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EXPLORATION OF RNA STRUCTURE SPACES

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Studies in molecular phylogeny have relied heavily on ribosomal RNAs to establish relationships between extant organisms and to identify the earliest branchings. These ribosomal RNAs are major components of the ribosomal machinery whose development was central in the early development of life. The role of RNA in early evolution may be even more general however, as even small RNAs have the potential to carry genetic information while exhibiting catalytic activity. At this stage it is essential to understand how an RNA molecule can respond to the evolutionary processes. This will provide insight to the reliability of early branching patterns detected in phylogeny studies and reveal how more complex structures can evolve from simpler ones.

RNA molecules of any particular function are perceived as a set of primary sequences that satisfy the biological conditions for that RNA. The set of such sequences for any particular RNA is a subset of sequence space that is referred to as structure space. Over evolutionary time, an RNA molecule explores its current structure space as long as it retains its biological function. Changes in function and/or increases in complexity require transition to a different structure space. In order to understand RNA evolution, it is essential to understand how the sequences comprising a typical structure space are related. If, for example, they are homogeneously distributed, then each has essentially the same number of equally accessible neighbors and the rate of the evolutionary process would be uniform. In contrast, in a heterogeneous structure space the probability of mutations being accepted is strongly dependent on current sequence and hence rates are not necessarily uniform.

In order to examine the structure of real structure spaces we are studying the 5S rRNA structure space experimentally. A plasmid containing a synthetic 5S rRNA gene, two rRNA promoters, and transcription terminators has been assembled. Assays are conducted to determine if the foreign 5S rRNA is expressed, and to see whether or not it is incorporated into ribosomes. Evolutionary competition is used to determine the relative fitness of strains containing the foreign 5S rRNA and a control 5S rRNA. By using site directed mutagenesis a number of mutants can be made in order to study the boundaries of the structure space and how sharply defined they are. By making similar studies in the vicinity of several points of structure space, it will be possible to determine how homogeneous the 5S rRNA structure space is. Useable experimental protocols have been developed, and a number of mutants have already been studied. Initial results suggest an explanation of why single stranded regions of the RNA are less subject to mutation than double stranded regions.