

ADAPTIVE EVOLUTION AND DIVERSITY OF AN ARTIFICIAL RNA SELF-REPLICATION SYSTEM

R. Mizuuchi¹, N. Ichihashi¹ and T. Yomo^{1,2,3}, ¹Department of Bioinformatics Engineering, Graduate School of Information Science and Technology, Osaka Univ., Japan, mizuuchi-ryo@bio.eng.osaka-u.ac.jp, ²Graduate School of Frontier Biosciences, Osaka Univ., Japan, ³Earth-Life Science Institute, Tokyo Institute of Technology, Japan

Introduction: The adaptive evolutionary ability is one of the remarkable characteristics of living things, which allows them to survive in various environments by changing gene expression and gene function. It also should enable a particular species, even primitive life-forms, to become extremely diverse, as can be found all over the world. The *in vitro* reconstitution of these adaptive abilities from biological molecules is a major challenge and certainly provides important insights into the origin of life and early evolution.

For years, we have explored the evolutionary ability of an artificial life-like system, translation-coupled RNA replication (TcRR) system, in which the genomic RNA replicates through the translation of the genetic information. The TcRR system contains an artificial genomic RNA encoding Q β replicase (RNA-dependent RNA polymerase) and a reconstituted *Escherichia coli* translation system[1]. To date, by repeating TcRR reactions in cell-like compartments, it has been proved that the genomic RNA spontaneously evolved to be highly replicable according to Darwinian principles, due to mutations introduced by replication error[2].

Recently, we have tested the adaptive evolutionary ability of the TcRR system. We continued the evolution experiment with a unique starting genomic RNA clone, an evolved RNA that emerged in the previous study[2], in five different severe translation environments in which different translation proteins were reduced. We already described the results of the evolution in reduced ribosome environment[3]. Here we report on the further analysis, a combination of the results observed in the five different environments. This is the first attempt of *in vitro* adaptive evolution experiment in a variety of environments using an artificial self-replication system although some in a particular environment were conducted[3][4][5].

Results & Discussion: Translation reaction consists of three steps: initiation, elongation and termination (and recycling). To make the severe translation environments, we reduced the concentration of some initiation-related proteins: (i) MTF, IF1 and IF3; (ii) IF1 and IF3; (iii) IF2, termination-related proteins: (iv) RF1, RF2, RF3 and RRF, and (v) ribosome, the pivotal factor involved in entire process of translation. After a long-term TcRR experiment with the unique starting genomic RNA clone under the five environments, the replicated RNA concentration was generally increased

in all environments. This result indicated that the genomic RNA adapted to the five different severe translation environments by evolution.

We sequenced some clones of the evolved genomic RNA. The patterns of common mutations, which were present in more than half of clones in each environment, clearly showed the considerable diversity. In particular, the genomic RNA evolved in the environments of reduced initiation-related proteins have markedly different mutations from ones evolved in the environment of reduced termination-related proteins. Moreover, the phylogenetic tree based on the mutations exhibited the slight yet obvious diversity among the genomic RNA evolved in the three environments in which the different initiation-related proteins were reduced. Besides, the mutations observed in reduced ribosome environment are roughly the same as found in the other environments. The order of the mutations, which we identified from RNA clones in the middle of evolution, suggested one possible evolutionary pathway in reduced ribosome environment, that is, evolving to improve the termination, then the initiation efficiency, which would increase in the translation efficiency as a whole.

The above results demonstrated that the TcRR system, composed of simple components and having no complex biological functions, still had some ability to undergo adaptive evolution and diversify. This study is a step toward constructing an artificial system harboring the same evolutionary ability as living things.

In addition, despite the mutation accumulation in every environment, the speed of evolution (degree of increase in the RNA replication per TcRR reaction) was more than 10 times higher in reduced ribosome environment than in the other four environments. One of the possible reasons for this rapid evolution is that entire translation process was inhibited in reduced ribosome environment, while a specific process was inhibited in the others. Given that, potential of rapid evolution may be greater in environments where a number of related reactions are simultaneously inhibited.

Reference: [1] Kita et al. (2008) *ChemBioChem*, 9, 2403–2410. [2] Ichihashi et al. (2013) *Nat. Commun.*, 4, 2494. [3] Mizuuchi et al. (2014) *ACS. Synth. Biol.*, DOI: 10.1021/sb5000884. [4] Kramer et al. (1974) *J. Mol. Biol.*, 89, 719–736. [5] Paegel and Joyce (2010) *Chem. Biol.*, 17, 717–724.