1996 PROGRAM

9:00 a.m. **Registration and Coffee - Room 137, Chemistry-Physics Building**

9:30 a.m. **Welcome by Dr. Fitzgerald Bramwell, Vice President for Research and Graduate Studies, University of Kentucky - Room 139, Chemistry-Physics Building**

9:35 a.m. **Introductory Remarks - Dr. Allan Butterfield, University of Kentucky**

9:40 a.m. **Dr. Phillip A. Sharp, Massachusetts Institute of Technology**
RNA Splicing

The genes of mammals contain an average of 10 introns whose length can extend 100,000 nucleotides. Precise excision of these introns is essential for gene expression. The sequence specificity for intron excision can be considered in two classes, microspecificity directing the chemistry of the splicing process, and macrospecificity defining the exon sequences to be joined during splicing. The conserved consensus sequences at the boundary of introns and at the branch site are the microspecificity. Interestingly the sequences at the 5' splice site are recognized twice during the splicing process, once by U1 snRNP and subsequently by U6 snRNA and other components of the spliceosome. During reactions in vitro it is possible to bypass the U1 recognition during formation of a spliceosome. By inference the utilization of this bypass pathway could be important in regulation in vivo. Surprisingly, the U1 snRNP independent pathway for splicing is probably also not dependent upon the activity of another splicing factor U2AF. A subclass of proteins that regulate RNA splicing contain a diamino repetitive sequence, Ser-Arg (SR). This repetitive subdomain is partially phosphorylated in vivo and is thought to associate with other SR domains in mediating formation of splicing complexes. Recent experiments suggest that there is an extended family of proteins with SR subdomains. These proteins are concentrated in subregions of the nucleus where RNA splicing occurs. Some members of the SR family are best pictured as lattice proteins which associate with multiple SR proteins bound to RNA. It is proposed that some aspects of both nuclear structure and gene structure are related to the activities of SR proteins in RNA splicing.

10:25 a.m. **Discussion**

10:35 a.m. **Dr. Olke C. Uhlenbeck, University of Colorado**
Mechanisms of the Hammerhead Ribozyme

The hammerhead ribozyme is a small RNA motif that undergoes autolytic cleavage at a unique site to give 5' hydroxyl and 2',3' cyclic phosphate termini. The secondary structure of the hammerhead consists of three RNA helices joining in a central core of 11 nucleotides. Two recent x-ray crystal structures of the hammerhead reveal that the core consists of two different folding domains. Domain I contains of the cleavage site and a four-nucleotide tight turn that resembles the "U-turn" in the tRNA antiloop. Domain II is a double stranded helix containing four non-canonical base pairs. Numerous biochemical experiments have shown that the most of the nucleotides in both domains are essential for full activity of the hammerhead. Biochemical and crystallographic experiments also indicate that both domains contain one or more divalent metal ions bound to specific sites. The general goal is to understand how these two domains collaborate to cause the approximately 10^6-fold enhancement of the rate of chemical cleavage. Our laboratory has developed a kinetic framework to analyze the properties of hammerheads with defined functional group substitutions at unique sites. The current focus is on examining the cleavage site nucleotide which forms a tertiary interaction that appears to prevent the structure from adopting an active configuration. We will also present experiments examining the role of various metal ion binding sites in the hammerhead.

11:20 a.m. **Discussion**

11:30 a.m. **Dr. James R. Williamson, Massachusetts Institute of Technology**
Multidimensional Heteronuclear NMR Studies of RNA - Protein Interactions to HIV

Formation of RNA-protein interactions is important for regulation of all levels of gene expression, including transcription, mRNA processing, and translation. In order to understand the structural basis for the interaction of RNA with proteins, we are studying RNA-protein and RNA-peptide complexes using multidimensional heteronuclear NMR spectroscopy. The first step in the structure determination is to prepare a minimal RNA-protein complex that is suitable for NMR studies. This usually entails the detailed biochemical characterization of the complex. The second step is to apply NMR methods to obtain distance and dihedral information that permits the structure to be determined. Two different RNA-protein complexes will be presented. First, the ribosomal S15-rRNA complex will be described to illustrate the biochemical characterization of an RNA-protein complex. Second, the structure of the HIV Rev-Rev Responsive Element (RRE) complex will be described, as determined by NMR spectroscopy. In both of these complexes, the RNA adopts a particular structure that is critical for recognition by proteins. By studying a series of complexes, we hope to begin to understand how the unusual structures that abound in RNA contribute to the specific recognition by proteins.

12:15 p.m. **Discussion**

12:30 p.m. **Buffet Lunch, Faculty Club (Please return registration form by April 8, 1996 for reservations. Cost $10.00 to be paid at registration.)**

2:30 p.m. **Discussion with Graduate Students - Room 137, Chemistry-Physics Building**

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Twenty-Second Annual
Symposium on

Chemistry & Molecular Biology

established in the memory of
Anna S. Naff

Biochemistry of RNA

SPEAKERS
Phillip A. Sharp
Olke C. Uhlenbeck
James R. Williamson

Monday, April 15, 1996

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