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Darwinian Evolution of Mutualistic RNA Replicators with Different Genes

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Introduction: The formation of cooperative relationships between RNA replicators is one of the major transition in early evolution, enabling the expansion of genetic information capacity[1]. A representative model of the cooperative replicators is hypercycle, in which distinct replicators mutually aid each other's replication[2]. Theoretical studies extensively examined the hypercycle and pointed out that the cooperative behavior is susceptible to the emergence of parasitic replicators that provide less or no cooperation[1]. For RNA replicators, parasitic RNAs spontaneously appear with selfish mutations that make the RNAs better templates to be replicated faster by exploiting other cooperative replicators. A possible solution is compartmentalization, which provide parasite-free compartments [1], although there has been no direct evidence. In this study, we experimentally investigated the sustainability of a cooperative RNA replication system that consists of two mutualistic RNA replicators encoding two different genes. We also examined the evolution of the cooperative behaviors through a long-term replication.

Results & Discussion: We combined a reconstituted *E.coli* translation system with two RNA replicators (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. The structure of NDK-RNA was modified with 49 mutations so that Qβ phage replicase can replicate it. In this system, NDK is translated from NDK-RNA and synthesizes CTP from CDP in the solution. Then the replicase, translated from Rep-RNA, replicates both NDK-RNA and Rep-RNA with the synthesized CTP. Thus, the RNA replicators are mutualistic.

We repeated the replication reaction in cell-like compartments (water-in-oil emulsion) 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 at high or low RNA concentrations, the cooperative relationship was destructed due to excess parasites generation or dispersion of the two RNAs into different compartments, as previously suggested[3]. On the other hand, in a certain range of total RNA concentrations, both Rep-RNA and NDK-RNA have synchronously and sustainably replicated more than 150 generations (10^{45}-fold replications). The synchronicity of NDK-RNA and Rep-RNA concentrations is surprising because we could not control each concentration independently.

Next, we obtained 32 evolved clones for both the RNAs and analyzed their cooperativities, which we defined as how efficiently proteins expressed from each RNA support the other's replication. In Rep-RNAs, most of the evolved clones showed parasitic behaviors, which provided less or no cooperation. This result is consistent with theoretical predictions that natural selection favors selfish mutations that increase the replication rates of templates but may impair encoded protein functions[1,3]. In contrast, however, we found that most of NDK-RNAs evolved to maintain or increase the cooperativity. This distinctive trait may have stabilized the long-term replication by preventing the spread of parasites that constantly arise from the cooperative RNAs.

References: [1] J. M. Smith and E. Szathmary (1995) *Oxford University Press.* [2] M. Eigen (1971) *Naturwissenschaften* 58:465-523. [3] U. Niesert et al. (1981) *Journal of Molecular Evolution* 17:348-353.