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Exploring Connectivity in Sequence Space of Functional RNA

Chenyu Wei[1,2], Andrew Pohorille[1,2], Milena Popovic[2,3], and Mark Ditzler[2]
[1]Department of Pharmaceutical Chemistry, UCSF; [2]Exobiology Branch, NASA Ames Research Center, Moffett Field, CA 94035; [3]Blue Marble Space Institute of Science, Seattle, WA 98145
*chenyu.wei@nasa.gov

Abstract: Emergence of replicable genetic molecules was one of the marking points in the origin of life, evolution of which can be conceptualized as a walk through the space of all possible sequences. A theoretical concept of fitness landscape helps to understand evolutionary processes through assigning a value of fitness to each genotype. Then, evolution of a phenotype is viewed as a series of consecutive, single-point mutations. Natural selection biases evolution toward peaks of high fitness and away from valleys of low fitness [1,2], whereas neutral drift occurs in the sequence space without direction as mutations are introduced at random. Large networks of neutral or near-neutral mutations on a fitness landscape, especially for sufficiently long genomes, are possible or even inevitable [1,3,4]. Their detection in experiments, however, has been elusive. Although a few near-neutral evolutionary pathways have been found [5-7], recent experimental evidence indicates landscapes consist of largely isolated islands [8.9]. The generality of these results, however, is not clear, as the genome length or the fraction of functional molecules in the genotypic space might have been insufficient for the emergence of large, neutral networks. Thorough investigation on the structure of the fitness landscape is essential to understand the mechanisms of evolution of early genomes.

RNA molecules are commonly assumed to play the pivotal role in the origin of genetic systems. They are widely believed to be early, if not the earliest, genetic and catalytic molecules, with abundant biochemical activities as aptamers and ribozymes, i.e. RNA molecules capable, respectively, to bind small molecules or catalyze chemical reactions. Here, we present results of our recent studies on the structure of the sequence space of RNA ligase ribozymes selected through in vitro evolution. Several hundred thousands of sequences active to a different degree were obtained by way of deep sequencing. Analysis of these sequences revealed several large clusters defined such that every sequence in a cluster can be reached from any other sequence in the same cluster through a series of single point mutations. Sequences in a single cluster appear to adopt more than one secondary structure. The mechanism of refolding within a single cluster was examined. To shed light on possible evolutionary paths in the space of ribozymes, the connectivity between clusters was investigated. The effect of length of RNA molecules on the structure of the fitness landscape and possible evolutionary paths was examined by way of comparing functional sequences of 20 and 80 nucleobases in length. It was found that sequences of different lengths shared secondary structure motifs that were presumed responsible for catalytic activity, with increasing complexity and global structural rearrangements emerging in longer molecules.

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