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Current Opinion in Systems Biology

How prebiotic complexity increases through Darwinian evolution

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Abstract

Present-day life is amazingly diverse and complex owing to Darwinian evolution. Despite the simplicity of the principle of Darwinian evolution, the process and its outcomes are largely unpredictable. Evolutionary simulation and experiments are useful methods for gaining insights into the process and outcomes of Darwinian evolution. In this short review, we discuss recent progress in theoretical and experimental approaches to understanding the possible evolutionary processes of prebiotic self-replicators. We especially focus on research addressing how a prebiotic self-replicator increases complexity through evolution, including our recent experiments, in which a complex replication network consisting of multiple self-replicating molecules spontaneously evolved from a single replicating RNA.

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Current Opinion in Systems Biology 2023, **34:**100456

This review comes from a themed issue on **Systems Ecology & Evolution (2023)**

Edited by **Edu Kussell** and **Nobuto Takeuchi**.

For a complete overview see the Issue and the Editorial

Available online 26 April 2023

https://doi.org/10.1016/j.coisb.2023.100456

Main text

Self-replicating systems and Darwinian evolution

Darwinian evolution is a repeating process of phenotypic (also genetic) diversification and natural selection, which has produced a diverse and complex living world. In addition to all present-day living organisms, the last universal common ancestor (LUCA), probably a bacteria-like cell containing more than 300 protein families [1], would be a product of prebiotic Darwinian evolution. A molecule or set of molecules with self-replicating ability is believed to have evolved to become LUCA at the culmination of prebiotic evolution [2]. To understand the feasibility of this hypothesis, many types of self-replicating molecules have been developed or are being developed in laboratories using various kinds of molecules, including DNA [3], RNA [4–10], an RNA/protein system [11], DNA/RNA/protein systems [12–14], peptides [15,16], peptide-based chemicals [17–20], lipid-based systems [21–25], and inorganic compounds [26] (recently reviewed in Refs. [27,28]).

Autocatalytic reactions are not necessarily subject to Darwinian evolution, which requires the production of phenotypic diversity in self-replicating molecules and the inheritance of phenotypes associated with the replicating molecules [29]. The requirements can be satisfied if a molecule self-replicates with moderate accuracy via a template copying reaction, in which the sequence of the template molecule is inherited by the copied complementary molecule (recently reviewed in Ref. [30]). During the copying process, errors produce diversity in the sequence (genotype), which produces various unique phenotypes among the molecules. Changes in the sequence and phenotype are inherited by their copies. If an error produces a change in the sequence that enhances copying activity, the changed molecule synthesizes more copies than other molecules and gradually dominates the population (i.e. natural selection occurs). In this manner, a template-copying molecule can undergo Darwinian evolution. A plausible prebiotic molecule that could have such evolutionary ability is a ribozyme polymerase. Recent studies have reported ribozymes with template copying ability [9,10,31,32], although their accuracy and polymerization efficiency are still insufficient for complete self-replication.

Template copying self-replication and continuous Darwinian evolution have been achieved only in systems that use modern proteins, such as RNA or DNA polymerases [11–14]. In these systems, RNA or DNA evolves through a continuous process of diversification driven by replication error and subsequent natural selection of more replicable mutant RNA or DNA.

Although these systems are not prebiotically plausible because they use polymerases from modern bacteria and viruses, they can be useful experimental models to understand how molecules can change through the process of Darwinian evolution.

In previous evolutionary experiments using RNA or DNA polymerases, the molecules indeed evolved, but their evolution did not produce any complex features or new functions, as would be required for the emergence of life [11,12]. In the case of RNA replication, Spiegelman and colleagues used the RNA-dependent RNA polymerase (RNA replicase) to perform self-replication of the genomic RNA of a bacteriophage as a template [11]. After many serial dilution cycles, efficiently replicable RNA evolved, but the evolved RNA was much shorter than the original RNA, having lost most of its encoded information. This result is reasonable because Spiegelman's system did not contain translation machinery and thus most of the encoded information was useless for RNA replication. It is widely believed that the acquisition of the ability of Darwinian evolution is a crucial step for prebiotic molecules to reach the emergence of life [2]. However, Spiegelman's experiment implies that Darwinian evolution does not necessarily generate complexity. How can complexity evolve from simple replicating molecules? This is an important question for understanding possible scenarios toward the origins of life.

Here, we define complexity based on two different categories, namely ecological and functional complexities. Ecological complexity is associated with the complexity in the network of interacting replicators and is positively correlated with the number of nodes and/or edges in the network. Functional complexity is associated with the complexity of the replicator's function and positively correlated with the level of difficulty and the number of functions performed by a replicator.

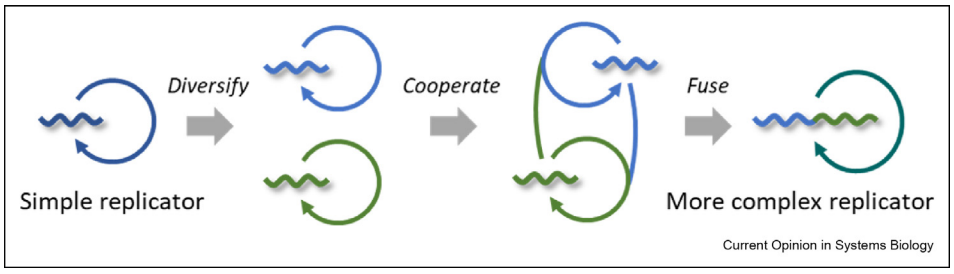
Theoretical studies for the evolution of complexity

A possible process for the prebiotic development of complexity was investigated using theoretical analysis. A pioneering model that could increase complexity in a prebiotic replication system is Eigen's hypercycle, which explains how information can be maintained in a cooperative replication network with low replication fidelity [33]. Szathmary and Maynard Smith proposed that the formation of a similar cooperative network between different self-replicating molecules is a general complexification pathway for biological systems as explained in more detail below [34]. A large obstacle for the hypercycles and other cooperative systems is selfish or parasitic replicators because they destroy cooperation among replicators [35]. This parasite problem can be circumvented by various types of compartmentalization (recently reviewed [36]) through group-level selection [37]. Group-level selection allows the coexistence of a large number of genes, leading to the evolution of cooperation and physical association of cooperative replicators [38,39]. Physical association between cooperative enzymatic molecules can be established in the absence of distinct compartmentalization, assuming that the cooperative molecules interact locally prior to dispersal [40]. More generalized multilevel selection has been studied recently by Takeuchi et al. who reported important parameters that balance the multilevel selection [41]. In another theoretical study, Takeuchi and Hogeweg demonstrated the evolution of ecological (organismal) complexity with RNA-like replicator that interacts via hybridization [42]. In the following recent study, Hickinbotham et al. proposed that parasitic entities are not just an obstacle to the evolution of complexity but drive the evolution of complex replication strategies [43].

Szathmary and Maynard Smith proposed a possible evolutionary pathway by which a simple replicator could develop ecological and functional complexities. In this pathway, these complexities could increase through cooperation and division of labor among previously independent self-replicating molecules or organisms [34,44]. For example, a eukaryotic cell is a product of symbiotic cooperation between a bacterium and archaeum, and multicellular organisms are a product of cooperation among different types of eukaryotic cells. If a similar event occurs among prebiotic self-replicating molecules, the self-replicating molecules can increase their complexity. Based on this idea, a possible scenario for the accrual of complexity by a simple molecular replicator is schematically shown in Figure. 1. In this scenario, a molecular replicator first diversifies into two replicators possessing different functions. These two replicators then start to cooperate to achieve the replication of both replicators (i.e., division of labor occurs) to form a complex replication network. If cooperation is beneficial for the replication of both replicators, the cooperative system should be positively selected. Next, the cooperating replicators may develop interdependency and fuse into a single molecule with the two functions [39]. Repeating this complexification process several times may convert a simple replicator into a complex multifunctional replicator.

It should be noted that there are additional difficulties for the primitive self-replicating molecules to develop complexity, such as a higher mutation rate and the absence of a cell membrane. These difficulties can be overcome by the compartmentalization provided by the environment and the stochastic correction principle [37]. In addition, the complexification process proposed here is only one of several possible processes. Other processes to increase ecological and functional complexity have been proposed in theoretical studies,

Figure 1



A possible complexification pathway for a primitive replicator. First, a simple replicator is diversified into two replicators (e.g., via co-evolution with parasites) that have different functions. Next, the diversified replicators begin to help each other replicate via complementary functions, forming an interdependent replication network. Finally, the two cooperatively replicating molecules fuse to form a single, multi-functional, more complex replicator.

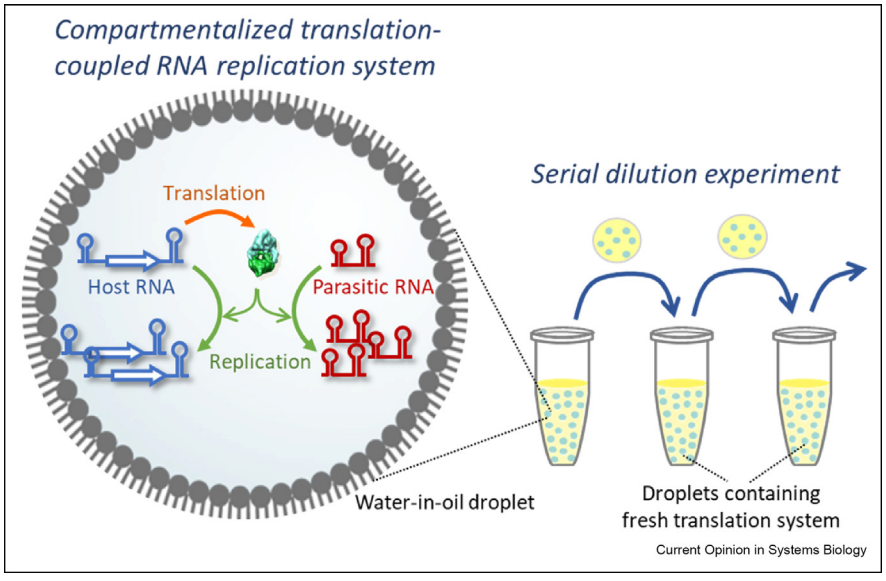
such as the structure of quasispecies [45] and the modification of folding [46].

An advantage of these theoretical studies is the degrees of freedom. Researchers can define all factors and conditions as they wish. A disadvantage is the lack of restrictions associated with realistic biological molecules. Biological molecules, such as polynucleotides and proteins, have limited functions owing to their physicochemical properties, restricting their possible evolutionary pathways. To investigate the plausibility of an evolutionary process under the restrictions emplaced by the physicochemical properties of biological molecules, experiments with realistic molecules are required.

Experimental studies to understand the evolution of complexity

In Spiegelman's RNA replication experiment using an RNA replicase, RNA evolved to become shorter and simpler without diversification. This result implies that another factor is required to drive complexification. One possibility is translation machinery, without which, most of the RNA region that encodes genes is useless for RNA replication and could be lost during evolution. Based on this idea, we introduced a reconstituted translation system, PURE (Protein synthesis Using Recombinant Elements) system [47], consisting of all RNAs and proteins required for translation in *Escherichia coli*, into Spiegelman's system [48]. In this new system, RNA

Figure 2

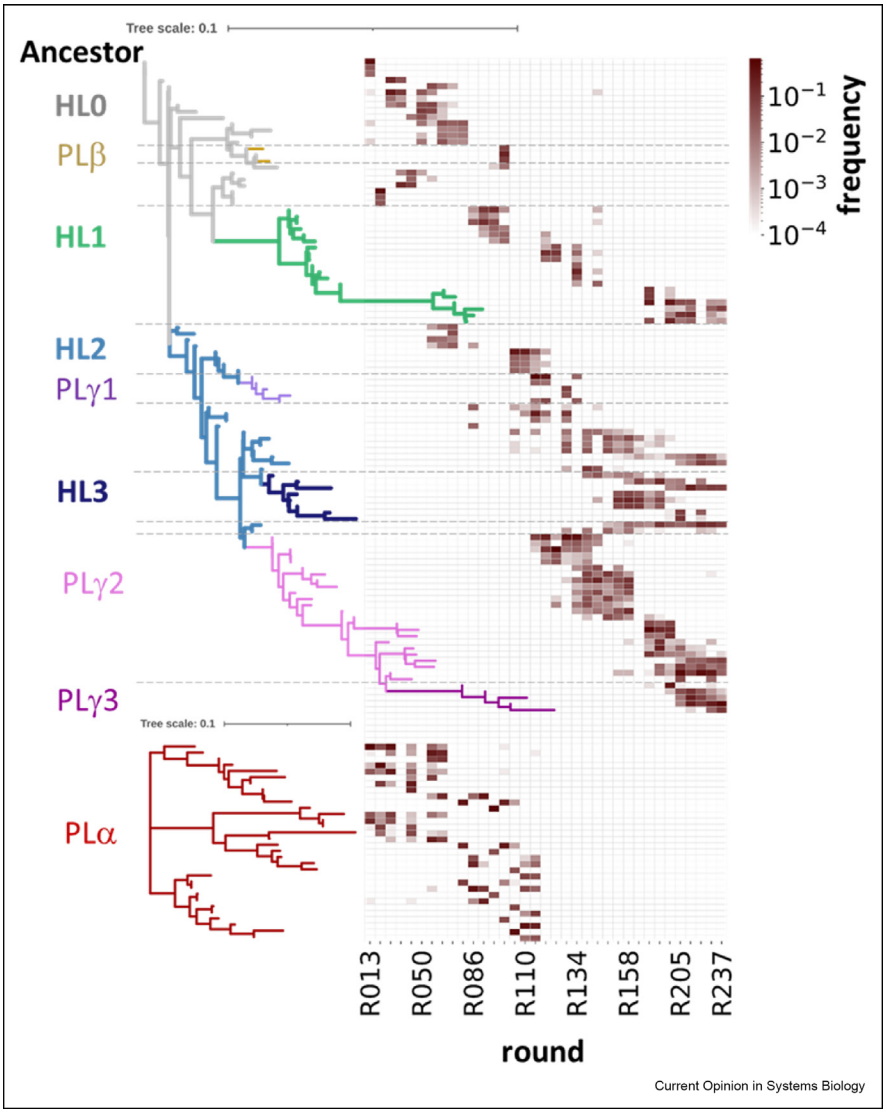


Compartmentalized translation-coupled RNA replication, and a serial dilution experiment. In translation-coupled RNA replication, a host RNA replicates via translation of the self-encoded RNA replicase (Qβ replicase). A parasitic RNA does not encode a replicase, but replicates using replicase translated from other host RNAs. The reaction mixture was encapsulated in water-in-oil droplets, and translation-coupled replication was continued through a serial dilution cycle with droplets containing a fresh translation system.

replication requires translation of the self-encoded RNA replicase, and thus a large fraction of the RNA retains the original RNA size, unlike Spiegelman's experiment. Some RNA, however, lost the replicase gene through recombination in the translation-coupled system. Such smaller RNAs replicate in a parasitic manner by relying on the replicase produced from other gene-encoding RNA [50] (Figure. 2). To repress excess amplification of the parasitic RNAs, continuous RNA replication in this system requires compartmentalization [51].

To date, we have been continuously performing compartmentalized translation-coupled RNA replication for many generations. Initially, we performed 96 rounds of serial dilution cycles at higher dilution rates to keep host and parasite RNAs out of the same compartment and

Figure 3



Complete phylogenetic tree and frequencies in the population of the previous evolutionary experiment using the translation-coupled RNA replication system. A phylogenetic tree was constructed containing the three most frequent genotypes in each sequence round. All data were obtained from the previous studies [49,50]. This tree includes all RNA lineages that disappeared during the experiment, such as parasite lineage α (PLα) and parasite lineage β (PLβ), which were omitted from the tree in a previous study [50]. Host (HL0-3) and parasitic (PLα, PLβ, and PLγ1-3) RNA lineages are shown as thick and thin lines, respectively. PLγ1-3 were the same as PL1-3 in a previous study [50]. The origin of parasite lineage α was uncertain and thus is represented as a separate tree. The tree was constructed using the NJ method with maximum composite likelihood and pairwise deletion using MEGA X [54]. The frequencies of the leaves in each host or parasitic population are shown in a heat map (i.e., the summation of the frequencies of all parasite (or host) RNAs in a round is one).

thus suppress parasite RNA replication [52]. Then we performed 240 rounds of serial dilution cycles at a low dilution rate allowing co-replication with parasitic RNAs [49,50]. The latter coevolutionary experiment with parasitic RNAs started from an evolved RNA clone obtained from the final round of the former experiment. Two biologically relevant events, diversification and ecological complexification, occurred in the latter co-evolutionary experiment [49,50]. RNA species that appeared in the co-evolutionary process are represented in phylogenic trees in Figure. 3, in which the existing period of each leaf in the RNA population is shown as a heat map. The evolutionary process is briefly explained as follows: Soon after starting replication using a single host RNA (ancestor), a small parasite RNA (parasite lineage α, PLα, ∼200 nt) appeared, and the original host lineage (HL0) diversified into parasite-resistant (HL1) and parasite-susceptible (HL2) host lineages [49]. We recently confirmed that both types of host lineages are sustainably co-replicated with a parasite by forming a stable three-member replication network [51]. HL1 accumulated further mutations (i.e., evolved) until the final round (240). In contrast, HL2 seemingly stopped evolving, while some HL2 RNAs persisted to the final round. From HL2, a new host species (HL3) and new parasitic RNAs (PLγ, ∼500 nt) were derived and existed in the final round. In the later stages of the experiment, PLα was rarely detected, but the new parasite lineages γ (PLγ1-3) dominated the parasite population. Biochemical analysis of the representatives of the five lineages in the final rounds, HL1, HL2, HL3, PLγ2, and PLγ3, showed that four of the RNAs (HL1, HL2, PLγ2, and PLγ3) form a stable replication network [50]. Computer simulations based on experimentally measured parameters support the interdependent replication of the four RNAs [50].

The evolutionary process we have observed so far partially supports the hypothetical primitive replicator complexification pathway shown in Figure. 1. First, the initial clonal RNA diversified into five lineages (host HL1, HL2, and HL3, and parasitic PL2 and PL3) in the final population, forming an interdependent (not cooperative yet) replication network [50]. In this experiment, we simply repeated the compartmentalized serial dilution process (i.e., no artificial selection for complexification), while the RNA replicator spontaneously evolved to form a more complex interdependent replication network. This result implies that RNA replicators coupled with protein translation can develop ecological complexity through Darwinian evolution. The universality of this phenomenon in other self-replication systems is the next important challenge.

Conclusions and future directions

Recent theoretical and experimental studies propose one possible answer to the question "How do molecules develop ecological complexity through Darwinian evolution?" That possible answer is the interaction between independently replicating molecules to form a replication network, which has been demonstrated as possible both in a theoretical study [42] and in our experiment [50]. In both cases, parasitic replicators function as a niche to allow the coexistence of different types of host replicators. Parasitic replicators might play an important role in the evolution of ecological complexity.

The next important question is "How does a molecular replicator develop functional complexity?" If cooperative replicators with different functions fuse and replicate as a single molecule, as shown in the last step of Figure. 1, this would be considered an increase in the functional complexity. However, in the evolution we have observed so far, the functions of the host RNAs in the interdependent network are still similar; they differ only in their template specificities. Diversifying the functions of co-replicating RNAs is the next important challenge. Recently, we found that the RNA polymerase encoded in the replicating RNAs tends to relax substrate specificity and start incorporating deoxyribonucleotides instead of legitimate substrates ribonucleotides after a long-term evolution, although the incorporation ratio is still low [52]. If we can establish an experimental condition in which deoxyribonucleotide incorporation is beneficial for replication, functional diversification may occur. We have also recently detected fusion of co-replicating RNAs in another experimental setup, although it is still at a low frequency [53]. Further evolutionary experiments using our and other experimental systems may also demonstrate functional complexification.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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