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A Clue for Prebiotic Era: Cross-Catalytic Replication of an RNA Ligase Ribozyme

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Summary

A self-replicating RNA ligase ribozyme was converted to a cross-catalytic format whereby two ribozymes direct each other's synthesis from a total of four component substrates. Each ribozyme binds two RNA substrates and catalyzes their ligation to form the opposing ribozyme. The two ribozymes are not perfectly complementary, as is the case for replicating nucleic acid genomes in biology. Rather, the ribozymes contain both template elements, which are complementary, and catalytic elements, which are identical. The specificity of the template interactions allows the cross-catalytic pathway to dominate over all other reaction pathways. In the presence of 2 μM each of the corresponding substrates, one ribozyme catalyzes the synthesis of the second ribozyme with an initial rate of 6.8×10^{-3} min^{-1}, while the second ribozyme catalyzes the synthesis of the first with an initial rate of 2.9×10^{-3} min^{-1}. As the concentration of the two ribozymes increases, the rate of formation of additional ribozyme molecules increases, consistent with the overall autocatalytic behavior of the reaction system. Here, I present results that possibly demonstrate a clue for a self-replicating molecule by showing an RNA ligase ribozyme, which is reminiscent of "Prebiotic Era".

Background and Significance

The process of self-replication has special significance in biology. It provides the basis for heritability of genetic information at the level of molecules, and heritability of form at the level of cells and whole organisms. Self-replication implies more than the

production of additional copies of the "self"; it also refers to the ability of the copies to behave in a similar manner, thereby exhibiting autocatalytic behavior with the potential for exponential growth. The means by which biological systems undergo self-replication is well understood. The genetic molecule, DNA or RNA, encodes and is replicated by a cellular machinery. The genetic molecule itself is not self-replicating, but together with the replicative machinery forms a self replicating system. The coding strand of the genetic material directs the synthesis of functional macromolecules that copy the coding strand to produce a non-coding strand, and copy the noncoding strand to produce a coding strand. Through this process of semi-conservative replication the genetic "self" becomes perpetuated [1].

Chemists have sought to capture self-replicative behavior in non-biological systems. Most commonly this has involved reactions of the form: A + B → T, where A and B are two substrates that bind to a template T and become joined to form a new copy of T. The reaction proceeds autocatalytically if the new copies of T also can direct the joining of A and B. Chemical selfreplication has been demonstrated for activated oligonucleotides [2,3], peptides [4,5], and even small organic compounds [6,7]. In one case a self-replicating oligonucleotide was formed by joining three substrates: A + B + C → T, with the rate of formation of both the intermediate A-B and the final product A-B-C (= T) being enhanced by T [8].

More complicated chemical self-replication systems have been devised that involve two templates that direct each other's synthesis: a template T directs the joining of A′ and B′ to form T′, while a template T′ directs the joining of A and B to form T [9,10]. Such systems more closely resemble biological self-replication, which involves the synthesis of crosscomplementary (rather than self-complementary) nucleic acid templates. Unlike biological systems, however, these chemical systems do not entail a replicative machinery. Once the substrates are bound at adjacent positions on the template they become joined through a favorable reaction between reactive groups at their opposed ends. There also is an example of a cross-catalytic amplification system involving two deoxyribozymes, each of which catalyzes a cleavage reaction, rather than a joining reaction [11]. Although not self-replicating, that system demonstrates

exponential amplification based on molecules that have both template and catalytic properties. It is difficult to design a self-replicating system that involves a separate replicative machinery because the machinery must also be copied and provided to each of the "progeny". One approach toward this goal has been to devise self-replicating molecules that function as both template and machinery. Recently, for example, a self-replicating ribozyme was developed that binds two RNA substrates through Watson-Crick pairing and catalyzes their joining to form another copy of the ribozyme [12]. The copies behave in a similar manner, resulting in autocatalytic behavior.

The self-replicating ribozyme was derived from the R3C ligase, which catalyzes attack of the 3′-hydroxyl of one RNA substrate on the 5′-triphosphate of another RNA substrate, forming a 3′,5′-phosphodiester linkage and releasing inorganic pyrophosphate [13]. The ligase ribozyme has a catalytic rate of 0.32 min^{-1}, corresponding to a rate enhancement of about 106-fold compared to the uncatalyzed, template-dependent rate of reaction [14]. The secondary structure of the ribozyme consists of a central three-way junction, with one arm containing the ligation junction surrounded by the P1 and P2 stems, a second arm containing the P3 stem, and a third arm containing the P4 and P5 stems and most of the unpaired residues that are essential for catalytic activity (Figure 1A). In the self-replicative format, the ribozyme (T) was made to ligate two substrates (A and B) that correspond to the 5′ and 3′ portions of the ribozyme itself (Figure 1B). The resulting enzyme-product complex must then dissociate to make available two ribozyme molecules that can enter the next cycle of replication. Dissociation of this complex was found not to be rate limiting, allowing the initial rate of formation of new ribozyme molecules to increase linearly with increasing starting concentration of ribozyme [12]. The self-replicating ribozyme does not exhibit sustained exponential growth, however, because the two component substrates can form a non-productive A.B complex whose dissociation becomes rate-limiting once the freely available reactants have been consumed. The 5′-terminal portion of A and the 3′-terminal portion of B, both of which are bound by T, are complementary to each other. This is necessary to maintain sequence identity between the ligated product A-B and the ribozyme T. However, it means that A and B can bind to each other in an

intermolecular fashion, and the corresponding portions of T can bind to each other in an intramolecular fashion, both events potentially limiting the rate of self-replication. The requirement for self-complementarity at the termini also is a significant design constraint that may limit the discovery of more efficient replicators.

The present study sought to break the symmetry of the self-replicating ribozyme by devising a cross-catalytic system involving two ribozymes that catalyze each other's synthesis from a total of four component substrates. This would replace the self-complementary relationship between A and B with cross-complementary relationships between A and B′ and between A′ and B. The ribozyme T would catalyze the ligation of A′ and B′ to form T′, and the ribozyme T′ would catalyze the ligation of A and B to form T (Figure 2). The ribozymes T and T′ would no longer be self-complementary at their termini, and there would be greater freedom to explore sequences that might be capable of more efficient replication.

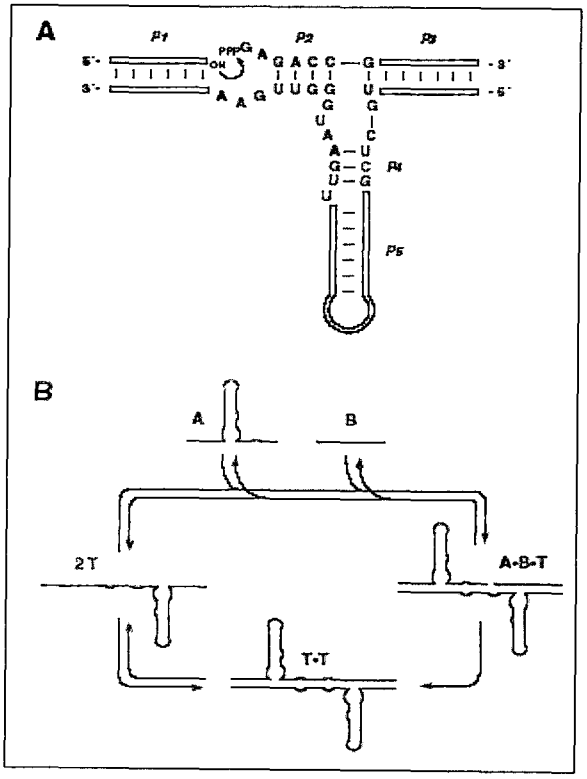

Fig. 1. Structure of the R3C ligase ribozyme and its self-replication cycle

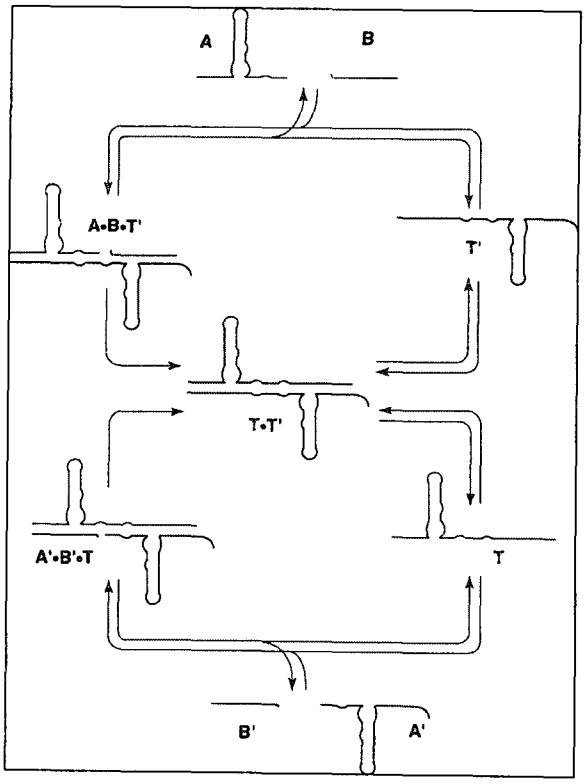

Fig. 2. Cross-catalytic replication of the R3C ligase ribozyme

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