DARWINIAN EVOLUTION OF TWO DISTINCT COOPERATIVE RNA REPLICATORS. R. Mizuuchi[1] and N. Ichihashi[1,2], [1]Graduate School of Information Science and Technology, Osaka Univ., Japan, [2]Graduate School of Frontier Biosciences, Osaka Univ., Japan. (E-mail of the first author: mizuuchi-ryo@ist.osaka-u.ac.jp)

Introduction: Molecular cooperation may have been a driving force for evolution of complexity in primitive life [1]. In the RNA world or the following RNA-protein world, there should have been a phase in which individual RNAs encoding different genetic information replicated in a cooperative manner, contributing to overall fitness. The cooperation among replicating RNAs (e.g. hyper-cycle) has been hypothesized to act as a link connecting distinct RNAs to expand information capacity through natural selection. However, there was no direct evidence that such a cooperation can be sustained and strengthened through Darwinian evolution overcoming spontaneously appearing selfish replicators. The primary aim of this study is to investigate whether the cooperation among distinct RNA replicators evolves to stabilize the relationship in a simple chemical system.

A previous study showed that the cooperation between two single-stranded DNAs can evolve through replication mediated by partial hybridization of those DNAs, although the DNAs encode no genetic information that is translated into a protein, different from the genome replication in natural organisms [2]. In contrast, in the present study we constructed a novel artificial RNA self-replication system, in which two distinct cooperative RNAs replicate via translation of encoded different proteins including RNA replicase. This system resembles two-component version of hypercycle, defined by Eigen more than four decades ago and extensively studied from the theoretical perspective [3][4]. Theoretically, cooperative RNA replicators in a hypercycle system mutually aid each other's replication, enabling the replicators to bypass mutation load, undergo group selection, and stabilize the concentrations. Here, we experimentally proved the stability of hypercyclic replication in a cell-like compartment through a long-term replication and have further investigated whether the cooperation between the RNAs evolves to stabilize.

Construction of an RNA self-replication system with two distinct cooperative replicators: We combined a reconstituted *E.coli* translation system with two distinct RNAs (Rep-RNA and NDK-RNA) encoding different genes: Qβ phage replicase (RNA-dependent RNA polymerase) for Rep-RNA, and *E.coli* NDK (nucleotide diphosphate kinase) for NDK-RNA. In this system, once NDK is translated from NDK-RNA, it synthesizes CTP from CDP. Then the replicase, translated from Rep-RNA, replicates both NDK-RNA and Rep-RNA with the synthesized CTP. Thus, these two RNAs should be cooperative and replicate interdependently (i.e., in a hypercyclic manner). However, the NDK-RNA sequence derived from *E.coli* was poorly replicated by Qβ phage replicase because of the lack of secondary structures. We therefore modified the NDK-RNA structure with 49 synonymous mutations to be rigid and less GC-rich loops. The modified NDK-RNA replicated 400-fold greater than the original one and the same amount as Rep-RNA, whose sequence has been optimized and replicates efficiently.

Long-term replication experiment: We encapsulated the translation system, Rep-RNA, and NDK-RNA in cell-like compartments (water-in-oil emulsion), and repeated the replication reaction with nutrients supply via manual fusion-division of compartments. During this cycle, mutations are spontaneously introduced into both the RNAs by replication errors. We found that the template RNAs, Rep-RNA and NDK-RNA, replicated synchronously and parasite RNAs (selfish RNA mutants) appeared after a short period of replication. The parasite RNA extensively replicated and competitively inhibit the template RNA replications, resulting in the destruction of the hypercycle system, as previously suggested [5]. However, when the RNA concentrations sufficiently decreased by dilution, the template RNAs start replication again overcoming the parasite existence. The replication in low concentration was sustainable; both Rep-RNA and NDK-RNA has synchronously replicated more than 100 generations (10^{30}-fold replications). We have already detected mutation fixation, indicating that the RNA templates are evolving. Currently, we are continuing the replication experiment to examine whether the cooperation between the two distinct RNAs develop.

Our study would provide direct evidence of evolution of cooperation between RNA replicators encoding different genetic information, which offers insights about how complexity in primitive life might have evolved and stabilized through Darwinian evolution.

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