico-chemical and evolutionary principles. As amply demonstrated, RNA binding residues are accessible on protein surface, tend to be positively charged and are highly conserved in sequence. The derived score is a combination of residue accessibility surface, electrostatics potential and conservation entropy. Importantly, the prediction score avoids comparison of all RNA binding residues and non-binding residues of different proteins together. Instead, it maximizes the prediction accuracy for each protein separately. It achieves similar or even better accuracy than the other best prediction programs. Our process achieves stable and high accuracies on both DNA and RNA binding proteins and illustrates how the main driving forces for nucleic acid binding are common. Because of the effective integration of the synergetic effects of the network of neighboring residues and the fact that the prediction yields a hierarchical scoring on the protein surface, energy funnels for nucleic acid binding appear on protein surfaces, pointing to the dynamic pathways occurring in the binding of nucleic acids to proteins.

Speaker: Yeo Gene (University of California San Diego, CA, USA)

Title: RNA binding proteins: large-scale mapping of binding sites and identifying functions of these proteins

Abstract: I will present an update on our efforts in generation and analyses of genome-wide RNA binding protein-RNA networks as part of the ENCODE consortium as well as developing large-scale functional assays for RNA binding proteins.