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Selection for the Spontaneous Appearance of Lifelike Chemistry *in vitro*

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Introduction: Among the most promising theories of life's origin are those that invoke the spontaneous formation of reflexively autocatalytic sets – sets of chemicals in which each chemical is created by at least one reaction in the set and each reaction is catalyzed by other members of the set (or occurs spontaneously)[1]. Such autocatalytic systems could be composed of diverse organic and inorganic components, perhaps including RNA[2-5,7-8]. Previous research has shown that given a sufficiently diverse enough mixture, reflexively autocatalytic sets are highly probable [9]. It has also been proposed that spontaneously emerging reflectively autocatalytic systems can be evolvable if either encapsulated in a membrane[10] or adsorbed onto a mineral surface[11]. We are using a novel class of experiments, modeled after microbial artificial ecosystem selection experiments[12] to evaluate whether evolvable autocatalytic systems can emerge spontaneously in the laboratory.

Research Approach: Mineral particles are incubated in an aqueous soup containing diverse potential building blocks of life and abundant free energy. Each generation we transfer a small aliquot of particles into a new container containing fresh soup and a population of virgin grains. By repeating this over many generations we impose selection for chemical systems that can self-propagate and can be efficiently transferred from generation to generation. Systematic changes over generations, for example in the amount of carbon attaching to grains or the amount of free energy dissipated during a period of incubation, could indicate that systems of chemicals have arisen that can collectively propagate themselves from generation to generation.

Methods: The experiment is conducted under sterile conditions using either iron pyrite or montmorillonite grains and a generation time of 2-3 days. As an additional aid to sterility, vials are autoclaved each generation. The chemical soup we have used includes transition metal ions (as possible catalytysts), ammonium nitrate (as a kinetically-slow source of redox potential energy), and diverse organic monomers including amino acids, sugars, nitrogenous bases, and diverse organic acids.

Currently we are using five analysis methods: (1) Nitrate/Ammonium assays to see if the rate of redox energy changes. (2) Energy dispersive spectroscopy (EDS)/X-ray photoelectron spectroscopy (XPS) to determine the amount of carbon and nitrogen adsorbed onto grains before and after incubation. (3) Assays of total primary amino nitrogen to assess amino acid compositional changes. (4) High performance liquid chromatography (HPLC) to track the formation of peptides and amino acid compositional changes. (5) We hope to soon add an additional assay, based on inclusion of a nonstandard amino acid FRET pair (p-cyanophenylalanine and 7-azatryptophan) to the solution so that FRET signal can be used to detect amino-acid-containing oligomers.

Results: We have completed >45 generations of selection, without evidence of bacterial contamination. Side-by-side comparison of experimental vials with control vials that had not been inoculated from the prior generation has, to date, failed to detect significant changes. We propose continuing this experiment for many additional generations and initiating additional experiments with different reagents and minerals.

References: [1]Joyce GF, *Nature* **338**, 217-224 (1989) [2]Dyson FJ *J Mol Evol* **18**, 344-350 (1982) [3]Dyson FJ *Origins of Life* (1985) [4]Sharov AA *J Cosmol* **5**, 833-842 (2010) [5]Hordijk W, Hein J, Steel M *Entropy* **12**, 1733-1742 (2010) [6]Shenhav B, Segrè D, Lancet D *Adv Comp Sys* **6**, 15-35 (2003) [7]Kaufman SA *J Theor Biol* **119**, 1-24 (1986) [8]Kaufman SA *The origins of Order* (1993) [9] Hordijk W, Steel M *J Theor Biol* **227**, 451-461(2004)[10]Shenav et al 2003 [11]Baum 2015 [12] Baum and Vestigian 2016