Diversity, stability and evolvability in models of early evolution

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Abstract

Based on the RNA world hypothesis we outline a possible evolutionary route from infrabiological systems to early protocells. To assess the scientific merits of the different models of prebiotic evolution and to suggest directions for future research we investigate the *diversity maintaining ability*, *evolutionary/ecological stability* and *evolvability* criteria of existing RNA world model systems for the origin of life. We conclude that neither of the studied systems satisfies all of the above criteria, although some of them are more convincing than the others. Furthermore, we found that the most conspicuous features of the proposed prebiotic evolutionary scenarios are their increasing spatial inhomogeneity along with increasing plasticity, evolvability and functional diversity. All of these characteristics change abruptly with the emergence of the protocells.

Keywords

diversity maintaining mechanisms, coexistence, ecological stability, evolutionary stability, evolvability, early evolution

Highlights

- The diversity maintaining ability, evolutionary/ecological stability and evolvability criteria of different models of early evolution are studied.
- Neither of the proposed models preceding the functional protocell phase satisfies all these criteria.
- Increasing spatial heterogeneity with increased evolvability and functional diversity are the main trends during the pre-protocell phase of evolution.

Introduction: The central aspects

Biological evolution must have started with the emergence of the first evolutionary units possessing the capacity for self-replication, inheritance and variability [1,2]. The conceptual basis of our arguments in this paper is the RNA world hypothesis [3,4], the dominant paradigm of origin-of-life research founded on the assumption that RNA molecules may have acted both as enzymes and as replicating information carriers [5-10] in prebiotic times. In the following we shall overview a possible scenario of evolutionary processes from simple communities of RNA molecules to protocells.

Our systematic analysis of potential prebiotic scenarios rests on four major pillars: *diversity maintenance*, *evolutionary and ecological stability* and *evolvability*. These pillars comprise essential criteria for any evolutionary system: models failing to meet all of these criteria have no, or have at best very limited, explanatory power in an evolutionary context. The criteria are defined as follows (cf. [11]).

Diversity maintenance: The copies of self-reproducing units are not always identical with their templates (variation). Some of the copies may differ in their fitness from that of the template. Long-term evolution and adaptability of a system of such units are guaranteed if selection benefits the fittest units while maintaining the production of functionally diverse mutant units.

Ecological stability: There are external factors (mainly abiotic, such as temperature, humidity, *p*H, etc.) that affect the mortality/replication rates of the units. We consider a community of evolutionary units to be ecologically stable if the natural/typical level of perturbations cannot decrease the diversity of the community.

Evolutionary stability: Even though the "ecological" and "evolutionary" time scales may have overlapped at this early stage of evolution, we require a distinct condition of evolutionary robustness. The community of such units is evolutionarily stable if the resident community is resistant to the emergence of any possible adverse mutant. If a mutant can destabilize the system (by reducing its functionality or diversity, or ruining the community altogether) the system is considered evolutionarily unstable.

Evolutionary potential (evolvability): The innate capability of evolutionary units that enable them to adapt to changing environments. Evolvability includes the ability to integrate new replicator types (originated principally, but not exclusively, from mutants) increasing system-level fitness.

In the following we will analyze the best known and/or the most promising models of the “replication first” scenario of the origin of life from the perspective of these criteria. We show that many of the most popular, heavily cited model frameworks suffer from lack of stability and/or diversity maintaining ability; others have limited or no evolutionary potential, while some other models simply have not yet been systematically investigated for all these criteria. On the basis of this analysis we suggest further investigations in specific model systems and we recommend a possible scenario of early evolution from the occurrence of short self-replicating macromolecules to that of the simplest protocells.

Prebiotic evolution I: self-replicating macromolecules

We consider chemical events in the RNA world era during which the first evolutionary units probably appeared. We suppose that replicator macromolecules (most probably RNA or some RNA-analogue) emerged in this phase as the result of chemical evolution, even if this step has the least empirical

support at the moment due to the complete lack of fossilized evidence and the scarcity of relevant chemical experiments. Manfred Eigen was the first to formulate a dynamical model of prebiotic replicator ecology [12]. In this model the amount of heritable information is measured as the length of the "master" sequence (the fittest – i.e., of highest replication rate – sequence attaining the highest concentration over generations). Eigen pointed out that high mutation rates (attributable to enzyme-free replication) pose a strict limit to the amount of information that can be maintained by selection. If the mutation rate remains below a critical value (the *error threshold*) then the master sequence and the diverse community of its mutants (the quasispecies) survive from generation to generation, otherwise the master will disappear from the system.

This type of system may adapt to environmental change, because if the external conditions changing then earlier mutant sequences could become masters. The diversity generated by sustained mutational events results in an evolvable community. The length of the "master" sequence represents the amount of information that can be stored in the system. Since the error rate of enzyme-free replication is assumed to be relatively high compared to that of modern enzymatic template replication, the maintainable information is less than the amount necessary for any "minimal cell", the simplest (biological) system meeting the criteria of life [13]. Splitting a long sequence of sufficient information content into shorter ones, each below the limiting sequence size maintainable at the feasible mutation rate, would be a straightforward solution. It would also be chemically more plausible: different sequences may have different chemical activities, and quite a number of such chemical functionalities are indispensable for running a minimal cell. However, splitting the information into smaller sequences does not necessarily increase the amount of dynamically maintainable information, due to the competitive exclusion principle which inevitably applies to replicator communities, each member of which uses the same resource (monomer supply) for its replication. The sequence with the highest effective replication rate will outcompete all the others, reducing the information content of the system far below the level required for sustaining a minimal cell. Despite the substantial research effort invested into solving this problem (some of the corresponding results will be discussed later, also see e.g. [11]), the question of how to avoid the "information gap" remains open.

The best known attempt at solving the information catastrophe puzzle is the hypercycle model of Eigen [14,15] which modified the original idea of pooling short sequences by introducing a circular heterocatalytic coupling of the different short replicators, assuming that each has a very specific chemical activity necessary for the replication of exactly one other member of the community. This system is capable of maintaining its diversity, but its evolvability is very limited [15], and it is unstable in both the ecological [16] and the evolutionary [17,18] sense. The spatial version of the hypercycle [19] does not perform much better either [20–22].

The diversity maintaining ability of prebiotic replicator models changes dramatically by taking an important chemical detail of replication into account: during template replication, the new copy of a replicator may remain bonded with its template. The resulting dynamics is the so called parabolic replication [23]. The bonded template-copy complex is inert for replication and, since the dissociation of this complex is less probable than the association of its components, the template-copy complex exhibits self-regulation: the more common the sequence the stronger the downregulation of its replication, due to more frequent pairing up and forming the inert double strand. The resulting "advantage of rarity" warrants the coexistence of, in principle, any number of different replicator types [23] in the parabolic regime. Depending on the concentrations of the replicators and other parameters of the dynamics (the influx of raw materials and decay rates of templates), the system can be either "parabolic" without selection ("survival of everybody") or exhibit Darwinian selection ("survival of the fittest") like in models where template-copy bonding is not assumed. This dynamically beneficial

feature of parabolic replication is also its major fault by itself regarding evolvability: lacking (Darwinian) selection adaptation is not possible in this system. These features remain unchanged in a model variant with extended chemical kinetics treating the dynamics of single and double strands separately [24] and spatially extended versions assuming surface binding of replicators [25].

For a sustained replicability ("chemical operability") of an RNA-based prebiotic system, it is necessary to maintain the catalytic activity of different replicators. Since catalytic activity is usually associated with certain structural motifs (active sites), it may be sufficient to maintain the critical part of the secondary structure, rather than the entire genotype. Knowing that some of the (genotypic) mutations are neutral on the phenotype [26] this approach poses a more permissive limit on the magnitude of the allowable mutation rate, the so-called phenotypic error threshold [27–29].

All previously discussed models assumed that replicators live in a well-mixed environment without any structure. Spatial (e.g. surface bound) or compartmentalized extensions of different systems can exhibit more stable dynamics with higher information integrating capacity, because spatial structures may act as further regulatory factors and open the route to multilevel selection. In the following section we extend the scope of our investigation in this direction.

Prebiotic evolution II: Metabolically coupled replicators

It is likely that the template-directed self-replication of RNA-like molecules was initially limited by the abiotic production of their activated monomers [9,30--32]. Since RNAs can have enzymatic activities, it is reasonable to assume that, in addition to self-replication, they had evolved to cooperate in maintaining a common metabolism supplying them with the necessary raw materials (Fig. 1) [33,34]. In well-mixed environments the products of metabolism are equally beneficial for all replicators, while all types of cooperating replicators are needed for the metabolism. In other words, metabolism aspecifically supports the replicators by supplying them with monomers, and the replicators specifically support metabolism by catalyzing certain reactions of it. This is the core idea of the metabolically coupled replicator system (MCRS), see [33]. However, if RNAs replicate at different speed the replicator with the highest effective replication rate will outcompete all the others, so that metabolism stops and the entire MCRS collapses. Therefore, the non-spatial version of the MCRS does not meet one of the basic dynamical criteria: it is ecologically unstable.

The situation is radically different if the dynamics occurs on a surface, on which replicators and monomers can diffuse and only neighboring molecules are involved in reactions. Due to their relatively high abundance replicators of high replication rates will find themselves in neighborhoods lacking at least one of the essential replicators with a high probability, thus missing out on monomers and stuck in replication. On the other hand, replicators that start to become rare because of lower replication rate will be members of neighborhoods with all necessary replicator types present more frequently, having a disproportionally higher chance to replicate. That is, local interactions and non-perfect mixing increase the replication success of locally rare replicators. This mechanism allows the coexistence of different replicators in spatial versions of the MCRS (sMCRS), as spatial inhomogeneities induce a kind of multilevel selection [33-35]. It is the same disadvantage of common types of replicators (and their local dilution effect) that prevents non-cooperative parasites (e.g. replicators that use the activated monomers but do not take part in the metabolism producing them) from destroying the MCRS or even becoming dominant in the system, though they coexist with it [33–35].

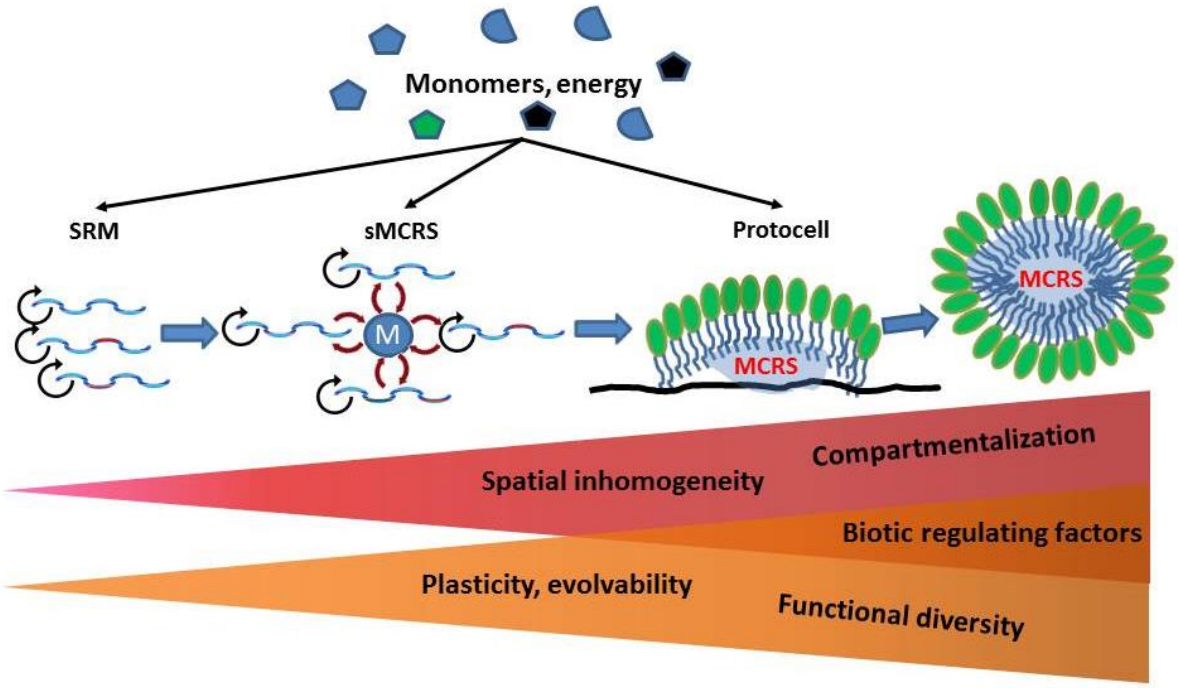

Figure 1. The scenario from self-replicating macromolecules (SRM), through the metabolically coupled replicator system (MCRS) to protocells. Before the emergence of the membrane subsystem MCRS should be bound to a surface (sMCRS). Self-replication is denoted by black circular arrows (SRM, MCRS) and interaction of replicators with the metabolism (M) by brown curved arrows. An MCRS system is surrounded by a membrane (green) either on a surface or later in a liquid phase. Widening arrows indicate the increasing spatial heterogeneity together with increasing plasticity, evolvability and functional diversity. Compartmentalization leads to the dominance of biotic regulating factors.

More comprehensive simulations revealed that a sMCRS is typically viable if replicators disperse more actively than monomers (or, alternatively and more realistically, if monomers more easily detach from the surface) and the number of different replicators could not be much higher than a dozen. The system's resistance against parasites depends also on the size of the interaction neighborhood of replicators and monomers, and naturally on the relative replication speed of parasites [34,35]. Parasites living together with cooperating replicators can mutate freely and thus have the opportunity to evolve to cooperating replicators, that is, to become a general ribozyme of the metabolism, or even to contribute to the synthesis of “membranogenic” molecules separating the system from its surroundings [35]. That is, a sMCRS has evolutionary potential, although the question how a complex and obligate metabolic network incrementally builds up and adapts to the environment is yet to be studied systematically.

In summary, the sMCRS is an evolvable, ecologically and evolutionary stable diverse community of replicators within a definite (but not necessarily chemically realistic) parameter space.

Protocells

We define the protocell as an entity integrating the metabolic, template and boundary subsystems in a functional and evolvable system [36,37]. In the original MCRS model system macromolecules have metabolic and template function as well. As we have seen, the diffuse spatial structure arising from surface attachment makes the MCRS a hopeful model of early evolution. Having a more definite

boundary (like a membrane compartment) around the MCRS would have many additional benefits both in the population dynamical and the evolutionary sense. It could maintain higher chemical concentrations of metabolites and macromolecules to increase the efficiency of reactions; it may sustain and stabilize a specific chemical milieu inside the compartment. Most importantly, if the MCRS is conveyed with a distinct physical boundary it constitutes a new evolutionary unit (the protocell) that is more definite than the metabolic neighborhoods of the sMCRS, thus opening the way for robust multilevel selection. It has been proposed that this encapsulated MCRS is the first living entity [36,37]. An MCRS functioning on a surface has a clear benefit by producing a boundary around itself, in terms of both metabolic and selection efficiency. Moreover, protocells later dissociating from the surface can occupy a purely liquid habitat (Fig. 1). While a "naked" MCRS hopelessly collapses in well-mixed liquid media (see above), it is not the case if the MCRS is wrapped within a protocell compartment. Experts think that fatty acids or mixtures of fatty acids and phospholipids may have constituted the first membrane of protocells [38,39]. The selective permeability of fatty acids suggests that the first protocells may have been heterotrophic, by taking up nutrients and other small extracellular molecules via passive diffusion. Fatty acid vesicles are much less permeable to larger polymers and highly charged molecules [40], so it is probable that the first protocells functioned without active transport by specialized ribozymes or other macromolecules.

The stochastic corrector model (SCM) of Szathmáry and Demeter [41] can be considered as a population of protocells each of which contains an MCRS with two types of competing replicators. When the amount of replicators within the cell reaches a critical value the cell divides and the replicators it harbors are distributed randomly between the daughter cells. According to the model assumption fission rate and thus the fitness of a cell is maximal if the copy number ratio of the two types of replicator is 1:1 in it. As we have emphasized above the non-spatial version of MCRS collapses, but the recurrent fissions in the SCM preclude the competitive exclusion of the inferior replicator type. Furthermore, natural selection acting on the daughter cells harboring randomly redistributed replicators can maintain a replicator distribution peaking close to the optimal copy number ratio [41]. This stochastic redistribution can also generate the necessary compositional variance within protocells. The optimal or nearly optimal compositions are selected from this, because selection acts on the protocell level, too. We might think that SCM as a simplest protocell model satisfies all the conditions to be a viable evolutionary unit. However, there are clear limits of the ecological and evolutionary robustness of the SCM. It is ecologically and evolutionary stable if two types of replicators are present in low copy numbers in the protocell [41,42]. As the number of different replicator types increases, the efficiency of SCM to maintain their coexistence, that is, even the ecological stability of the system decreases abruptly [43] because of the fast weakening stochastic correction effect controlling reassortment.

The key assumption of SCM is that the environment is well-mixed within the protocell, which would cause the collapse of MCRS system without random redistribution of replicators and selection on the cellular level. But this could not have been the case if the protocell evolved from the MCRS living on a surface (see Fig. 1). So we propose to study an SCM model within which the metabolically coupled replicators are living on a surface. We think that spatial structures within the protocell can robustly support the coexistence of cooperative replicators (ecological stability) and suppress harmful mutants (evolutionary stability).

As discussed above, it may have been the emergence of protocells that made the MCRS capable of occupying liquid phase habitats. This step opens the path for using alternative energy sources and different chemical representations of metabolism, so by the emergence of the first living cells new niches had become available. This means that protocells could have had a significantly increased

evolutionary potential compared to the spatial (surface-bound) MCRS. With the appearance and dominance of active transport, the selectivity of membrane permeability increased, so the total number of replicators and types of different replicators within the cells could also be increased. Then, stochastic correction could not maintain a complex metabolic replicator system of this size any more [43]. Therefore, selective permeability and active transport should have coevolved with the control over replication and/or increased replication fidelity [44].

Impermeable membrane with active transport has made increasing autotrophy possible [37,38]. Since the simplest biological transporters are short antibiotic peptides [45], it is reasonable to assume that chemical warfare among the cells evolved early together with active transport. It is a familiar argument that chemical warfare with nontransitive interference competition can maintain a diverse community of bacteria [46–48]. However, such communities are shown to be neither ecologically nor evolutionary stable in well-mixed habitats [47, 49]. Therefore, nontransitive interference competition was able to increase functional diversity of protocells (which is connected with our first and fourth criteria) only in habitats where some kind of spatial heterogeneity was present.

Presumably, in a way similar to extant bacteria, evolved protocells could have produced not only hostile extracellular chemicals but also exoenzymes to decompose, dissolve and uptake food. These enzymes are public goods for all the cells, thus not only the producers but the non-producing (cheater) cells can use them freely. In the classical public goods game situation the abundance of producers increases the concentration of the common good linearly, thus selection leads to the extinction of either the producer or the cheater strain [50]. However, the saturating effect of enzymes [51–53] and the extra benefit for the producer due to limited enzyme diffusion [54] generally lead to the coexistence of producers and cheaters [54,55] which increases the functional diversity even further. Suitable enzymes would have made the protocells capable of digesting concurrent cells [56,57]. The emergence of these first cannibalistic/predatory protocell strains increased again the functional diversity of cells, and acting as new regulating factors, also the number of different coexisting cells in a habitat [58-60]. In this way, besides the propensity of the protocells for robust ecological and evolutionary stability, their potential for maintaining diversity and speeding up evolutionary changes may have increased substantially.

Conclusions

We have reviewed RNA world models of early evolution from the point of view of their potential for diversity maintenance, ecological, evolutionary stability and evolvability. Table 1 summarizes the results of our considerations in relation to these four criteria with respect to the three most hopeful modelling approaches to prebiotic replicator dynamics. We think that these models deserve more attention and further investigations, since previous research has confirmed that the replicator systems they represent can behave as real evolutionary units while some of their important aspects require more comprehensive study.

We have pointed out that, in order to maintain ecologically and evolutionarily stable diverse communities, some kind of spatial heterogeneity and limited mixing are necessary in every model system of early evolution (Table 1). The only exception is the system of parabolic replicators, but this system is not evolvable. We emphasize that, despite the elegance, popularity and high impact of some models and mechanisms of early evolution discussed before, in well-mixed environments none of them is robust against ecological and/or evolutionary disturbances. Consequently, they have limited or no explanatory power in their present form and even spatial extensions of the corresponding systems lack one or more of the four essential criteria of feasibility. For example, a hypercycle of replicators,

or degradation resistance against antibiotics needs spatial heterogeneity to be viable at all, but even the spatial hypercycle is not stable against spatially heterogeneous perturbations by adding parasitic (cheating) mutants [19,61]. On the other hand, we have shown that spatial inhomogeneities can explain diverse, stable and evolvable communities of evolutionary units without specific interaction structures or mechanisms in some RNA world models.

	DIVERS	ECOSTAB	EVOSTAB	EVOLVAB
PR	+	(+)	+	-
sMCRS	(+)	+	+	(+)
SCM	(+)	?	(+)	+

Table 1. *The properties of the investigated models with respect to our "central criteria". Diversity maintaining ability (*DIVERS*), Ecological stability (*ECOSTAB*), Evolutionary stability (*EVOSTAB*) and Evolvability (*EVOLVAB*) of the parabolic replicator (*PR*), the spatial version of the metabolically coupled replicator system (*sMCRS*) and the stochastic corrector model (*SCM*). "+" sign: the model meets our criterion, (+): the model meets the criterion to a limited extent, "-": the system does not meet the criterion, "?": not investigated.*

We suggest that spatial inhomogeneity, stability, evolvability and functional diversity are positively correlated to each other, and they all tend to increase in biologically and chemically feasible prebiotic evolutionary models. These changes imply the emergence of new evolutionary units (Fig. 1). We argue that the emergence of protocells allows sophisticated control over spatial heterogeneity and mixing which, beside increasing ecological and evolutionary stability of the protocell populations, sharply increases their functional diversity and evolvability. After this major transition had taken place biotic regulating factors have become more important and adaptation mechanism more fine-tuned. The combination of the main ideas of different infrabiological models in Table 1 may also help to bridge the information gap burdening theoretical studies of evolution towards the first protocells. We are convinced that studies along these lines will significantly contribute to our theoretical understanding of, and possible later experimental approaches to, the origin of life.

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