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RNA-Catalyzed Polymerization and Replication of RNA

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Introduction: According to the RNA world hypothesis, ancestors of extant life stored and expressed genetic information in RNA molecules that were replicated by an RNA polymerase ribozyme. In an effort to reconstruct RNA-based life, *in vitro* evolution was used to improve the activity and generality of an RNA polymerase ribozyme by selecting for variants that can synthesize functional RNA molecules from an RNA template using the four nucleoside 5′-triphosphates (NTPs). The resulting polymerase can synthesize a variety of complex structured RNAs, including aptamers, ribozymes, and tRNA [1]. Furthermore, the polymerase can replicate and amplify short RNA templates in an RNA-catalyzed form of the polymerase chain reaction.

Enhanced RNA polymerase activity: The evolving population of polymerase ribozymes were challenged to synthesize two different RNA aptamers and to copy "difficult" templates that are either purine-rich or contain elements of stable secondary structure. The resulting ribozymes then were challenged to synthesize the hammerhead ribozyme, with selection based on the catalytic function of the synthesized product. This process placed strong selection pressure on the rapid and accurate synthesis of a complex RNA. The current best polymerase ribozyme is able to copy most template sequences, including pairs of complementary sequences. Thus it is able to function as an RNA replicase, achieving the exponential amplification of RNA molecules through repeated cycles of primer annealing, primer extension, and strand separation.

Reverse transcriptase activity: The evolved RNA polymerase ribozyme also has the ability to synthesize DNA molecules from an RNA template using the four dNTPs. This activity would have been crucial for the transition from RNA genomes to DNA genomes during the early history of life on Earth. Although the ribozyme prefers C-rich templates, it is able to incorporate all four dNTPs in good yield and with high fidelity. Further *in vitro* evolution experiments are being carried out to optimize reverse transcriptase activitity, as well as to explore the DNA-templated polymerization of both RNA and DNA.

Self-assembly of ribozyme fragments: The RNA polymerase ribozyme was divided into four fragments that can assemble non-covalently to form a functional enzyme. Two of the divisions have no significant effect on polymerase activity, but the third reduces activity significantly. *In vitro* evolution is being used to devise a better segmentation strategy for the third division to attain full catalytic activity. The aim is to use the non-covalently assembled ribozyme to synthesize each of the four component fragments, as well as their complements, to achieve the RNA-catalyzed exponential amplification of the ribozyme itself. Self-replication and self-sustained evolution of RNA has previously been achieved only in the special case where a ribozyme joins two pre-formed fragments to produce additional copies of itself [2]. Open-ended Darwinian evolution will require that the ribozyme also synthesize the component fragments, which then are either covalently ligated or non-covalently assembled to form a functional replicase ribozyme.

References: [1] Horning DB and Joyce GF (2016) *Proceedings of the National Academy of Sciences USA* 113: 9786–9791. [2] Lincoln TA and Joyce GF (2009) *Science* 323:1229–1232.