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:

Sept 6  Overview of RNA-Protein Machines


1-2: Discussion of Basic Methodology: primer extension, RT-PCR, RNA-Seq, CLIP-Seq (Anat-Chem 255)

Sept 20  RNA-folding I - Self-splicing: footprinting and NIAM/NAIS


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


Sept 27    RNA folding II: Riboswitches: in-line probing and SHAPE


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Oct 4    Structure and determinants of RNA-RBP interface: NMR and FRET


Agrawal et al (2016) An extended U2AF65-RNA binding domain recognizes the 3’ splice site signal. Nat Comm doi 10.1038 (these present competing structures so focus on methods and similarities /differences)


1-2: RNA binding proteins: Identifying and determining binding specificity by UV crosslinking, RNA affinity, EMSA (1-2 Anat Chem 255)


Oct 18 Ribosomes: Interplay of RNA folding and RBP binding


Oct 25 Spliceosome: From Biochemistry (Native gels, RNAse H, psoralen) to EM

Wahl et al (2009) The spliceosome: design principles of a dynamic RNP machine. Cell 136:701-18. (an excellent but mammoth review- Fig 2 is critical, the rest is helpful)


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Rauhut et al (2016) **Molecular architecture of the Saccharomyces cerevisiae activated spliceosome.** *Science* epub

Galej et al (2016) **Cryo-EM structure of the spliceosome immediately after branching.** *Nature* epub

*we could spend a entire semester on these papers. Just focus on the highlights and differences between the structures. See Kosmyna and Query (Nature N&V) for help. Other reviews are likely to come out before Oct 4. This is Nobel material.

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**Nov 1**

Regulation of Splicing: Site-specific labeling, psoralen, MS2 purification


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Sharma et al (2011) **U1 snRNA directly interacts with the polypyrimidine tract-binding protein during splicing repression.** *Mol Cell* 41:579-88 (use as background)


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**Nov 8**

12-1: Penn RNP Discussion Group  *(note: in JF Library, Anat-Chem 250)*

1-2: Nucleotide modifications in and by RNP complexes *(Anat-Chem 255)*


Zhao et al (2016) *Psuedouridylation of 7SK snRNA promotes 7SK snRNP formation to suppress HIV-1 transcription and escape from latency. EMBO Rep* epub
(this has lots of data, but just use as another example of H/ACA impact - and mention function of 7SK snRNP)

Nov 15 Telomerase and SRP: Applying concepts to additional RNP machines


(their is also back-to-back paper that looks at the same thing for those interested)

Nov 22 Helicases and understanding the dynamic nature of RNP machines: FRET and CoSMoS


Hoskins et al (2011) *Ordered and Dynamic Assembly of Single Spliceosomes Science* 331: 1289. (just read for basic method)

Nov 29      RISC and CRISPR: How understanding RNP Machines can inspire useful tools


Dec 6      Gideon Dreyfuss: The RNP world

Dec 13      *Penn RNP Discussion Group* (note: in JF Library, Anat-Chem 250) and Exam (Final Date TBD)