RNA ENZYME EVOLUTION FOR EARLY EARTH EXPLORATION.

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The RNA World Hypothesis posits that primitive life used RNA as both the genetic material and as catalyst for chemical reactions, unlike in today's biology where DNA carries the genetic material and proteins are the primary catalysts. The versatility of natural and synthetic RNA enzymes provides experimental evidence to support the RNA World Hypothesis. Because phosphoryl transfer is important in numerous biological processes, exploring ribozymes that can facilitate phosphoryl transfer could provide new insight to the chemical origins of life. A key feature of any incipient enzyme is that it form specific interactions with its substrates. All previously-studied kinase (deoxy)ribozymes have utilized nucleotide triphosphates (NTPs) as phosphoryl donors. However, modern biology utilizes a variety of non-NTP donors, some of which are also prebiotically plausible.

We are exploring the ability of RNA can form binding pockets for those non-NTP donors and exploiting them in phosphoryl transfer. Our selection experiments are constrained by early-Earth conditions to find kinase-like RNA molecules with the capability to transfer a phosphate to the free 5'OH region after incubation with a variety of phosphoryl donors; this includes simple, prebiotic compounds (non-NTP donors) that could plausibly have been available on the early Earth. RNA libraries are designed with various binding domains for donor recognition and RNA folding stabilization. Early experimental designs included dephosphorylation and ligation steps to prepare the library for phosphoryl transfer; however, this approach yielded a ribozyme population that phosphorylated its 5'OH end during the ligation step, before incubation with the donors. To account for this, a new selection is underway that uses a hammerhead ribozyme design to generate a free 5'OH acceptor. After the selection, we will use high throughput sequencing to correlate captured sequences to known structures and determine which features provide more successful ribozymes. Our analysis will help us better understand the role of RNA during Early Earth and find new synthetic RNA enzymes. We will present the results of this selection and initial characterization of the ribozymes at AbSciCon2017.