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SELF-ASSEMBLY OF MULTIPLE SMALL RNA FRAGMENTS INTO AN AUTOCATALYTIC PREBIOTIC SYSTEM

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Introduction: The RNA World is the theoretical idea that there was a period in the early history of life on Earth when RNA, or something chemically very similar, carried out most of the information processing and metabolic transformations needed for biology to emerge from chemistry. The finding of the catalytic nature of RNA molecules in 1982 by Thomas Cech and Sid Altman demonstrated that RNA could catalyze chemical reactions¹, which supports the “RNA World” hypothesis. One type of these catalytic RNAs, or ribozymes, is the group I intron, which splices itself out of a nascent transcript using RNA-RNA recombination reactions. Life is based on biopolymers that have the ability to replicate themselves. Ferris *et al.*² described the abiotic synthesis of long prebiotic oligomers from activated monomers on catalytic montmorillonite surface. The importance of that study² is that it also showed that oligomers with length in the range of 20-30 monomers were abundant than the longer ones under prebiotic conditions. Here, we consider how an autocatalytic self-replicating RNA system may have originated from inactive shorter RNA fragments. In an effort to find out how life emerged from chemistry, it would be useful to be able to demonstrate that even shorter RNA oligomers can form stable catalytically active contiguous ribozymes *in vitro*. This study describes a system that models prebiotic formation of a catalytically active ribozyme by the recombination of inactive RNA oligonucleotides. For a prebiotic system, we use the covalently self-assembling *Azoarcus* tRNA intron, which was previously described³. This system entails the self-assembly of *Azoarcus* ribozyme system from two, three or four inactive RNA fragments of about 50 nucleotides each: W, X, Y and Z. Here we show the fragmentation and covalent self-assembly of the *Azoarcus* group I intron from five shorter inactive RNA fragments. Experiments were performed by analyzing the self-assembly of a group I intron from RNA fragments as short as 18 nucleotides. Self-assembly reactions were tested at 48°C with 25mM Mg²⁺ concentration in aqueous solutions with 2µM RNA. Moreover, these self-assembly reactions are being tested under different hydration and dehydration conditions for a lengthy period of time to analyze how early Earth conditions such as evaporation, and rehydration could affect the replication of an autocatalytic system. Concentrating through evaporation helps the five-piece system to function efficiently when all the fragments come together. This kind of cooperation is potentially important for the emergence of life. Now we are able to show successfully that five small inactive RNA fragments can self-assemble into a catalytically active covalent contiguous ribozyme via RNA-directed recombination. Furthermore, this system illustrates that continuous cycles of hydration-dehydration enhance the chances that random shorter oligomers will recombine and cooperate well in an autocatalytic system fashion to initial reproduction.

References:

1. Kruger, K. et al. 1982. *Cell* 31:147–157.
2. Ferris, J.P. et al. 1996 *Nature* 381: 59-61.
3. Hayden, E. J. & Lehman, N. (2006) *Chemistry & Biology* 13: 909–918.