Amplification by Cross-catalysis in a Replicating Ligase Ribozyme System

Dong-Eun Kim, and Gerald F. Joyce*

Department of Biotechnology and Bioengineering, Dong-Eui University, Busan, Korea;

*Departments of Chemistry and Molecular Biology, The Scripps Research Institute, La Jolla, California, USA

Introduction

Self-replication of the molecules has been demonstrated in synthetic chemical systems based on oligonucleotides, peptides, and complementary molecules without natural analogues. A self-replicating system using a ribozyme that catalyzes the ligation of component molecules has been recently developed1, in which the R3C ligase ribozyme2 was redesigned so that it would ligate two substrates to generate an exact copy of itself. However, within a living cell nucleic acid replication occurs in a semi-conservative way, with a plus-strand template directing the synthesis of minus-strand, and *vice versa*. Thus, it would be more challenging and intriguing to generate a pair of self-replicating molecules that exclusively catalyze each other's production (*i.e.* cross-catalysis). Cross-catalytic amplification of self-replicating peptides has been reported by two groups3,4, but no one has demonstrated a cross-catalytic production of nucleic acid replication to date.Breaking the symmetry of the ternary complex of the template (T) and the substrates (A and B) designed in the previous study1can develop an exclusively cross-catalytic system with the self-replicating ribozyme. This requires two pairs of substrates (A and B; A' and B') that bind to their respective template (T' and T), resulting in reactions of the form A + B => T and A' + B' => T' (Fig. 1). On a condition that T:T'complex readily dissociates

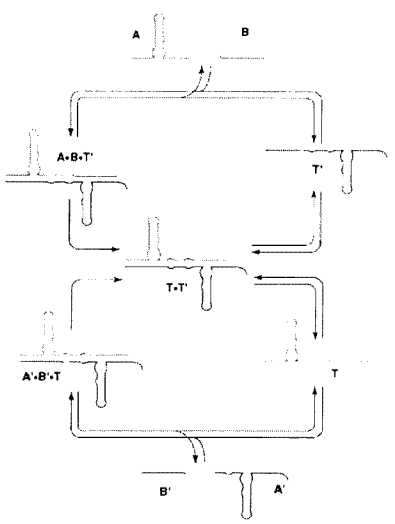

Figure 1.

into two templates, not limiting the subsequent cycles, self-replication with exponential growth could continue indefinitely.

Experimental Methods

Aself-replicating system based on the redesigned R3C ligase ribozyme has been developed, in which four different ligated products may be generated fromfour RNA fragments (A, A', B, and B'). Two of the four potential products, A-B and A'-B' are resulted from ligation by template T' and T, respectively (Fig. 2). As shown in Fig. 1, each cycle consists of the following three steps; 1) specific substrate binding, 2) ligation, and 3) product dissociation into each template. In order to have cross-catalytic cycles perpetuate successfully, these three steps should not limit the cycle. Based on these rationales, several modifications were introduced to the self-replicating ligase derived from R3C ligase ribozyme. It was found that 20 base pairs of consensus duplex sequence positioned in the middle stems in Fig. 2 is required for ligation reaction. Earlier attempt to remove a consensus sequences in 'P3' stem1forfeited ligation capability. Due to the selectivity dictated by the sequences in the P3 stems, both templates would not bind the undesirable substrate pairs, such as A:B' and A';B dimer, of which ligation will lead to accumulation of auto-catalytic products. Verification of the catalytic efficiency was accomplished by running individual reactions containing only two RNA fragments either with or without a complementary template. Note that the ligated products A'-B and A-B' would catalyze auto-catalyticpathway of self-replication. However, these ligated products might be less likely to undergo successive cycles due to re-annealing of P3 arms of template itself. Four substrates and two ligase templates were prepared as described elsewhere1. Time-courses of template production in reaction mixtures containing all four substrates (2.0 microM each) in the presence or absence of template ligase (1.0 microM) were monitored by 10 % denaturing Urea-PAGE analysis. Ligase reaction was initiated by adding all four substrates simultaneously without prior incubation with template at the room temperature.

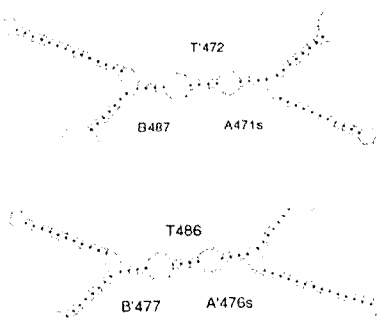

Figure 2

Result and Discussion

Initial attempts to observe the cross catalytic products with longer P3 stem was not successful. Failure to observe the cross-catalytic products indicates an obvious difficulty of separating a newly formed ligated product from the template, suggesting that a product dissociation step limit an entire cycle5. One of the ways to circumvent this difficulty is denaturing the RNA strands. A double-helical nucleic acid could easily be dissociated into single strands by heating. However, in the isothermal situation the temperature cycling is not feasible. The P3 stem was thus destabilized by putting pairs of bases exhibiting weak affinity, as shown in Figure 2.

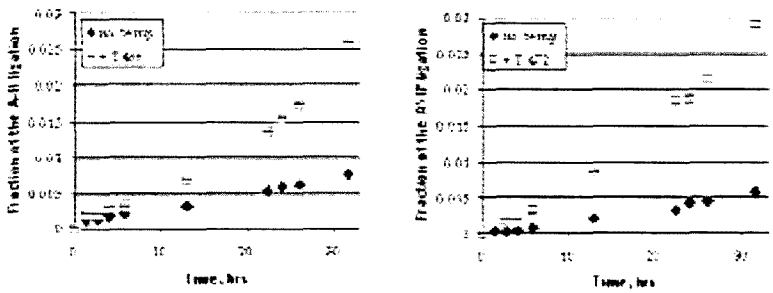

Figure 3.

In the absence of template ligase, no significant accumulation of templates was observed (see 'no template'products in Fig. 3). However, the addition of template ligase to the mixture of four substrates promotes the production of a single ligated product as anticipated from the design strategy. Very importantly, significant amount of template-promoted cross-catalytic ligation was observed as shown in Fig.3. These results indeed indicate that the template ligase produced from one cycle catalyzes the other cycle of ligation by cross-catalytic pathway (Fig. 1). Selective amplification in the informational replicating system5with RNA molecules will throw light in understanding the early evolution of living system.

References:

1. Paul, N. & Joyce, G. F. (2002) *Proc. Natl. Acad. Sci.* **99,** 12733 12740.
2. Rogers, J. & Joyce, G. F. (2001) *RNA* **7,** 395-404.
3. Lee, D. H., Severin, K., Yokobayashi, Y. & Ghadiri, M. R. (1997) *Nature* **390,** 591-594.
4. Yao, S., Ghosh, I., Zutshi, R. & Chmielewski, J. (1998) *Nature* **396,** 447-450.
5. Orgel, L. E. (1992) *Nature* **358,** 203-209.