Marginally Stable Chemical Systems as Precursors of Life

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Current research on the origin of life aims at finding the simplest entity that can undergo spontaneous Darwinian evolution toward increasing replication efficiency. Here I consider some of the models of self-replicating molecular systems, and I show that they exhibit a distinct feature, namely, an infinity of stationary states forming a continuous curve; i.e., they are only marginally stable. I show that, in marginally stable chemical systems, thermodynamic fluctuations induce a drift directed toward increasing replication efficiency. This drift represents a form of evolution, taking place slowly, cooperatively, in macroscopic volumes of water.

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The research on abiogenesis aims at finding a plausible model to explain how life could have arisen from nonliving matter. The likelihood of a model is assessed by reproducing in the laboratory some of the hypothetical basic steps, as in a bottom-up approach to the generation of artificial protocells. Experiments have shown that the building blocks of biological informational polymers, in particular, RNA, can spontaneously form [1,2] and polymerize [3]. Directed (artificial) evolution [4] has been used to create molecules that mimic some of the characteristic features of living entities, in particular, ribozymes, i.e., RNA molecules with catalytic activity. Among them, Refs. [5–7] describe ribozymes that can self-catalyze their formation, and Ref. [8] describes a ribozyme that can copy other RNA molecules, i.e., a replicase.

The presence of a replicase R, able to replicate generic templates T, is a necessary condition for inheritance, i.e., the ability of a living entity to transmit its mutations to its offspring, which is one of the key features required by Darwinian (spontaneous) evolution. It has been observed [9] that a purely chemical system including a replicase R lacks another key feature of Darwinian evolution, namely, selection: the ability to replicate other templates T gives R no advantage in being replicated, so the concentration of R does not increase over the other polymers whose templates are present. It has been argued that Darwinian evolution requires a physical process besides traditional chemistry, namely, the coupling of the replicating polymers with membranes [9], to introduce competition between different entities [10–14]. This argument presents a challenge, because the informational polymers must emerge simultaneously with the membrane structure that contains them.

Here I describe a different physical process that plays the role of selection in Darwinian evolution. I consider the effect of thermodynamic fluctuations, i.e., fluctuations of the number of molecules in a given volume, due to thermal motions. Recently, it has been noticed that fluctuations can induce "deviant nonclassical effects" on certain biochemical systems [15,16]. I will show that replicases make it possible to build systems that can be influenced by fluctuations, due to a distinct feature that I will call "chemical marginal stability." I will show the results of numerical calculations, concerning simulations of different marginally stable chemical systems where fluctuations are taken into account. Quite surprisingly, the fluctuations induce not only a random walk, but also a drift, directed towards increasing replication efficiency. The relevance of this phenomenon for the origin of life, and possible *in vitro* experiments are discussed.

Figure 1(a) shows a ribozyme L that is able to ligate two substrates, $B1$ and $B2$, i.e., a ligase. The outcome of the reaction is a new molecule L, identical to the ligase that

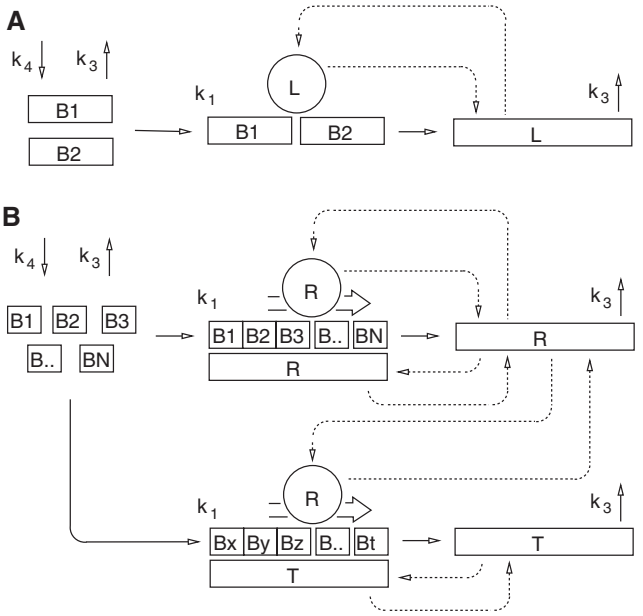

FIG. 1. Panel A, self-catalysis: the ribozyme L (a ligase) catalyzes the ligation of substrates $B1$ and $B2$, forming a new molecule L. Panel B, replication with inheritance: the ribozyme R (a replicase) copies a generic RNA template, including other molecules of R.

generated it. Such a reaction can be described as self-catalytic. It has actually been obtained in an artificial system [5,6]; a cross-catalytic version of the reaction, involving two ligases, was able to self-sustain and produce an exponential amplification of a small initial amount of the ligases [7].

The fact that the outcome of the ligation is the same as the ligase L that catalyzes the reaction relies on the peculiar choice of the substrates $B1$ and $B2$. In general, a mutant ligase $L2$ will not transmit its mutation to the newly formed ligase product. Inheritance, which is one of the features needed by Darwinian evolution, requires some enzyme performing a replication activity, i.e., a replicase. Figure 1(b) shows a toy model where a hypothetical replicase R can copy any generic template T, including other replicase molecules R, by polymerizing the monomers Bn. In such a system, a superior mutant replicase $R2$ would also be replicated. I will refer to this case as ''replication with inheritance,'' as opposed to the previous ''self-catalysis.'' It is worth noting that the ribozyme R can act both as a replicase and as a template, but the same molecule cannot play both roles simultaneously; the production of a new R thus requires two molecules of R, one as a replicase and one as a template.

The system shown in Fig. 1(b) is extremely simplified: in real experiments, partial success has been reached in producing, by directed evolution, a ribozyme R that creates a complementary strand T' of a generic RNA template T by extending a primer [8].

The fundamental difference between self-catalysis and replication with inheritance has been described; now I will show that this difference has a counterpart in kinetics. In the case of self-catalysis, I consider a ligase $L1$, together with a more active ligase $L2$; they both catalyze the ligation giving rise to $L1$. The corresponding mass-action equations are

$$\begin{aligned} \dot{L}_1 &= k_1 B^2 L_1 + k_2 B^2 L_2 - k_3 L_1 \qquad \dot{L}_2 = -k_3 L_2 \\ \dot{B} &= -k_1 B^2 L_1 - k_2 B^2 L_2 - k_3 B + k_4 \end{aligned} \tag{1}$$

where the concentrations of the substrates $B1$ and $B2$ are assumed to be equal to B. It is worth noting that the system is open: the term k_4 represents the source of substrates, and the term k_3 represents the loss of molecules.

In the case of replication with inheritance, I consider two replicases $R1$ and $R2$, with different activities. The mass-action equations are

$$\begin{aligned} \dot{R}_1 &= k_1 R_1^2 B + k_2 R1 R2 B - k_3 R_1 \\ \dot{R}_2 &= k_1 R_2 R_1 B + k_2 R_2^2 B - k_3 R_2 \\ \dot{B} &= -(k_1 R_1 + k_4 R_2)(R_1 + R_2) B - k_3 B + k_4. \end{aligned} \tag{2}$$

Since polymerization takes place by progressive extension, one monomer at a time, the rate is proportional to the concentration of monomers. The replicase $R2$ is more active than $R1$, so that $k_2 > k_1$.

Figures 2 and 3 show the kinetics obtained by integrating the differential equations (1) and (2). The trajectories in the concentration space are shown as black arrows. In the case of self-catalysis (Fig. 2), all the initial states a, b, c, d, e, and f eventually lead to the same stationary state S, corresponding to the vanishing of the more active ligase $L2$. In the case of replication with inheritance (Fig. 3), the initial states a, b, c, a', b', and c' lead to the different stationary states a_S, b_S, and c_S. The presence of many different stationary states is the kinetic counterpart of ''inheritance'': the newly produced molecules are of the same species of their ancestors, and the two populations can coexist in different proportions.

The stationary states form a continuous curve, shown as a black line. The presence of a manifold of stationary states is referred to as ''marginally stable stationarity'' in dynamic system theory, but until now has never been detected in chemical systems.

In mechanics, an example of a marginally stable system is a marble on a horizontal track. The marble can remain in any point of the track, since any point is a nonunstable equilibrium state. On the other hand, a disturbance will result in a displacement of the marble along the track; the marble will not return to the original position, but will reach a different stationary point, since there is no restoring force. Analogously, the stationary-state curve of Fig. 3 allows the chemical system to move smoothly under the effect of spontaneous concentration fluctuations, passing from one stationary point to one of the surrounding sta-

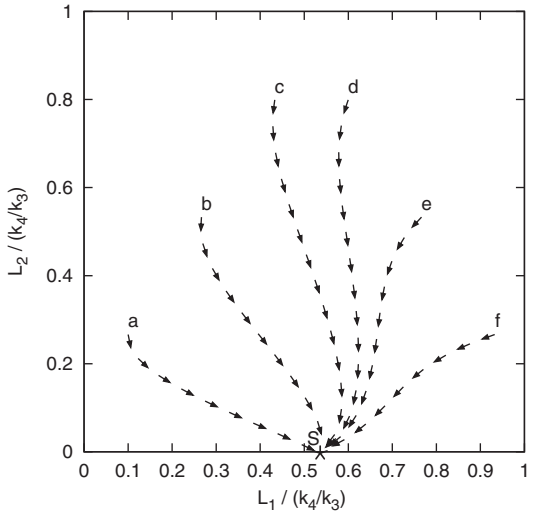

FIG. 2. Kinetics of the self-catalytic chemical system described by Eq. (1). The ligase $L2$ is more active than the ligase $L1$, but is not produced in the reaction. The arrows represent the kinetics obtained from the mass-action equations, Eq. (1). The symbol * represents the stationary state. The parameters are $k_1 = 4.5 k_3^3/k_4^2$ and $k_2 = 18 k_3^3/k_4^2$; the values of k_3 and k_4 are irrelevant under the adimensionalization used.

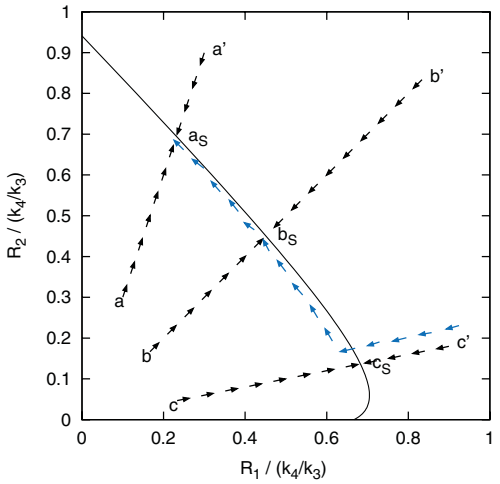

FIG. 3 (color). Kinetics of the chemical system that self-replicates with inheritance described by Eq. (2), with two replicases, $R1$ and the more active $R2$. The black arrows represent the kinetics obtained from the mass-action equations Eq. (2). The black line represents the stationary-state curve. The trajectory shown by the blue arrows is calculated by means of the reaction-diffusion master equations. The parameters are $k_1 = 4.5k_3^3/k_4^2$, $k_2 = 18k_3^3/k_4^2$, $D_{R1} = D_{R2} = k_3(k_3/k_4/\Xi)^{2/3}$, $D_B = 10k_3(k_3/k_4/\Xi)^{2/3}$, and $l = 3(k_3/k_4/\Xi)^{1/3}$; the values of k_3, k_4 and Ξ are irrelevant under the adimensionalization used.

tionary points, without being prompted to return. This is not possible in a system like the one in Fig. 2, where the displaced system eventually comes back to the stationary state S, which is an attractor.

Concentration fluctuations are always present in any solution: in the simplest case, they coincide with the Poisson fluctuations of the number of solved molecules in a given volume of solvent, which fluctuates due to the Brownian motion of molecules entering and exiting from the volume. In order to describe the fluctuations, the reaction-diffusion equations must be considered:

$$\frac{\partial}{\partial t} c_s(\underline{x}, t) = F_s[c_r(\underline{x}, t)] + D_s \nabla^2 c_s(\underline{x}, t), \qquad (3)$$

where the concentrations c_s of the species s depend both on spatial position $\underline{x}$ and time t; D_s are the diffusion coefficients, and $F_s[c_r(\underline{x}, t)]$ represent the mass-action aspects, as in Eqs. (1) and (2). Fluctuations can be studied by considering the reaction-diffusion master equations (RDME) [17]. Following [18], I divide the reaction volume into $N \times N \times N$ cubic cells, with a side length of l. The dynamic variables $n(s, i, j, k)$ are the number of molecules of the species s in the cell with integer indices i, j, k; a constant Ξ is defined so that $\Xi l^3 c(s, i, j, k) = n(s, i, j, k)$. The random variables $n(s, i, j, k)$ change as the result of a Markovian process, including chemical reactions, modeled as "birth and death" processes, and diffusion, modeled as a random walk. The probability that a molecule will move from a cell to a neighboring one during a time interval Δt is $D_s \Delta t/l^2$. Periodic conditions are imposed so that $n(s, i + N, j, k) = n(s, i, j, k)$, and so on.

The solution of the RDME is obtained numerically by a Monte Carlo approach. The trajectories obtained with RDME are shown in Fig. 3 as blue arrows. At first, the system approaches the stationary-state curve, but then a motion along the stationary-state curve is observed. Quite surprisingly, the motion is not a random walk, but a drift pointing toward the increase of the most active replicase $R2$. This drift has a straightforward interpretation: in volumes with a higher concentration of $R2$, more templates will be replicated, and, in turn, a larger fraction of them will be $R2$. The observed drift can constitute the required effect for obtaining evolution: the concentration of the more efficient replicase $R2$ increases and becomes dominant; afterward, a third, still more active replicase can arise due to random mutations, and its concentration will increase, and so on, in analogy with Darwinian evolution.

The directionality of the drift implies that the stationary states cannot be thermodynamic equilibrium states. The systems described in Fig. 1 are manifestly open, since they exchange molecules with the environment; in general, the nonequilibrium can be represented by an energy flow, for example, in the form of nucleoside triphosphates [5–8].

In order to quantitatively evaluate the speed at which the amplification of the best replicase takes place, it must be noted that the average rate dR_2/dt obtained by the numerical model depends on the linear dimension of the cell, l; the limit as $l \to 0$ must be taken. The graph of Fig. 4 shows the dependence of the speed dR_2/dt on l for two different sets of parameter values. Two regimes can easily be observed. At longer l, the drift speed decreases as l^3: this behavior can be explained by observing that the drift is a second-order effect of the numerical fluctuations and thus is proportional to the average number of molecules in a cell, which is in turn proportional to the volume l^3. At shorter l, saturation is reached, representing the limit as $l \to 0$ of the speed. The roll-off value of l is compatible with the reactive mean free path [19,20], that is, the value of l at which microscopic simulations with RDME must be performed to find a quantitative agreement with observations.

To observe the drift shown in Fig. 3 experimentally, a replicase $R2$ must be found that can self-sustain its self-replication. The other template $R1$ can be nonactive, $k_1 = 0$. The reaction starts with a solution containing a concentration B_0 of the nucleotides Bn and an equal quantity of the replicase $R2$ and template T. At fixed time steps ΔT, a fraction $f\Delta T$ of the solution is removed and replaced by a new solution of nucleotides at concentration B_0. This operation gives the terms $k_3 = f$ and $k_4 = fB_0$ shown in Fig. 1. Given the activity of $R2$, namely k_2, the refresh rate must be $f = k_2 B_0^2/9$. Under these conditions, the system is represented by the numerical simulations shown in Fig. 4. From the graph, it can be observed that the drift speed is roughly some percentage of k_4, which, in turn, is of the

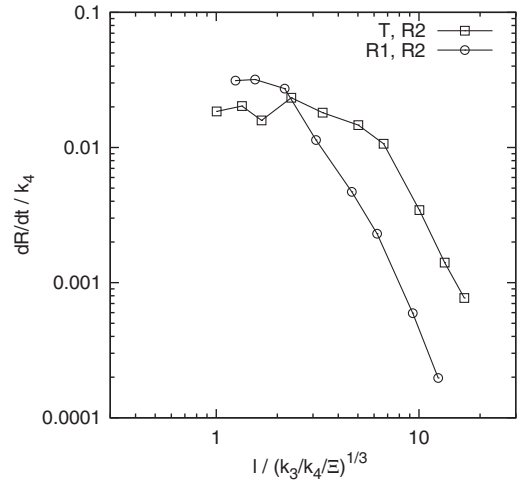

FIG. 4. Dependence of the drift speed dR_2/dt on cell linear dimension l. Results for two different systems are shown: a replicase and a nonactive template ($k_1 = 0$ and $k_2 = 9k_3^3/k_4^2$, squares), and two replicases with different activities ($k_1 = 4.5k_3^3/k_4^2$ and $k_2 = 18k_3^3/k_4^2$, circles). The other parameters are $D_{R1} = D_{R2} = k_3(k_3/k_4/\Xi)^{2/3}$ and $D_B = 10k_3(k_3/k_4/\Xi)^{2/3}$; the values of k_3, k_4, and Ξ are irrelevant under the adimensionalization used.

same order of the replication speed, and should be easily observable.

It is worth noting that not all chemical systems including a replicase show marginal stability; on the contrary, the aim of this work is to show that some peculiar system, that can actually exist, can be marginally stable and that such a system undergoes spontaneous evolution. On the other hand, any chemical system showing marginal stability could be involved in abiogenesis, even if it does not include replicases.

Observation of the drift shows that an evolutionary advantage is indeed present, pushing the system toward increasing replication efficiency. It takes place slowly, cooperatively, in macroscopic volumes of water, but plays the same role as selection in Darwinian evolution, which, in contrast, is based on competition between small entities. I propose that this kind of evolution took place before the compartmentalization induced by membranes, which led to smaller and more efficient self-replicating entities in competition with one another [9].

The reproduction of marginal stability in the laboratory would be the first experiment in which spontaneous evolution takes place in a chemical system, and the first "*in vitro*" model of the primordial chemical reactions that led to life.

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[1] M. W. Powner, B. Gerland, and J. D. Sutherland, Nature (London) **459**, 239 (2009).
[2] J. W. Szostak, Nature (London) **459**, 171 (2009).
[3] J. P. Ferris, A. R. Hill Jr., R. Liu, and L. E. Orgel, Nature (London) **381**, 59 (1996).
[4] O. Kuchner and F. H. Arnold, Trends Biotechnol. **15**, 523 (1997).
[5] N. Paul and J. F. Joyce, Proc. Natl. Acad. Sci. U.S.A. **99**, 12 733 (2002).
[6] T. A. Lincoln and G. F. Joyce, Science **323**, 1229 (2009).
[7] D. E. Kim and J. F. Joyce, Chem. Biol. **11**, 1505 (2004).
[8] W. K. Johnston, P. J. Unrau, M. S. Lawrence, M. E. Glasner, and D. P. Bartel, Science **292**, 1319 (2001).
[9] J. W. Szostak, D. P. Bartel, and P. L. Luisi, Nature (London) **409**, 387 (2001).
[10] M. M. Hanczyc, S. M. Fujikawa, and J. W. Szostak, Science **302**, 618 (2003).
[11] I. A. Chen, R. W. Roberts, and J. W. Szostak, Science **305**, 1474 (2004).
[12] I. A. Chen, Science **314**, 1558 (2006).
[13] D. W. Deamer, Nature (London) **454**, 37 (2008).
[14] S. S. Mansy, J. P. Schrum, M. Krishnamurthy, S. Tobé, D. A. Treco, and J. W. Szostak, Nature (London) **454**, 122 (2008).
[15] M. S. Samoilov and A. P. Arkin, Nat. Biotechnol. **24**, 1235 (2006).
[16] H. Qian, S. Saffarian, and E. L. Elson, Proc. Natl. Acad. Sci. U.S.A. **99**, 10 376 (2002).
[17] D. T. Gillespie, *Markov Processes: An Introduction for Physical Scientists* (Academic Press, New York, 1992).
[18] M. M. Mansour, J. Dethier, and F. Baras, J. Chem. Phys. **114**, 9265 (2001).
[19] F. Baras and M. M. Mansour, Phys. Rev. E **54**, 6139 (1996).
[20] M. M. Mansour, J. Dethier, and F. Baras, J. Stat. Phys. **101**, 425 (2000).