A Chemical Ecosystem Selection Approach for Generating Evolvable Chemical Systems *in Vitro*

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Abstract

Many attempts to ascertain the physicochemical processes governing the emergence of life have focused on studying the synthesis of particular biomolecules and their precursors or on designing simple systems that manifest life-like properties. In this paper, we present the methodological components of an experimental framework designed to generate and detect spontaneously forming chemical systems capable of collective propagation and adaptive evolution. The *chemical ecosystem selection* paradigm involves incubating complex mixtures of organic compounds with populations of mineral grains to promote the appearance of autocatalytic sets that interact with the mineral surface and perform serial transfers to favor surface-associated systems that are better at being transmitted from grain to grain. This approach has the potential to serve as a novel tool for screening a vast array of experimental conditions and determine the likelihood that they will produce life-like chemical systems.

Introduction

The capacity to self-propagate and evolve adaptively are two defining features of life (Joyce, 1989). Rather than assuming that the onset of evolution required the spontaneous appearance of a self-replicating entity, such as a protocell or an RNA molecule, some theorists have suggested that adaptive evolution may have initiated in a much simpler state when autocatalytic chemical sets became spatially localized on a mineral surface (Wächtershäuser, 1988). These sets are characterized by series of reactions that produce catalysts for other reactions in the set, such that the entire ensemble collectively propagates over the mineral surface. Such systems would tend to become better at colonizing new mineral surfaces by *neighborhood selection,* a process similar to group selection but acting in the absence of individually bounded units (Nunney, 1985). Under this model, life-like chemical systems (LCSs) that can propagate and evolve adaptively might arise quite easily. However, the empirical challenge might lie in finding conditions that allow evolvable systems to emerge and detect them once they have arisen.

Motivated by this theory, we have developed an experimental paradigm analogous to artificial ecosystem selection, *chemical ecosystem selection* (CES), to find life-like systems based on their capacity to respond to selection. The CES framework can be viewed as a bridge between prebiotic synthesis experiments demonstrating that complex molecules can be generated from simpler ones, such as pyruvate, under high temperature and pressure conditions (Hazen and Deamer, 2007) with an artificial selection approach to enrich for life-like behaviors in already complex molecular systems (Spiegelman et al., 1965). In CES experiments, selection is applied to stimulate the formation of increasingly complex molecular assemblages from simple chemical building blocks.

Here, we outline the general CES approach, consider several important experimental parameters, and briefly comment on our progress in deploying the CES protocol thus far.

The Chemical Ecosystem Selection Paradigm

The CES approach involves incubating complex mixtures of organic compounds with populations of mineral grains and performing serial transfers to select for surface-associated systems that are better at being transmitted from grain to grain (Baum, 2015; Baum and Vetsigian, 2017). The rationale is to use a chemical mix of simple molecules that is diverse enough that the likelihood of an autocatalytic set being present is high (Kauffman, 1986; Virgo, et al., 2013). Such sets would be adsorbed on the mineral surface, which would help spatially confine the system and provide catalytic functions. If multiple sets were present or arose over time (e.g., through addition of new side-reactions) they might differ in colonizing ability and, thereby, compete for available mineral surface. Selection is imposed by transferring a small amount of colonized grains to a new vessel containing virgin mineral grains and would be expected to enrich for LCSs that propagate faster.

Protocol

To begin a CES experiment, a fixed amount of mineral grains is placed into serum vials which are then sealed and autoclaved. Solutions containing a diverse mixture of organic compounds are prepared by dissolving solids in sterile water and filter-sterilized. High-energy compounds are added to the solutions immediately prior to dispensing them into sealed reaction vessels. Serial transfers are performed at regular intervals by moving a small volume of mineral suspension to a new vial containing virgin mineral grains and freshly prepared organic solutions.

Experimental Parameters

The composition of the chemical mix and the choice of mineral substrates used in a CES experiment can be altered to accommodate different hypotheses about what sets of conditions are conducive to the emergence of LCSs. We are currently carrying out two parallel CES iterations designed to test two origins of life scenarios; hydrothermal vents and geothermal pools. In both cases, the chemical mix is composed of organic monomers reminiscent of the results of Miller-Urey-type experiments. In the vent experiment, the salt composition mimics oceanic concentrations, with Na^+ and Cl^- as the dominant ions. The geothermal pool version uses typical cytosolic ion concentrations instead, characterized by a relatively high molar concentration of K^+ (Mulkidjanian, 2012). We are using these in combination with iron sulfide, iron oxide, carbonate, and phyllosilicate minerals.

Our current experiments use diverse chemical energy sources, which have the added advantage of being trackable, allowing us to determine the rate at which free energy is consumed in a given reaction vessel. Energy sources we have explored include inorganic ions out of equilibrium (e.g., NH_4^+ and NO_3^-), activating compounds such as ammonium persulfate, and chemicals containing high-energy phosphate bonds (e.g., ATP).

The CES framework is amenable to different environmental conditions; reaction vessels can be maintained at an elevated temperature or exposed to brief period high pressure/high temperature conditions through autoclaving.

Chemical Analysis and LCS Detection

We have identified a number chemical proxies for energy dissipation as first-line indicators that interesting reactions are occurring in a given vessel. To date, we have optimized protocols to track changes in redox state using NADH as a reporter and to quantify the concentrations of inorganic ions, such as ammonium, nitrate, or inorganic phosphate released by hydrolysis of ATP and trimetaphosphate. These methods allow us to look for directional changes over generations and compare a set of samples after many generations of selection to a control set established just one generation earlier. Statistical analyses can employ linear mixed-effects models.

If the chemical proxies suggest that LCS might have emerged in any conditions, we will search for further evidence of their capacity to propagate and evolve adaptively. For this we are exploring high-performance liquid chromatography (HPLC) with mass spectrometry (MS) to analyze solution and X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopy (FTIR) to the identify the elemental composition and functional groups of molecules interacting with mineral surfaces.

Discussion

A key deliverable of these experiments will be a systematic protocol that can be modified to accommodate specific hypotheses as to the chemical and physical environment in which LCSs evolve. There are an almost infinite number of different chemical mix, energy source, mineral surface, and environment combinations that can be tested. We have already deployed the CES framework to evaluate conditions representative of two environments (hydrothermal vents and geothermal pools) and are working on increasing the throughput of the CES protocol to facilitate a more efficient search of chemical parameter space.

In parallel with the CES procedure just described, which entails strict vertical inheritance through "lineages" of reaction vials, we are also exploring an approach that includes artificial selection and recombination among vessels. We have developed a protocol to select among chemical communities for those that maximize a chemical trait (such as use of a potential energy source) and then combining "winning" grains before reiterating selection. By seeing if the average value of the chemical trait changes over generations, we will know if there is a response to the imposed selective pressure. Overall, the results of these experiments are expected to reveal new insights into the origin of life problem by advancing our understanding of how evolvable chemical systems could have emerged on prebiotic Earth and how readily they might arise elsewhere in the universe.

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