Mathematical modeling reveals spontaneous emergence of self-replication in chemical reaction systems

Received for publication, May 10, 2018, and in revised form, September 29, 2018. Published, Papers in Press, October 3, 2018, DOI 10.1074/jbc.RA118.003795

© Yu Liu and David J. T. Sumpter. Published by The American Society for Biochemistry and Molecular Biology, Inc.

Yu Liu1 and David J. T. Sumpter
From the Department of Mathematics, Uppsala University, 75105 Uppsala, Sweden

Editored by John M. Denu

Explaining the origin of life requires us to elucidate how self-replication arises. To be specific, how can a self-replicating entity develop spontaneously from a chemical reaction system in which no reaction is self-replicating? Previously proposed mathematical models either supply an explicit framework for a minimal living system or consider only catalyzed reactions, and thus fail to provide a comprehensive theory. Here, we set up a general mathematical model for chemical reaction systems that properly accounts for energetics, kinetics, and the conservation law. We found that 1) some systems are collectively catalytic, a mode whereby reactants are transformed into end products with the assistance of intermediates (as in the citric acid cycle), whereas some others are self-replicating, that is, different parts replicate each other and the system self-replicates as a whole (as in the formose reaction, in which sugar is replicated from formaldehyde); 2) side reactions do not always inhibit such systems; 3) randomly chosen chemical universes (namely random artificial chemistries) often contain one or more such systems; 4) it is possible to construct a self-replicating system in which the entropy of some parts spontaneously decreases, in a manner similar to that discussed by Schrödinger; and 5) complex self-replicating molecules can emerge spontaneously and relatively easily from simple chemical reaction systems through a sequence of transitions. Together, these results start to explain the origins of prebiotic evolution.

Self-replication is one of the central properties of life (1), and to explain life’s origins, we need to explain how self-replication arose. It is widely accepted that before DNA, life replicated through RNA molecules (2). However, it remains unclear how this set can be produced from a small food molecule set. RAF modeling reveals spontaneous emergence of self-replication in chemical reaction systems that do not rely on a single complex template molecule (as is the case for DNA and RNA molecules). These include the malic acid cycle (a metabolic path of some bacteria and plants for synthesis of malates), the Calvin cycle in photosynthesis (10), the reductive citric acid cycle for a certain group of chemautotrophs (11, 12), the artificially designed myokinase–pyruvate kinase cycling system (13), the metabolic pathways of ATP (as well as some other coenzymes such as NAD+ and CoA) found in many different living organisms (14), and the whole metabolic reaction network of Escherichia coli (15). Self-replication has also been identified in nonliving systems, such as the formose reaction (10, 16), and experiments in laboratories (17–19), including self-replication of nucleotide-based oligomers (20).

Many models have been put forward to explain the origin of self-replication in terms of a system of coupled chemical reactions (21). Some of these can be categorized as artificial chemistry models, as reviewed by Dittrich et al. (22) and by Banzhaf and Yamamoto (23). For example, the chemoton model describes a system composed of three coupled quasi-self-replicating subsystems (metabolism, membrane, and template), which as a whole is able to self-replicate (10). The chemoton can be considered as a model of a minimal living system, but cannot explain how this system spontaneously develops from a soup of simple molecules. The metabolically coupled replicator system is another type of model in this direction (24, 25). But it mainly focuses on the information heredity and ecological stability, that is, how to maintain coexistence of different replicators and constrain parasitism, rather than investigating why self-replication can occur in the first place. The most influential model in this direction is the reflexively autocatalytic and food-generated (RAF)2 theory (26–28), extended from Kauffman’s autocatalytic sets theory (29). A set of chemical reactions is RAF if 1) every reaction in this set is catalyzed by at least one molecule involved in this set and 2) every molecule involved in this set can be produced from a small food molecule set. RAF

The authors declare that they have no conflicts of interest with the contents of this article.

Author’s Choice—Final version open access under the terms of the Creative Commons CC-BY license.

This article contains sections S1–S10.

1 Supported by the Erasmus Mundus Action 2 program (Lotus III Scholarship), funded by the European Commission. To whom correspondence should be addressed. Tel: 46-76-5635-008; E-mail: yu.ernest.liu@hotmail.com.

2 The abbreviations used are: RAF, reflexively autocatalytic and food-generated; ODE, ordinary differential equation.
sets are shown to be able to readily emerge from a set of chemical reactions and always consist of smaller RAF sets, demonstrating the capability to evolve (30, 31). Other similar models also contributed to this theory (32–36), including the chemical organization theory (37) and the graded autocatalysis replication domain model (38). The former is closely related to RAF theory (see Hordijk et al. (39) for a detailed comparison), whereas the latter suffers from lacking evolvability (see Vasas et al. (40, 41) for detailed critical analyses). In addition, many of the biological observations mentioned in the previous paragraph can be put into the framework of RAF theory (15, 17–20).

Although RAF theory is influential, it has limitations as an explanation of self-replication. First, the theory stipulates that every reaction in the set is catalyzed. Even though catalyzed reactions are very common in living systems, not every reaction involves a catalyst (e.g. condensation reactions (8, 42)). In the early stages of biological evolution, probably no reaction required sophisticated biotic catalysts (e.g. enzymes) (11, 15), so there is a strong motivation not to include these enzymes (20, 43, 44) or even catalysts as given in a model of the origins of self-replication. Uncatalyzed reactions have significant effects on the dynamics of the whole system (e.g. “innovating” new species of molecules and then triggering other RAF sets) (45, 46). The second concern with RAF theory is that it is a purely graph-theoretic approach. As a result, there is no constraint on how chemical reactions are constructed and coupled (although it gives the theory much freedom, the construction of reaction systems is too arbitrary to investigate the model systematically). As another result, extra assumptions need to be made about chemical kinetics to investigate the dynamics of how populations of molecules change over time (47). Here, we see another reason to relax the assumption of studying only catalyzed reactions: catalyzed reactions are never elementary reactions, so the kinetics cannot be simply calculated from the reaction stoichiometry (48).

Any theoretical approach to the origin of self-replication should explicitly include energetics (15, 49), an aspect that is missing from all of these models and theories above. There are several reasons for this. First, energetics (e.g. Gibbs energy) determines whether a chemical reaction is spontaneous, so to investigate the spontaneity of the emergence of life, it has to be considered. Second, to concretely discuss the issue—famously put forward by Schrödinger—that life maintains its order by feeding on “negative entropy” (50), energetics has to be explicitly taken into account, as entropy is negatively related to the thermodynamic free energy (which is Gibbs energy in the scenario of constant pressure and temperature (51)). It should be noted that the relationship between life and entropy is investigated in different ways and contexts (52–54). For example, Branscomb and Russell (53) explained the specific mechanisms to increase thermodynamic free energy in two real-world biochemical scenarios: the hypothesized alkaline hydrothermal vent (55) on the prebiotic Earth and the system where the ferredoxin I protein translocates protons. In the context of statistical physics, a lower limit was derived for the amount of heat generated in a nonequilibrium system where a process of self-replication occurs (54).

In this paper, we set up an artificial chemistry model, in the form of a general mathematical framework for chemical reaction systems, that properly accounts for energetics, kinetics, and the conservation law. Catalysts are not explicitly included in the model, but we later find that catalysis can emerge, along with self-replication and potentially complex molecules, in our system.

In what follows, we give an overview of our model. The specifications are given under “Theory” at the end of this paper. We would encourage the reader, on first reading, to watch a short movie included in the supporting information that illustrates how our model works, and see “Theory” directly afterward.

We model a well-mixed soup of molecules, each of which is defined by its integer mass, i. A molecule’s type is thus denoted i. Only synthesis reactions and decomposition reactions are possible. Only reactions that conserve mass can occur (i.e. the total mass on the reactant side adds up to that on the product side). Each type of molecule i has its own standard Gibbs energy of formation $G_i^\circ$, that decides whether a reaction is spontaneous. Moreover, a reaction has to overcome an energy barrier (namely Gibbs energy of activation) to occur. It is called low-barrier if its energy barrier is low and its reaction rate is thus high or high-barrier otherwise. To define a specific chemical reaction system, we show a list of low-barrier (and spontaneous) reactions only (e.g. Scheme 1).

$$\begin{align*}
\frac{5}{2} + \frac{4}{6} & \rightarrow \frac{1}{6} + \frac{4}{5} \\
\frac{2}{3} + \frac{4}{6} & \rightarrow \frac{5}{7}
\end{align*}
$$

SCHEME 1

Many other reaction systems can also be constructed. Some of them are physically possible—meaning that there are proper $G_i^\circ$ values for all molecules involved so that all reactions listed are spontaneous, whereas some others are not. We are only interested in physically possible systems.

After that, we assume that there is an unlimited reservoir of resource molecules (e.g. molecule $2$ for system shown in Scheme 1). Then we use the standard Gillespie algorithm to simulate the dynamics, namely $N_i(t)$, the time series of molecule i’s population. In addition, we construct ordinary differential equations (ODEs) to describe the mean-field dynamics.

Results

Collectively catalytic system

We first investigate the system shown in Scheme 1, which is actually a model of the citric acid cycle in cellular respiration (10). Specifically, as shown in Fig. 1a, molecule 1 represents carbon dioxide, 2 represents acetyl-CoA, 4 represents oxaloacetic acid, 5 represents $\alpha$-ketoglutaric acid, and 6 represents citric acid, respectively.

Given an unlimited reservoir of resource molecules 2, the simulation shows that $N_i(t)$ increases linearly. Also, molecules 4, 5, and 6 are involved in a cycle of reactions, but the total number is constant. Section S4 shows the dynamics and ODE solutions in more detail. We also observe that each reaction in Scheme 1 occurs approximately the same number of times. We
Modeling spontaneous self-replication

**Figure 1.** Sketches of three chemical reaction systems specified within our model. Details are described under “Theory.” **a,** a simplified model of the citric acid cycle. In both simplified models, we only consider carbon-changing reactions and do not consider isomers of some molecules. Note that the integer representation of mass can be thought of as a rough ordering of the molecule’s mass or relative complexity. **b,** a physically impossible chemical reaction system (Scheme 7).

Now we investigate the formose reaction, which involves the formation of sugars from formaldehyde (10).

Specifically, as shown in Fig. 1b, molecule 1 represents formaldehyde, 2 is glycolaldehyde, 3 is glyceraldehyde, and 4 is tetrose, respectively.

Given the resource molecule 1, its dynamics are shown in Fig. 2. All three intermediate molecules (N1(t), N2(t), and N3(t)) increase exponentially. The solutions of the corresponding ODEs are consistent with the simulations (section S6).

The fact that 2 grows exponentially can be seen directly by adding up the three low-barrier reactions to obtain \( \bar{I} + \bar{I} + \bar{I} \Rightarrow 2 + 2 \). It indicates that if one 2 is present beforehand, one extra 2 can be produced, by transforming two of 1. Then the additional 1 is further used by the system, and more 2s are produced. Although the molecules 3 and 4 are canceled out when we add up the reactions, they also grow exponentially (with 4 increasing much more slowly than the other two). The reason is that the actual reaction rate of each low-barrier reaction is not the same (see section S6). This observation is important because it illustrates that it is not just one type of molecule that grows exponentially in such systems, but all of the intermediate molecules.

We define a self-replicating system, of which Scheme 2 is an example, to be a system in which at least one type of molecule is replicated. By investigating various self-replicating systems, we find that not only exponential but also superexponential growth is observed (see examples in section S7). The dynamics indicate that the reactions in self-replicating systems become faster and faster. This is a very special property compared with the collectively catalytic system (e.g. the citric acid cycle (Scheme 1)), where the overall reaction rate keeps constant.

In general, the following stoichiometric criteria are sufficient (but not necessary) to show that a physically possible chemical
reaction system, given supplies of resource molecules, is self-replicating: 1) the criterion for self-driven (mentioned above) is satisfied; 2) there are some types of intermediate molecules, and the number of times they appear on the reactant side is less than that on the product side (called the criterion for overproduction); 3) there is no type of intermediate molecule for which the number of times it appears on the reactant side is larger than that on the product side (called the criterion for no-overintake). The formose reaction (Scheme 2) satisfies all of these criteria.

**Effect of side reactions on self-replicating systems**

Let us consider the following system (Scheme 3), which is the formose reaction coupled with an additional reaction that transforms 4 and 5 to 9.

\[
\begin{align*}
1 + 2 & \rightarrow 3 \\
1 & \rightarrow 4 \\
4 & \rightarrow 2 + 2 \\
2 & \rightarrow 9 \\
\end{align*}
\]

(Equation 1)

**SCHEME 3**

The last reaction can be thought of as a side reaction, which consumes the intermediate molecules.

When there is an infinite reservoir of molecule 1, the dynamics of this system are exactly the same as in Fig. 2, because the side reaction 4 + 5 → 9 cannot proceed without 5. Even with an infinite reservoir of both molecules 1 and 5, the dynamics of this system are unaffected, because reaction 4 → 2 + 2 occurs so fast that the side reaction 4 + 5 → 9 cannot obtain molecule 4 to proceed. In general, by observing Equations 1 and 3 under “Theory,” decomposition reactions, such as 4 → 2 + 2, occur faster than synthesis reactions, because the reaction rate for synthesis, \( \gamma_{+S} \), has an extra term \( N/(S + N) \) (compared with \( \gamma_{-O} \)), which is always smaller than 1. In our experimental setting, the number of solvent molecules S is very large, so this extra term is much smaller than 1. In such settings, the self-replicating system outcompetes the side reaction.

Nonetheless, self-replication can be inhibited by some other side reactions. Consider the following system (Scheme 4).

\[
\begin{align*}
1 + 2 & \rightarrow 3 \\
1 + 3 & \rightarrow 4 \\
4 & \rightarrow 2 + 2 \\
2 & \rightarrow 7 \\
\end{align*}
\]

(Equation 2)

**SCHEME 4**

Given infinite reservoirs of both molecule 1 and 5, we no longer observe self-replication (i.e. \( N_1(t) \) and \( N_2(t) \) do not grow exponentially), because this side reaction consumes the newly produced molecule 2 before it can be used by reaction 1 + 2 → 3.

Note that we always assumed \( \psi_\beta = 10 \) for every low-barrier reaction (see “Theory”), which means that the reaction rate constant \( \beta \exp(-\kappa G_{+O}) \), or \( \beta \exp(-\kappa G_{+S}) \), for every low-barrier reaction is identical, denoted as \( \omega \). But for now, we relax this assumption. Let the reaction rate constants for the first three reactions in Scheme 4 be identical, \( \omega \) and the rate constant for the side reaction be \( \eta \omega \) where \( \eta \geq 0 \). Firstly, when \( \eta = 0 \), the Scheme 4 system goes back to the original formose reaction. Secondly, when the side reaction occurs at low rate (i.e. \( 0 < \eta < 1 \)), the onset of self-replication is delayed but still occurs eventually (the larger \( \eta \) is, the more delayed the onset of self-replication becomes; figures not shown). Lastly, when the side reaction has a sufficiently high rate (i.e. \( \eta \geq 1 \)), it consumes the newly produced molecule 2 before it can be used by reaction 1 + 2 → 3, so self-replication is completely inhibited. \( \eta = 1 \) is the critical value in this case.

In the real chemical universe we are living in, the formose reaction is often inhibited by side reactions. Many products further react into a “browning tar” so that the formose reaction has a very low yield of sugars (56). However, this does not preclude the existence of a similar reaction system in the prebiotic world, because, in general, the stability of a self-replicating system depends on the existence and reaction rates of other low-barrier reactions. Whether self-replication is inhibited depends on how side reactions are coupled with the system and what the specific condition is. Also note that all of the arguments above apply to collectively catalytic systems.

**Collectively catalytic and self-replicating systems are common**

Another natural question to ask is how common these reaction systems are, such as the citric acid cycle and the formose reaction. Specifically, if we construct alternative chemical universes, or, in another term, different artificial chemistries, where we systematically choose which reactions are low-barrier, we can ask how many of the resulting artificial chemistries contain collectively catalytic or self-replicating systems. To answer this question, we start by using the criteria shared by both of them, that they are self-driven. Table 1 shows the numbers for different \( L \), which is set to be the mass of the largest molecule in this particular artificial chemistry in question, to have a measurement of the number of all chemical reaction systems. For example, when \( L = 6 \), there are in total 1, 2, . . . , 6,
Modeling spontaneous self-replication

Table 1
Number of physically possible artificial chemistries that contain self-driven, collectively catalytic, or self-replicating systems

All of the percentages are calculated with respect to the number of all physically possible artificial chemistries. AC, artificial chemistry; CC, collectively catalytic; SR, self-replicating.

| L  | No. of physically possible ACs | No. of ACs containing self-driven systems | Lower bound ACs containing CC systems | Lower bound ACs containing SR systems |
|----|-------------------------------|------------------------------------------|-------------------------------------|-------------------------------------|
| 4  | 79                            | 8 (10%)                                   | 0 (0%)                              | 2 (25.3%)                           |
| 5  | 681                           | 152 (22%)                                 | 5 (7.3%)                            | 10 (14.7%)                          |
| 6  | 16,825                        | 6,886 (41%)                               | 21 (1.2%)                           | 74 (4.4%)                           |
| 7  | 401,445                       | 232,552 (58%)                             | 184 (0.5%)                          | 642 (1.6%)                          |

* Number of physically possible artificial chemistries.
* Number of physically possible artificial chemistries that contain self-driven systems.
* Lower bound on the number of physically possible artificial chemistries that contain collectively catalytic systems.
* Lower bound on the number of physically possible artificial chemistries that contain self-replicating systems.

six types of molecules. By choosing which reaction is low-barrier, we can construct \( \sum_{i=0}^{6} 2^i = 19,683 \) different artificial chemistries, 16,825 of which turn out to be physically possible. Using the criterion for self-driven given above, we find that 6886 (41%) of all of the physically possible artificial chemistries contain self-driven systems. This percentage increases with \( L \), which indicates that self-driven systems are common and are more common in systems involving more types of molecules.

However, we cannot be sure that these self-driven systems are collectively catalytic or self-replicating, and there is even a third type of self-driven system, the nonsustaining system, which makes things more complicated (see section S8 for details of the nonsustaining system and section S9 for more details of classification for the self-driven system). Nevertheless, we can use the stoichiometric criteria mentioned above (note that these criteria are sufficient but not necessary) to give a lower bound on the number of artificial chemistries that contain collectively catalytic or self-replicating systems. That is, for a self-driven system, a system is collectively catalytic if it satisfies the criterion for balanced-canceling, or self-replicating if it satisfies both the criteria for overproduction and no-overintake.

Using the stoichiometric criteria, we find that the lower bound on the number of artificial chemistries containing collectively catalytic or self-replicating systems increases with \( L \), although the percentage decreases (Table 1). However, because it is the lower bound, it does not mean that the actual number of artificial chemistries containing collectively catalytic or self-replicating systems decreases. Establishing a firm relationship between the number of chemicals (\( L \)) and self-replication will involve simulating dynamics of all systems.

How can life maintain low entropy?

According to the second law of thermodynamics, the total entropy of an isolated system never spontaneously decreases over time. Life, thought of as an open system as opposed to an isolated one, is able to maintain order (i.e. maintain a relatively low entropy level). Schrödinger suggested that this is achieved by life “feeding on negative entropy” (50). His question is how this can happen spontaneously. But before we answer this, we first need a way to discuss this question concretely and more quantitatively.

Under the framework of our model, if we simply consider life as some self-replicating entity, we should then ask if it is possible for a self-replicating system to spontaneously increase its Gibbs energy or at least keep it unchanged. (We first note that in the scenario of constant pressure and temperature, the decrease of entropy corresponds to the increase of Gibbs energy, because \( G = H - TS \), where \( G \) is Gibbs energy, \( H \) is enthalpy, \( T \) is temperature, and \( S \) is entropy (51).) Let us consider the self-replicating system in Scheme 5, given the resource molecule 2.

\[
\begin{align*}
5 & \rightarrow 1 + 4 \\
2 + 3 & \rightarrow 5 \\
2 + 4 & \rightarrow 6 \\
6 & \rightarrow 3 + 3
\end{align*}
\]

**SCHEME 5**

The simulation shows that \( N_1(t) \), \( N_2(t) \), and \( N_4(t) \) increase exponentially, as well as \( N_3(t) \) and \( N_6(t) \) (although they are very small; see section S10). Molecule 1 is the end product, whereas 3, 4, 5, and 6 are replicated through the whole system. We then consider that the “living” system consists of the self-replicating part (namely all of molecules 3, 4, 5, and 6) and the resource molecules in the system.

Now we investigate how Gibbs energy of the system changes. We set Gibbs energy of the initial system to zero (as the reference point), because only relative quantity matters. Therefore, Gibbs energy of the self-replicating part is \( G_{\text{replicating}}(t) = \sum_{i=1}^{6} N_i(t) \cdot G_i \). Gibbs energy of the resource molecules in the system is \( G_{\text{resource}}(t) = -F_2(t) \cdot G_2 \), where \( F_2(t) \) is the number of resource molecules 2 ever consumed until time \( t \). Gibbs energy of the waste is \( G_{\text{waste}}(t) = N_1(t) \cdot G_1 \). Then the Gibbs energy of the living system in time \( t \) is \( G_{\text{living}}(t) = G_{\text{living}}(0) + G_{\text{replicating}}(t) + G_{\text{resource}}(t) + G_{\text{waste}}(t) \). As shown in Fig. 3, \( G_{\text{living}}(t) \) increases, whereas \( G_{\text{total}}(t) = G_{\text{living}}(t) + G_{\text{waste}}(t) \) decreases.

We have thus given an explicit example of a self-replicating “living” system that spontaneously consumes the resources to increase its own Gibbs energy. Note that our system, as defined, is a well-mixed gas system. So the waste molecules 1 are not automatically separated from other molecules, and Gibbs energy of the gas-mixing process is neglected in the calculation above. However, the contribution of the fact of well-mixed gas is relatively small (for details, see section S10). So it is still possible for a self-replicating system to spontaneously increase Gibbs energy or at least keep it unchanged.

Spontaneous evolution from simple toward complex

Nature provides many examples of the spontaneous evolution from simple toward complex. Is it possible to construct a
Modeling spontaneous self-replication

An example chemical reaction system that is able to evolve from simple toward complex

| A self-replicating system (formose reaction SCHEME 2): | A self-replicating system: |
|------------------------------------------------------|---------------------------|
| \( \bar{1} + 2 \rightarrow \bar{3} \)                 | \( \bar{1} + 12 \rightarrow \bar{13} \) |
| \( \bar{1} + 3 \rightarrow 4 \)                       | \( \bar{1} + 13 \rightarrow 14 \) |
| \( 4 \rightarrow 2 + \bar{2} \)                      | \( \cdots \)                |
| \( 3 + 5 \rightarrow 8 \)                            | \( \bar{1} + i \rightarrow \bar{i} + 1 \) |
| \( 3 + 8 \rightarrow 11 \)                           | \( i = 14, 15, \ldots, 22 \) |
| \( \bar{11} \rightarrow 5 + 6 \)                     | \( \cdots \)                |
| **SCHEME 8**                                          | **SCHEME 9**               |

They constitute a collectively catalytic system. Then the reaction \( \bar{1} + 3 \rightarrow 4 \), which is the overall reaction of the three, can still be considered as low-barrier, and the formose reaction system is still self-replicating. The only complication is that we have to wait for the complex molecule \( \bar{30} \) to appear.

Discussion

We have set up a general mathematical model for chemical reaction systems in which molecules are represented by integers, roughly corresponding to the molecules’ mass. All chemical reactions are defined by addition operations, which automatically guarantees mass conservation. Each molecule has a predefined standard Gibbs energy of formation, whereas each reaction has a predefined Gibbs energy of activation, which defines the rate constant for each reaction. By changing the Gibbs energy of molecules and reactions, we have investigated a range of artificial chemistries, some of which are like chemical systems found in our real universe. The strength of our approach is that it accounts for the conservation law, energetics, and kinetics while being general enough to investigate the origins of self-replication and the emergence of life.

Although our model did not explicitly include catalysts, as other models did (26, 27, 28), catalysis and autocatalysis emerge in a number of systems. We found three distinct types of self-driven system (i.e., systems that “feed” themselves). Both collec-
Modeling spontaneous self-replication

tively catalytic and self-replicating systems are vital in biology, whereas the third (nonsustaining) system appears less important. In terms of generating complexity, the self-replicating system plays a more important role, because it is able to replicate innovations. For example, in the self-replicating formose reaction (Scheme 2), after the first molecule 2 is produced by a high-barrier reaction, more 2s are easily replicated. In a biological setting, if this molecule spreads to other places, it can trigger more self-replicating systems. In contrast, in the collectively catalytic citric acid cycle (Scheme 1), for example, after the innovation (the first molecule 5), the second 5 will not appear until the responsible high-barrier reaction occurs once again.

In biology, most metabolic reactions are catalyzed by sophisticated and highly specific enzymes, and thus avoid disturbance from undesired side reactions. In the very early stage of life, however, probably no reaction required enzymes (11, 15). We found that whether side reactions prevent a system from self-replicating depends on how they are coupled with the system and the specific conditions. There appear to exist examples where self-replication can be sustained in the presence of side reactions. However, how sophisticated enzymes develop de novo so that side reactions are avoided is another question, which deserves further investigation.

By arbitrarily constructing different artificial chemistries, we found that lots of them contain self-driven systems, and the lower bounds on the number of artificial chemistries containing collectively catalytic or self-replicating systems increase with more types of molecules. This result suggests that in a random artificial chemistry, it would not be too surprising to observe the emergence of self-replication, one of the central properties of life (1). Although it is not the first theory to propose that self-replication is relatively easy to emerge, as the RAF theory did (29–31), it is the first one requiring no catalyst.

We provided a general model explicitly showing that high thermodynamic free energy molecules can be produced exponentially from low free energy molecules, whereas specific mechanisms in specific real-world scenarios have been investigated before (52, 53). The example system we showed is a metaphor of why high free energy ATP molecules are constantly produced in organisms (14). In addition, as our model takes energetics (corresponding to entropy) into account, it provides a more concrete way to discuss the issue—famously put forward by Schrödinger (50)—why life is able to spontaneously maintain a relatively low entropy level, although it cannot give the full answer. Answering the questions of how the molecules in living systems can be placed in an ordered structure (namely a low entropy state) would require extending our model to include spatial effects.

Our model explicitly shows that complexity evolves from extreme simplicity stage by stage. It gives insights into three issues related to the origin of life. First, the first RNA molecule is much more likely to be produced de novo by this stage-by-stage procedure, rather than a magic event (3). It provides theoretical supports to metabolism-first theories (3), such as Wächtershäuser’s iron-sulfur world hypothesis (6) and Szathmáry’s theory (7–9). Second, before life, Earth should have gone through many stages in which different self-replicating systems existed, and consequently Earth’s compositions were different in each stage. The raw materials for life that we should look for are those for the first self-replicating system rather than those for the extant life (6). That is why, in the current theoretical framework, the raw materials for life (e.g. nucleotides) seem not to be available on the primordial Earth (3, 4). Third, collectively catalytic and self-replicating systems generate more types of new molecules, and in return, more types of molecules make more reactions feasible (in the form of catalysis and autocatalysis). This could explain why metabolic reactions in extant life always require sophisticated enzymes (15), whereas no reaction is expected to involve catalysts in the very early stage of life (20, 43, 44).

As we noted when introducing the model, in order to be as simple as possible, we currently do not fully account for different isomers or for the impossibility of certain chemical transformations. Nevertheless, the current model can be considered as a simpler version of a more general model, with a more intricate way of “ordering” the complexity of molecules (e.g. accounting for different isomers).

Our model provides a convenient platform to construct artificial chemistries and investigate general properties of chemical reaction systems. It may provide a theoretical guideline for systematically searching for other chemical paths toward life (or at least self-replicating entities), as pursued in astrobiology (57, 58) and xenobiology (59) for example.

Theory

Constructing chemical reaction systems

We model a well-mixed soup of molecules, each of which is defined by its integer mass, i. A molecule’s type is thus denoted i. Only two types of reaction are possible: synthesis of two molecules to create a molecule of greater mass (e.g. $2 + 4 \rightarrow 6$) and decomposition into two molecules to create two molecules of lower mass (e.g. $6 \rightarrow 1 + 5$). All reactions that conserve mass—the total mass on the left-hand side of the equation adds up to those on the right-hand side—can occur. For convenience, we define a reaction pair to be a reaction and its corresponding reverse reaction.

Each type of molecule i has its own standard Gibbs energy of formation $G_i$'s. Then, as illustrated in Fig. 4, a reaction is either spontaneous, meaning that the total standard Gibbs energy of formation of the reactants is greater than that of the products (i.e. $G_i + G_j > G_{i+j}$), or nonspontaneous (i.e. $G_i + G_j \leq G_{i+j}$).
If one reaction in a reaction pair is spontaneous, the other is nonspontaneous, and vice versa.

According to transition state theory (48), the reactants have to overcome the Gibbs energy of activation (namely $\Delta G^\ddagger_{i,j}$ in Fig. 4) for the reaction to occur. In the model, any reaction pair is either low-barrier—the energy barrier (corresponding to $\psi_{ij}$ in Fig. 4) is low and the reaction rate is thus high—or high-barrier. In our system, all reactions are possible, but to define a specific chemical reaction system, we write a list of low-barrier (and spontaneous) reactions only (e.g. the citric acid cycle shown in Scheme 1 and the formose reaction shown in Scheme 2).

Although the real citric acid cycle and the formose reaction involve further reactions, in the simplified model above, only carbon-changing reactions are considered. Specifically, in the Scheme 1 system, we consider each reaction uncatalyzed and neglect the fact that each reaction is catalyzed by specific enzymes. Similarly, in the formose reaction, tetrose further aggregate into a “browning tar” whereby the system would be inhibited to some extent (56). We set aside these complexities to have two easy-to-follow special cases, but our framework does, in general, allow us to describe additional side reactions where necessary.

To be as simple as possible, a strong assumption we make is that molecules with the same mass are identical. In real chemistry, molecules with the same mass may have different atomic compositions (e.g. CO and N$_2$), and even when molecules have the same atomic compositions, they can be different isomers (e.g. ethanol and methoxymethane). Furthermore, our model implies that any type of molecule can be produced by some others (because an integer can always be written as a sum of two integers). But in real chemistry, certain chemical transformations are impossible (e.g. organometallic compounds can never be produced by a chemical reaction system involved with only carbohydrates). Our model currently excludes these real-world possibilities and limitations. However, we emphasize that the “integer molecule mass” in our model does not necessarily correspond to the physical mass, but should be thought of as a rough ordering of the mass or the relative complexity of molecules.

Note that some chemical reaction systems are not physically possible (e.g. the following, shown in Fig. 1c).

\[
\begin{align*}
\frac{2}{2} & \rightarrow \frac{1}{1} + \frac{1}{1} \\
\frac{1}{1} \frac{1}{2} & \rightarrow \frac{2}{2} \\
\frac{1}{1} \frac{1}{2} \frac{1}{2} & \rightarrow \frac{1}{1} \frac{1}{2} \\
\frac{1}{4} & \rightarrow \frac{2}{2} + \frac{2}{2}
\end{align*}
\]

SCHEME 7

By adding up these low-barrier reactions, all molecules are cancelled out. As a consequence, one cannot find proper Gibbs energy for each molecule such that each reaction above is spontaneous. We are only interested in physically possible systems.

**Kinetics**

We now specify a general model for the kinetics of our chemical system, under the following assumptions: 1) all molecules are ideally gaseous, 2) the whole system is kept at constant pressure and temperature, and 3) every possible reaction is elementary. The derivation follows the law of mass action and transition state theory (48). Here we cover the key points, and a full derivation is given in **section S1**.

For any synthesis reaction $i + j \rightarrow i + j$, the reaction rate in unit s$^{-1}$ is as follows,

\[
\gamma_{i,j} = \beta \exp(-\kappa \Delta G^\ddagger_{i,j}) \cdot N_i \cdot N_j / (S + N)
\]

where the subscript $+ij$ stands for the synthesis reaction, $\beta$ and $\kappa$ are constants, $N_i$ is the number of molecules $i$ in the system, $N_j$ is the number of $j$, $S$ is the number of solvent molecules, determining the global rate at which molecules interact, $N$ is the number of all the molecules except for solvent molecules, and $\Delta G^\ddagger_{i,j}$ as shown in Fig. 4, is defined as follows.

\[
\Delta G^\ddagger_{i,j} = \left\{ \begin{array}{ll}
\psi_{ij} - (G_i + G_j) & \text{if } G_i + G_j > G_i + G_j \\
\psi_{ij} - (G_i + G_j) & \text{if } G_i + G_j \leq G_i + G_j
\end{array} \right.
\]

When implementing the model, we set values of $\psi_{ij}$ (positive and finite) for each reaction pair and $G_i$ for each molecule. Together, these give a unique value for $\Delta G^\ddagger_{i,j}$.

Likewise, for any decomposition reaction $i + j \rightarrow i + j$, the reaction rate in unit s$^{-1}$ is as follows,

\[
\gamma_{i,j} = \beta \exp(-\kappa \Delta G^\ddagger_{i,j}) \cdot N_i + j
\]

where the subscript $-ij$ stands for this decomposition reaction, and the following is true.

\[
\Delta G^\ddagger_{i,j} = \left\{ \begin{array}{ll}
\psi_{ij} - (G_i + G_j) & \text{if } G_i + G_j > G_i + G_j \\
\psi_{ij} - (G_i + G_j) & \text{if } G_i + G_j \leq G_i + G_j
\end{array} \right.
\]

Because the transition state of a reaction pair $i + j \rightarrow i + j$ and $i + j \rightarrow i + j$ is identical, by setting $\psi_{ij}$ both $\Delta G^\ddagger_{i,j}$ and $\Delta G^\ddagger_{-ij}$ are uniquely determined. In addition, by setting $\psi_{ij}$ large or small, we can easily make the reaction pair low-barrier or high-barrier.

**Simulation of dynamics**

We take the citric acid cycle (Scheme 1) as an example to illustrate how to set up the simulation experiment. 1) Set up all of the constants. Assume that the constant pressure the system is kept at is 100 kilopascals and the constant temperature is 298.15 K, so we obtain that $\beta \sim 6.21 \times 10^{-10}$ s$^{-1}$ and $\kappa \sim 0.403$ mol/kJ. 2) Set $G_i$ for each type of molecule (up to 6 in this case), to make sure that the three reactions are spontaneous (i.e. $G_5 > G_2 + G_4$, $G_5 > G_2 + G_4$, and $G_2 + G_4 > G_3$). Here, we set $G_i = -780$, $G_2 = -500$, $G_3 = -490$, $G_4 = -190$, $G_5 = -830$, and $G_6 = -900$, all in unit kJ/mol. These values are set in the range of normal chemical substances’ Gibbs energy of formation (48, 60), roughly in the range between $-1500$ and 300. Note that the choice is not unique, and a wide range of choices can be made to allow the system to work. 3) Set $\psi_{14} = \psi_{15} = \psi_{24} = 10$ for these three low-barrier reactions and $\psi_{ij} = 100$ for all other reaction pairs (all in units kJ/mol). 4) Assume that
there is an unlimited reservoir of resource molecules. In principle, the resource molecule can be assumed to be any type of molecule, but often to be the types that only appear on the reactant side. In this case, we assume it to be molecule \(2\). In other words, whenever a molecule \(2\) is consumed or produced, it is replenished or removed so that the number of \(2\) always keeps as a constant \(Q = 1000\). This setting makes biological sense if we consider a system separated from the unlimited reservoir by a “membrane” or some type of “wall.” As long as some resource molecules are consumed, more will enter the system driven by the chemical gradient. 5) We denote the number of molecule \(i\) at time \(t\) as \(N_i(t)\). Initially, \(N_2(0) = 1\), and \(N_1(0) = N_2(0) = N_6(0) = N_7(0) = 0\). Molecule 4 triggers the chemical reaction system. We also set \(S = 1 \times 10^9\), which is much larger than \(Q\), so that we are in the dilute limit. We then use the standard Gillespie algorithm to simulate the dynamics (see details in section S2), and the code is accessible (https://github.com/yuenestliu/Self-replication-simulator). In addition, we construct ODEs to describe the mean-field dynamics (for details, see section S3).

Author contributions—Y. L. conceptualization; Y. L. software; Y. L. formal analysis; Y. L. and D. J. T. S. supervision; Y. L. funding acquisition; Y. L. validation; Y. L. investigation; Y. L. visualization; Y. L. formal analysis; Y. L. and D. J. T. S. supervision; Y. L. funding acquisition; Y. L. writing-review and editing.

Acknowledgment—We thank Dr. Wim Hordijk for helpful discussions and comments on RAF theory.

References
1. Szathmáry, E. (2006) The origin of replicators and reproducers. Philos. Trans. R. Soc. Lond. B Biol. Sci. 361, 1761–1776 CrossRef Medline
2. Gilbert, W. (1986) Origin of life: the RNA world. Nature 319, 618 CrossRef
3. Robertson, M. P., and Joyce, G. F. (2012) The origins of the RNA world. Cold Spring Harb. Perspect. Biol. 4, a003608 Medline
4. Kim, H.-J., Ricardo, A., Illangkoon, H. I., Kim, M. J., Carrigan, M. A., Frye, F., and Benner, S. A. (2011) Synthesis of carboxylates in mineral-guided prebiotic cycles. J. Am. Chem. Soc. 133, 9457–9468 CrossRef Medline
5. Li, L., Francklyn, C., and Carter, C. W. (2013) Aminocyclizing enzymes challenge the RNA world hypothesis. J. Biol. Chem. 288, 26856–26863 CrossRef Medline
6. Wächtershäuser, G. (1988) Before enzymes and templates: theory of surface metabolism. Microbiol. Rev. 52, 452–484 Medline
7. Szathmáry, E. (1999) in Levels of Selection in Evolution (Keller, L., ed) pp. 31–52, Princeton University Press, Princeton, NJ
8. Szathmáry, E. (1995) A classification of replicators and lambda-calculus models of biological organization. Proc. Biol. Sci. 260, 279–286 CrossRef Medline
9. Maynard Smith, J., and Szathmáry, E. (1995) The Major Transitions in Evolution, pp. 41–44, Oxford University Press, Oxford, UK
10. Gánti, T. (2003) The Principles of Life, pp. 4–100, Oxford University Press, Oxford, UK
11. Morowitz, H. J., Kostelnik, J. D., Yang, J., and Cody, G. D. (2000) The origin of intermediary metabolism. Proc. Natl. Acad. Sci. U.S.A. 97, 7704–7708 CrossRef Medline
12. Wächtershäuser, G. (1990) Evolution of the first metabolic cycles. Proc. Natl. Acad. Sci. U.S.A. 87, 200–204 CrossRef Medline
13. Kopp, L. E., and Miech, R. P. (1972) Nonlinear enzymatic cycling systems: the exponential cycling system. ii. Experimental cycling system. J. Biol. Chem. 247, 3564–3570 Medline
14. Kun, A., Papp, B., and Szathmáry, E. (2008) Computational identification of obligatorily autocatalytic replicators embedded in metabolic networks. Genome Biol. 9, R51 CrossRef Medline
15. Sousa, F. L., Hordijk, W., Steel, M., and Martin, W. F. (2015) Autocatalytic sets in E. coli metabolism. J. Syst. Chem. 6, 4 CrossRef Medline
16. Szathmáry, E., Santos, M., and Fernandez, C. (2005) in Prebiotic Chemistry (Walse, P., ed) pp. 167–211, Springer, Berlin
17. Vaidya, N., Manapat, M. L., Chen, I. A., Xuβi-Bruneť, R., Hayden, E. J., and Lehman, N. (2012) Spontaneous network formation among cooperative RNA replicators. Nature 491, 72–77 CrossRef Medline
18. Lincoln, T. A., and Joyce, G. F. (2009) Self-sustained replication of an RNA enzyme. Science 323, 1229–1232 CrossRef Medline
19. Ashkenasy, G., Bagasia, R., Yadv, M., and Ghadir, M. R. (2004) Design of a directed molecular network. Proc. Natl. Acad. Sci. U.S.A. 101, 10872–10877 CrossRef Medline
20. Sievers, D., and von Kiedrowski, G. (1994) Self-replication of complementary nucleotide-based oligomers. Nature 369, 221–224 CrossRef Medline
21. Dittrich, P., Ziegler, J., and Banzhaf, W. (2001) Artificial chemistries—a review. Artif. Life 7, 225–275 CrossRef Medline
22. Banzhaf, W., and Yamamoto, L. (2015) Artificial Chemistries, pp. 111–136, MIT Press, Cambridge, MA
23. Czárn, T., Könyû, B., and Szathmáry, E. (2015) Metabolically coupled replicator systems: overview of an RNA-world model concept of prebiotic evolution on mineral surfaces. J. Theor. Biol. 381, 39–54 CrossRef Medline
24. Sziågyi, A., Zschar, I., Scheuring, I., Kun, Á., Könyû, B., and Czárn, T. (2017) Ecology and evolution in the RNA world dynamics and stability of prebiotic replicator systems. Life (Basel) 7, E49 CrossRef Medline
25. Hordijk, W., and Steel, M. (2017) Chasing the tail: the emergence of autocatalytic networks. Biosystems 152, 1–10 CrossRef Medline
26. Hordijk, W., and Steel, M. (2004) Detecting autocatalytic, self-sustaining sets in chemical reaction systems. J. Theor. Biol. 227, 451–461 CrossRef Medline
27. Steel, M. (2000) The emergence of a self-catalysing structure in abstract origin-of-life models. Appl. Math. Lett. 13, 91–95 CrossRef Medline
28. Kauffman, S. A. (1986) Autocatalytic sets of proteins. J. Theor. Biol. 119, 1–24 CrossRef Medline
29. Hordijk, W., Steel, M., and Kauffman, S. (2012) The structure of autocatalytic sets: evolvability, enablement, and emergence. Acta Biotheor. 60, 379–392 CrossRef Medline
30. Stadler, P. F., Fontana, W., and Miller, J. H. (1993) Random catalytic reaction networks. Phys. D Nonlinear Phenom. 63, 378–392 CrossRef Medline
31. Hordijk, W. (2016) Evolution of autocatalytic sets in computational models of chemical reaction networks. Orig. Life Evol. Biospheres 46, 233–245 CrossRef Medline
32. Vasas, V., Fernando, C., Santos, M., Kauffman, S., and Szathmáry, E. (2012) Evolution before genes. Biol. Direct 7, 379–392 CrossRef Medline
33. Jain, S., and Krishna, S. (2001) A model for the emergence of cooperation, interdependence, and structure in evolving networks. Proc. Natl. Acad. Sci. U.S.A. 98, 543–547 CrossRef Medline
34. Wills, P., and Henderson, L. (2000) Self-organisation and information-carrying capacity of collectively autocatalytic sets of polymers: ligation systems. in Proceedings from the International Conference on Complex Systems on Unifying Themes in Complex Systems, pp. 613–623, Perseus Books, Cambridge, MA
37. Dittrich, P., and di Fenizio, P. S. (2007) Chemical organisation theory. Bull. Math. Biol. 69, 1199–1231 CrossRef Medline
38. Segre´, D., Lancet, D., Kedem, O., and Pilpel, Y. (1998) Graded autocatalysis replication domain (GARD): kinetic analysis of self-sustaining reaction networks at the origin of life. New J. Phys. 20, 015011 CrossRef Medline
39. Hordijk, W., Steel, M., and Dittrich, P. (2018) Autocatalytic sets and chemical organizations: modeling self-sustaining reaction networks at the origin of life. Proc. Natl. Acad. Sci. U.S.A. 107, 1470–1475 CrossRef Medline
40. Vasas, V., Szathmáry, E., and Santos, M. (2010) Lack of evolvability in self-sustaining autocatalytic networks constraints metabolism-first scenarios for the origin of life. Proc. Natl. Acad. Sci. U.S.A. 107, 1470–1475 CrossRef Medline
41. Vasas, V., Fernando, C., Szilágyi, A., Zachár, I., Santos, M., and Szathmáry, E. (2015) Primordial evolvability: impasses and challenges. J. Syst. Chem. 5, 2 CrossRef Medline
42. Fontana, W., and Buss, L. W. (1994) “The arrival of the fittest”: toward a theory of biological organization. Bull. Math. Biol. 56, 1–64 CrossRef Medline
43. Rasmussen, S., Constantinescu, A., and Svaneborg, C. (2016) Generating minimal living systems from non-living materials and increasing their evolutionary abilities. Philos. Trans. Royal Soc. Lond. B Biol. Sci. 371, 20150440 CrossRef Medline
44. Levy, M., and Ellington, A. D. (2003) Exponential growth by cross-catalytic cleavage of deoxyribozymogens. Proc. Natl. Acad. Sci. U.S.A. 100, 6416–6421 CrossRef Medline
45. Hordijk, W., and Steel, M. (2016) Autocatalytic sets in polymer networks with variable catalysis distributions. J. Math. Chem. 54, 1997–2021 CrossRef Medline
46. Farmer, J., Kauffman, S. A., and Packard, N. H. (1986) Autocatalytic replication of polymers. Phys. D Nonlinear Phenom. 22, 50–67 CrossRef Medline
47. Smith, J. L., Steel, M., and Hordijk, W. (2014) Autocatalytic sets in a partitioned biochemical network. J. Syst. Chem. 5, 2 CrossRef Medline
48. Atkins, P., and de Paula, J. (2014) Atkins’ Physical Chemistry, 10th Ed., pp. 185, 823. Oxford University Press, Oxford, UK
49. Martin, W. F., Sousa, F. L., and Lane, N. (2014) Energy at life’s origin. Science 344, 1092–1093 CrossRef Medline
50. Schrödinger, E. (1944) What Is Life? The Physical Aspect of The Living Cell, Cambridge University Press, Cambridge, UK
51. Albert, R. A. (2004) A short history of the thermodynamics of enzyme-catalyzed reactions. J. Biol. Chem. 279, 27831–27836 CrossRef Medline
52. Amend, J. P., LaRowe, D. E., McCollom, T. M., and Shock, E. L. (2013) The energetics of organic synthesis inside and outside the cell. Philos. Trans. R. Soc. Lond. B Biol. Sci. 368, 20120255 CrossRef Medline
53. Branscomb, E., and Russell, M. J. (2013) Turnstiles and bifurcators: the disequilibrium converting engines that put metabolism on the road. Biochim. Biophys. Acta 1827, 62–78 CrossRef Medline
54. England, J. L. (2013) Statistical physics of self-replication. J. Chem. Phys. 139, 121923 CrossRef Medline
55. Russell, M. J., Daniel, R. M., and Hall, A. J. (1993) On the emergence of life via catalytic iron-sulphide membranes. Terra Nova 5, 343–347 CrossRef Medline
56. Ricardo, A., Carrigan, M. A., Olcott, A. N., and Benner, S. A. (2004) Borate minerals stabilize ribose. Science 303, 196–196 CrossRef Medline
57. Cavicchioli, R. (2002) Extremophiles and the search for extraterrestrial life. Astrobiology 2, 281–292 CrossRef Medline
58. Billings, L., Cameron, V., Claire, M., Dick, G. J., Domagal-Goldman, S. D., Javax, E. J., Johnson, O. J., Laws, C., Race, M. S., Rask, J., Rummel, J. D., Schelble, R. T., and Vance, S. (2006) The astrobiology primer: an outline of general knowledge—version 1, 2006. Astrobiology 6, 735–813 CrossRef Medline
59. Schmidt, M. (2010) Xenobiology: a new form of life as the ultimate biosafety tool. Bioessays 32, 322–331 CrossRef Medline
60. Mavrovouniotis, M. L. (1991) Estimation of standard Gibbs energy changes of biotransformations. J. Biol. Chem. 266, 14440–14445 CrossRef Medline
Mathematical modeling reveals spontaneous emergence of self-replication in chemical reaction systems
Yu Liu and David J. T. Sumpter

J. Biol. Chem. 2018, 293:18854-18863.
doi: 10.1074/jbc.RA118.003795 originally published online October 3, 2018

Access the most updated version of this article at doi: 10.1074/jbc.RA118.003795

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 52 references, 15 of which can be accessed free at http://www.jbc.org/content/293/49/18854.full.html#ref-list-1