

February 17 & 24: **Biochemistry of catalytic RNAs** – Dave Brow & Sam ButcherGoals of the unit:

In addition to its informational role, RNA has important biochemical functions in the cell. For example, essential RNA subunits are found in the spliceosome, the ribosome, telomerase and the signal recognition particle. This session will focus on the molecular characteristics of RNA that are relevant to its biochemical function. The specific goals are to:

- A) Discuss the biophysical and biochemical properties of RNA in solution.
- B) Consider selected techniques currently used to identify structure in RNA, noting their strengths and limitations.
- C) Using the group II self-splicing intron as a model:
  - i) Review the common structural elements in RNA.
  - ii) Examine how these elements combine to produce complex structures.
  - iii) Relate RNA structure to function (catalytic activity).
- D) Discuss current issues in the study of RNA function, including the nature of tertiary and quaternary interactions, the kinetics of binding and dissociation of RNAs, and the changes in RNA structure that accompany function.

Reading assignment for session 1 (February 17):

In preparation for class on the 17th, you should read these two reviews, which can be downloaded from the course web site at: [www.biochem.wisc.edu/courses/biochem710/](http://www.biochem.wisc.edu/courses/biochem710/)

**Fedorova, O. and N. Zingler.** (2007). Group II introns: structure, folding and splicing mechanism. *Biological Chem.* 388:665-678.

**Valadkhan.** (2007). The spliceosome: a ribozyme at heart? *Biological Chem.* 388:693-697.

The first review will introduce you to group II introns. You may skip the sections titled "Unusual splicing reactions", "Maturases" and "Host-encoded splicing factors", if you wish. The second review describes the similarities between group II introns and the spliceosomal RNAs that are postulated to have evolved from them.

There is no written assignment due on February 17. During session 1 we will spend the first hour or so discussing general approaches for the analysis of RNA structure, and the second hour discussing group II introns.

Reading and written assignment for session 2 (February 24):

In preparation for class on the 24th, you should read the following research article:

**Hamill, S. and A.M. Pyle.** (2006). The receptor for branch-site docking within a group II intron active site. *Molecular Cell* 23:831-840.

At the first session you will receive a homework assignment on this paper to complete for session 2. At the second session we will discuss the paper and your answers to the assigned questions, which you should turn in at the end of the session.

## Methods of RNA Structure Determination (for future reference)

[See also Jaeger JA, SantaLucia J and Tinoco I (1992) Determination of RNA structure and thermodynamics. *Annu. Rev. Biochem.* 62:255]

## I. Primary structure (nucleotide sequence)

- A. Base-specific nuclease digestion of end-labeled RNA.  
Donis-Keller H, Maxam AM, and Gilbert W (1977) Mapping adenines, guanines and pyrimidines in RNA. *Nucl. Acids Res.* 4:2527.
- B. Base-specific chemical degradation of end-labeled RNA.  
Peattie DA (1979) Direct chemical method for sequencing RNA. *PNAS* 76:1760.
- C. Dideoxy sequencing of RNA with reverse transcriptase.  
Godson GN (1980) Primed synthesis methods of sequencing DNA and RNA. *Federation Proc.* 39:2822.

## II. Secondary structure (base-pairing)

- A. Computer modeling by energy minimization.  
Zuker M (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucl. Acids Res.* 31:3406. <http://www.bioinfo.rpi.edu/applications/mfold>
- B. Phylogenetic analysis of base covariation.  
Gautheret D and Gutell RR (1997) Inferring the conformation of RNA base pairs and triples from patterns of sequence variation. *Nucl. Acids Res.* 25:1559.  
<http://www.rna.cccb.utexas.edu>
- C. Probing with chemicals and enzymes sensitive to base-pairing.  
Stern S, Moazed D, Noller HF (1988) Structural analysis of RNA using chemical and enzymatic probing monitored by primer extension. *Methods Enzymol.* 164:481.
- D. Thermal denaturation.  
Puglisi JD and Tinoco, I (1989) Absorbance melting curves of RNA. *Methods Enzymol.* 180:304.

## III. Tertiary structure (long-range base-pairing and non-Watson-Crick interactions)

- A. Nucleotide base-specific chemical structure probes.  
Peattie DA and Gilbert W. (1980) Chemical probes for higher order structure in RNA. *PNAS* 77:4679.
- B. Hydroxyl radical cleavage.  
Latham JA and Cech TR. (1989) Defining the inside and outside of a catalytic RNA molecule. *Science* 245:276.
- C. In-line base-catalyzed cleavage.  
Soukup GA, Breaker RR. (1999) Relationship between internucleotide linkage geometry and the stability of RNA. *RNA* 5:1308.
- D. NMR.  
Latham MP, Brown DJ, McCallum SA, Pardi A. (2005) NMR methods for studying the structure and dynamics of RNA. *Chembiochem.* 6:1492.
- E. X-ray crystallography.  
Doudna JA and Cate JH. (2000) Solving large RNA structures by X-ray crystallography. *Methods Enzymol.* 317:169.

Selected events in the history of RNA studies\*

- 1946 It is proposed that "ribonucleoprotein granules" are the "agents of protein synthesis".
- 1953 Proteins are shown to be synthesized in ribonucleoprotein granules (a.k.a., "microsomes").
- 1955 Rat liver extracts with added ATP are shown to covalently link leucine to soluble RNA (tRNA).
- 1958 The name "ribosome" is coined.
- 1960 Messenger RNA is discovered.
- 1961 The first codon (UUU = phe) is discovered.
- 1965 Nucleotide sequence of the first natural RNA (yeast tRNA<sup>Ala</sup>) is determined.
- 1966 Entire genetic code is determined.
- 1968 Discovery of the U-rich small nuclear and small nucleolar RNAs (U1, U2, etc.).
- 1974 Crystal structure of the first RNA (yeast tRNA<sup>Phe</sup>) is determined to 3.0 Å.
- 1977 Introns are discovered, first in mRNA then in tRNA.
- 1978 Complete sequence of 16S ribosomal RNA determined (by sequencing gene).
- 1982 Group I self-splicing introns are discovered.
- 1983 The tRNA processing "enzyme" RNase P is found to be a ribozyme.
- 1985 The spliceosome is identified and named.
- 1985 Self-cleaving plant viroid RNAs discovered.
- 1986 Group II self-splicing introns are discovered.
- 1993 MicroRNAs are discovered.
- 1998 Discovery of double-stranded RNA interference (RNAi).
- 2000 Crystal structures of the 30S, 50S and 70S ribosome are determined, and the ribosome is seen to be a ribozyme.
- 2004 Crystal structure of a complete group I intron determined to 3.1 Å.
- 2008 Crystal structure of a complete group II intron determined to 3.1 Å.

\*Early events based in part on "The Eighth Day of Creation" by Horace Freeland Judson.