Oscillating population dynamics and evolution of host and parasitic RNAs in an artificial cell

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Abstract

The *in vitro* reconstitution of complex biological behavior from simple components is challenging in wet ALIFE. Here, we present the reconstitution of host-parasite oscillation dynamics *in vitro* using artificial RNA replicators. We used a translation-coupled RNA replication system encapsulated in water-in-oil droplets. This system used an artificial genomic RNA that replicates using the own encoded replication enzyme. During replication, a parasitic RNA, which lost the replicase gene, spontaneously appeared and co-replicated with the genomic "host" RNA, representing an oscillation in population dynamics. The presence of the parasitic RNA induced the evolution of the host RNA and produced diversity in the host RNA. These results demonstrate the important role of a parasitic entity on the evolution of life.

Introduction

Recently, various artificial cell-like systems have been constructed *in vitro* to study the origin and principles of life (Szostak et al. 2001; Jewett and Forster 2010; Forlin et al. 2012; Stano and Luisi 2013; Caschera and Noireaux 2014). One of the fundamental functions of life is the replication of genetic information. In all living organisms, the genetic information (genome) is replicated through the translation of information into proteins. We have previously reported an artificial life-like system that functionally mimics genome replication—translation-coupled RNA replication (TcRR) (Ichihashi et al. 2013). This system consists mainly of two parts, the artificial genomic RNA, which encode an RNA replicase, and the translation machinery of *Escherichia coli* (Shimizu et al. 2001). When we incubate the system, RNA replicase is translated and replicates the RNA. Mutations are spontaneously introduced by replication error, and faster replicable RNA mutants evolved according to the Darwinian principle.

We noticed that during the replication of the TcRR system, a parasitic RNA spontaneously appeared (Bansho et al. 2012) and dominated the population, competitively inhibiting the genomic "host" RNA replication (Fig. 1). This parasite was a large obstacle to achieve continuous replication and evolution.

Theoretically, the parasitic entity has been considered a serious problem for primitive self-replication systems because once present, it amplified selfishly by exploiting the host replication system. As a possible solution, compartmentalization or equivalent spatial structures has been proposed (Maynard Smith 1979; Niesert et al. 1981; Szathmary and Demeter 1987; Takeuchi and Hogeweg 2009), while experimental verification remains to be done.

In this report, we demonstrate that TcRR reaction continued, under compartmentalized conditions, even in the presence of a parasitic RNA. The presence of parasitic RNA produces diversity in host RNA species through evolution. This report is based on a previous work (Bansho et al. 2016), but presents a new analysis and discussion.

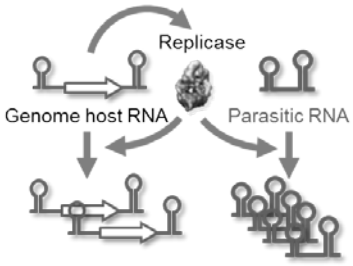

Figure 1. Schematic drawing of the replications of genomic host and parasitic RNAs. The genomic host RNA replicates using the replicase translated from itself. The parasitic RNA replicates using the host replicase.

Result

To examine the effect of compartmentalization on the parasite problem, we performed long-term replication experiments with and without compartmentalization. As compartments, we used water-in-oil emulsions, small droplets dispersed in an oil phase. When we performed the TcRR reaction under a usual bulk condition (i.e., without compartment), a parasitic RNA appeared and the replication of the host genomic RNA was inhibited. In the presence of compartmentalization, the replication of the genomic host RNA continued even after the appearance of parasitic RNA, and the population dynamics of both the host and parasite oscillated (Fig. 1A).

Further continuation of the replication revealed that the oscillation dynamics gradually changed. In the early rounds (round 2–20), the parasitic RNA (squares) overwhelmed the

host population (diamonds), while it reverted in the later rounds (round 42–50) (Fig 1A). The sequences of the host and parasitic RNAs showed that mutations were predominantly accumulated in the host RNA. Population analysis revealed that some mutant host RNA appeared and increased in frequency successively during long-term replication (Fig 1B). The appearance of the mutant RNAs did not fully replace the original RNA, which survived as a minor population.

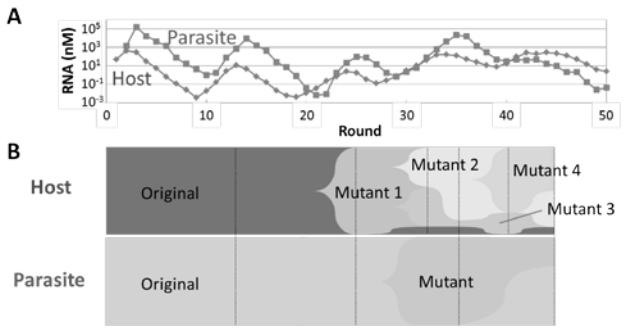

Figure 2. A long-term replication experiment
A) Population dynamics of the host and parasitic RNAs. The average concentrations of the host RNA (diamonds) and parasitic RNA (squares) with compartmentalization are shown. B) Mutations and frequency in the RNA population. Frequencies of each mutant RNA are schematically shown at each round. These figures were reproduced from data previously presented (Bansho, Furubayashi et al. 2016).

We also analyzed the biochemical properties of the mutant host RNA from the later rounds. The original host RNA did not replicate in the presence of the parasitic RNA, while the mutant RNA replicated efficiently, indicating that the host RNA acquired resistance to the parasitic RNA through evolution.

Discussion

This study demonstrated that when the reaction system is compartmentalized, the genomic host RNA replication continued after parasitic RNA appeared. These results provide experimental evidence to support compartmentalization as a means to overcome the effects of parasitic RNA, which was previously just theoretically proposed.

We have previously demonstrated the evolution of genomic RNA in the absence of parasitic RNA (Ichihashi et al. 2013). A comparison of this evolutionary process with that in the presence of the parasite was performed here. Diversity was maintained in the presence of parasitic RNA; in the absence of the parasitic RNA, a mutant RNA that replicates faster dominated the host population. The presence of the parasitic RNA allowed the original RNA to survive as a minor population even after mutant RNAs appeared. These results show that parasites play an important role in host evolution and RNA replication is a useful tool to understand the evolutionary relationship between host and parasitic species.

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