BMB 632
Probing structure and function of complex RNA-protein machines
Director: Kathy Liu Co-director: Kristen W Lynch
Mondays, 10 am – noon
JF library

Summary:

RNA-Protein complexes or RNPs can range from simple assemblies to megadalton enzymatic machines. The latter include two of the most abundant and essential enzymatic complexes for converting genes to functional protein – the ribosome and the spliceosome. Understanding the molecular interactions that hold these RNPs together and how these complexes function has required the development of new techniques and pushed the boundaries of quantitative biochemistry. In this course we will take an in-depth look at general concepts common to many RNA binding proteins, the methods used to study protein-RNA and RNA-RNA interactions, and how the complex nature of large RNPs uniquely allow them to achieve their precise functions. The course will be a combination of both lectures and student-lead discussion of recent literature. Students will be evaluated based on their presentations of primary literature and their participation in class discussion and a final oral exam.

Syllabus:

Jan 31 Overview of RNA-Protein Machines and Discussion of Basic Methodology: primer extension, RT-PCR, RNA-Seq, CLIP-Seq **(Liu)**

Hentze et al. (2018) **A brave new world of RNA-binding proteins.** *Nat Rev. Mol. Cell Biol.* 19: 327-341.

Feb 7 Ribozymes and Riboswitches: foot-printing and SHAPE **(Lynch)**

Watcher (2014) **Gene Regulation by structures mRNA elements**. *Trends Genet* 30: 172-181

Konforti et al (1998) **A map of the binding site for catalytic domain 5 in the core of a group II intron ribozyme.** *EMBO J* 17: 7105-7117

Johnson JE et al. (2012) **B12 cofactors directly stabilize an mRNA regulatory switch.** *Nature* 492: 133-7.

Feb 14 Ribosomes: RNA folding and the role of RNA binding proteins **(Liu)**

Priya and Woodson (2009) **S16 throws a conformational switch during assembly of the 30S 5' domain**. *Nat Struct Mol Biol* 4:438-445 (use as background)

Soper et al (2013) **In vivo x-ray footprinting of pre-30S ribosomes reveals chaperone-dependent remodeling of late assembly intermediates.** *Mol Cell* 52:506-516

Feb 21 Ribosomes: Ribosome assembly and rRNA modifying enzymes' roles **(Liu)**

Singh et al. (2021) **Nucleolar maturation of the human small subunit processome.** *Science* 373, 1216

Mackereth CD, et al. (2015) **The human 18S rRNA base methyltransferases DIMT1L and WBSCR22-TRMT112 but not rRNA modification are required for ribosome biogenesis**. *Mol Bio Cell* 26(11):2080-95.

Feb 28 Nucleotide modifications by and in RNP complexes: snoRNAs and mRNA mods **(Liu)**

McMahon et al (2015) **Small RNAs with big implications: New insights into H/ACA snoRNA function and their role in human disease**. *WIRES RNA* 6:173-189.

Jack K, et al. (2011) **rRNA pseudouridylation defects affect ribosomal ligand binding and translational fidelity from yeast to human cells.** *Mol Cell* 44:660-6.

Cole L, et al. (2017) **RNA modifications and structures cooperate to guide RNA–protein interactions.** *Nat Rev Mol Cell Biol.* 18:31–42

Mar 7 Spring break, no Class

Mar 14 Nucleotide modifications by and in RNP complexes: tRNA mods **(Liu)**

Tao Pan (2018) **Modifications and functional genomics of human transfer RNA**. *Cell Res.* 28, 395–404 (2018).

Takakura, et al. (2019) Biogenesis and functions of aminocarboxypropyluridine in tRNA *Nat Commun* 44:660-6.

Mar 21 Spliceosome and the study of large RNPs: Native gels, gel filtration, EM **(Lynch)**

Shi (2017) **Mechanistic insights into precursor messenger RNA splicing by the spliceosome.** Nat Rev Mol Cell Biol. 18:655-670.

Konarska and Sharp (1987) **Interactions between small nuclear**

ribonucleoprotein particles in formation of spliceosome. *Cell* 49: 763-774.

Fica et al., (2019) **A human postcatalytic spliceosome structure reveals essential roles of metazoan factors for exon ligation.** *Science* 363:710-714

Mar 28 Regulation of Splicing and Identifying RBPs: Site-specific labeling, UV crosslinking, EMSA, psoralen, MS2 purification **(Lynch)**

Fu and Ares (2014) **Context-dependent control of alternative splicing by RNA-binding proteins**. *Nat Rev Genetics* 15:689-701.

Rothrock et al., (2005) **HnRNP L represses exon splicing via a regulated exonic splicing silencer.** *EMBO J* 24:2792-2802.

Chiou et al. (2013) **HnRNP L and hnRNP A1 induce extended U1 snRNA interactions with an exon to repress spliceosome assembly.** *Mol Cell* 49: 972-82

Apr 4 Single-Molecule study of Spliceosome and RISC: FRET, CoSMoS **(Lynch)**

Serebov and Moore (2016) **Single Molecule Approaches in RNA-Protein Interactions.** *Adv Exp Med Biol* 907:89-106.

Semlow et al (2016) **Splicoeosomal DEAH-box ATPases Remodel pre-mRNA to Activate Alternative Splice Sites**. *Cell* 164:985-998.

Yao et al (2015) **Single-Molecule Analysis of the Target Cleavage Reaction by the *Drosophila* RNAi Enzyme Complex.** *Mol Cell* 59:125-132.

Apr 11 CRISPR: use of RNP as tools **(Liu)**

Koonin and Makarova (2013) **CRISPR-Cas.** *RNA Biol* 10:679-686

Jinek et al (2012) **A Programmable dual-RNA guided DNA Endonuclease in Adaptive Bacterial Immunity.** *Science* 337:816-821

Apr 18 Highthroughput methods to study RNA and RNPs: RNA-Seq, Ribosome footprinting, Mass Spec, etc **(Liu)**

Helm and Motorin (2017) **Detecting RNA modifications in the epitranscriptome: predict and validate** *Nat. Rev. Genet.* 18:275–291

Hussain et al (2013) **Characterizing 5-methylcytosine in the mammalian epitranscriptome** *Genome Bio*. 14:215

Li et al (2017) **Base-Resolution Mapping Reveals Distinct m1A Methylome in Nuclear- and Mitochondrial-Encoded Transcripts** *Mol*. *Cell* 68(5):993-1005

EXAM DATE TBD (Likely later April)