Darwinian evolution in a translation-coupled RNA replication system within a cell-like compartment. N. Ichihashi[1], K. Usui[2], and T. Yomo[3], [1]JST ERATO and Graduate School of Information Science and Technology, Osaka University, [2]JST ERATO, [3] JST ERATO, Graduate School of Information Science and Technology, Osaka University, and Graduate School of Frointier Bioscience, Osaka University (Yamadaoka 1-5, Suita-shi, Osaka-fu, Japan, ichihashi@ist.osaka-u.ac.jp).

Introduction: The construction of an artificial cell or model protocell is hypothesized to provide important insights into the emergence of life from an assembly of non-living molecules[1]. To date, various cellular functions have been reconstituted from purified biological polymers. However, the creation of an artificial cell that harbors the same level of evolutionary ability as natural organisms remains a major challenge.

The evolution of living organisms is a result of the error-prone replication processes of genetic material, either DNA or RNA, by the replication enzyme translated from its own information. In this study, we attempted to construct an artificial system that replicates and evolve in the same manner as natural organisms.

Result: *Translation-coupled RNA replication system.* To construct an artificial system that replicates in the same manner as natural organisms, through the translation of a replication enzyme, we combined an artificial genomic RNA that encodes an RNA-dependent RNA polymerase, the Qβ replicase, with a reconstituted translation system[2]. In this translation-coupled RNA replication system, the genomic plus-strand RNA (2125 nt) replicates using an RNA replicase translated from its own sequence via the synthesis of the complementary minus-strand. This type of replication requires a cell-like compartment to ensure interaction between the translated replicase and the original genomic RNA. In this study, we encapsulated the TcRR system into a micro-scale 1–6 μm cell-like compartment, a water-in-oil emulsion

Long-term replication. We perfomed a long-term continuous replication in the compartments[2]. First, the RNA amplification was assisted by reverse transcription and PCR due to the inefficient replication during the initial stage. We then simplified the cycle and performed the cycle through fusion-division cycle of nutrient emulsion containing the fresh translation system for another 100 rounds. Through all the long-term replication process, approximately 600 generations, the replication ability improved more than 100-fold.

Analysis of the evolved RNAs. To test whether the improvement in the replication ability is the consequence of evolution, we analyzed sequences of RNA clones during each round. The average number of mutations per clone increased constantly. We defined the mutations observed in more than half of the analyzed clones as "fixed." These fixed mutations increased intermittently and ultimately reached a total of 38 mutations, which included 34 point mutations, 1 insertion, and 3 deletions. These results (the increased replication ability, or fitness, and the successive fixation of the mutations) provide evidence of RNA evolution according to Darwinian principles.

We further characterized the biochemical properties of the evolved RNAs, such as the activity of the encoded replicase, the activity of the RNA as a template for replicase, the translation activity of the replicase, and so on. This biochemical analysis revealed that the RNA improved mainly the ability as a template for replication, and consequently the evolved RNA acquired the resistance against a parasitic replicator that spontaneously appear during the replication process through RNA recombination.

Discussion: This artificial cell-like system provide a useful platform to understand how an assembly of chemical molecules could become "alive" through an evolutionary process. In principle, the genomic RNA obtained in this study has an unlimited potential to acquire new functions and develop a more complex network by encoding additional genes, including translation factors, that are currently supplied externally. Examining whether the genomic RNA could (with additional replication cycles) evolve to create a system that resembles a natural living organism or whether the evolution would be halted by other obstacles such as an error catastrophe[3] would be of interest. The TcRR provides a novel platform for the experimental investigation of evolutionary scenarios that may lead to the emergence of a "living state" from the assembly of non-living molecules.

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