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Emergence of Life in the lab?

How Darwinian evolution can emerge from scratch is one of the fundamental questions of biology - and a major question for humankind. The Origin of Life field addresses such a second, biological big bang that has reshaped our own planet Earth and possibly many others in the universe.

Biochemistry has taught us a great deal about biological evolution and established powerful experimental methods. Yet, the start of evolution remained a mystery. We are convinced that focused, interdisciplinary lab experiments will be able to recreate the emergence of life in the lab at a pace much faster than many would expect. In the next five years, microscale experiments based on geoscience, chemistry and physics will hopefully create and confirm a complete hypothesis of how life can emerge. (Fig. 1)

This sounds like a bold claim, but we think it is possible to recreate the core processes of emergent life with a set of well-defined and highly specific lab experiments that run on the time scale of weeks, not thousands of years. In the following, we will describe our current working hypothesis and why we are optimistic to find answers to this big question. Our target is to recreate a sustained, open ended Darwinian evolution to form ever increasing complexity starting with a minimal amount of only three molecules inside a plausible geological boundary condition and without human intervention. Our approach focuses on evolution as a molecular process that maintains and increases its information content through feedback loops using continuous selection and molecular recycling. Only then can molecules be considered to become “alive” and avoid the death of equilibration.

A minimal set of molecules maximizes the probability of the emergence of life. At this time our experiments strongly suggest three starting molecules: 2’,3’-cyclic GMP which self-polymerizes in the dry state, 2’,3’-cyclic CMP that co-polymerize into matrices of polyG [1] and trimetaphosphate to trigger 2’3’ phosphorylation. The oligomerization of mixed GC mers with a length of >10 mers is optimal at low or zero magnesium concentration, slightly elevated pH and 20–40 °C. These conditions are almost capable for the strands to bind to each other and form double stranded RNA. The trimetaphosphate for phosphorylation is produced with 95% yield at 450-500 °C, likely reachable in volcanic conditions and provided as highly soluble feeding deposit.

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We targeted first the replication machinery where we found that the core reactions for early life such as phosphorylation, polymerization, templated ligation and physical sequence-selection and hydrolysis surprisingly work under identical conditions. Side products are avoided by using only mildly activated polymerization and ligation. We expect that the central role and hence the reactive bottleneck of G polymerization can select for chirality. Initial chirality biases in the starting pool can be enhanced by the nonlinear concentration dependence of polymerization and the tight base-stacking in the dry state. This chirality selection can then by propagated to the much slower copolymerization of the other bases C, A and U. We also explore how the polymerization of RNA can enhance a preceding synthesis pathway for the formation of G and C nucleosides.

The templated ligation of short strands, as opposed to base-by-base templated polymerization is our preferred mode of replication due to its simple chemical and physical requirements. When the polymerized, mixed oligomers are long enough, templated replication will start from remaining or recycled 2’,3’-cyclic ends under the same reaction conditions on the time scale of a day. Using the higher speed of ligating proteins, we could show that templated ligation creates complementary networks of sequence structures from random sequences and offer a concentration dependent replication dynamics very similar to Hypercycles [2, 3]. We think all of this is crucial to prevent the informational drift towards random sequences. In previous experiments, ligation from random sequences showed a selection towards a smaller sequence space [4]. As the binding of complementary sequences exponentially depends on the sequence space, we expect a selection of the smaller sequence space of two bases compared to the sequence space of four or more bases.

Under the same conditions, hydrolysis of double stranded RNA can select for 3’-5’-linkages over 2’-5’ linkages [5–7]. Furthermore, since the hydrolyzed sequences originated from already selected sequences, the evolution will increasingly operate within a localized sequence space [8]. In situ phosphorylation by trimetaphosphate will help to recycle monomers and oligomers to maintain the active 2’,3’-cyclic phosphate end. With longer strands emerging, ribozyme based templated replication [9, 10] could have eventually taken over from ligation-based replication.

We have shown experimentally how this chemistry is boosted by various non-equilibrium settings provided by a thermal gradient [11–13]. Most notably, wet-dry cycles are triggered at an air-water interface, adjacent to a bulk volume of constant salt concentration and pH. In the presence of a CO_2-water interface, recondensed dew droplets resulted in acidic pH and

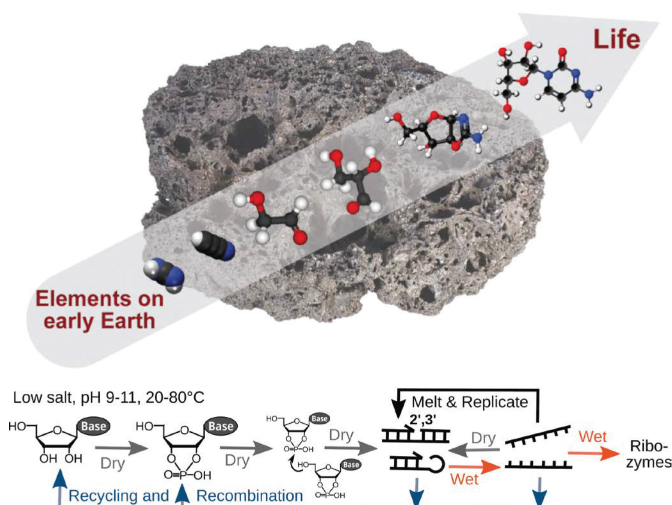

Fig. 1: Rocky start of RNA-based life. How can the process of life be triggered by a chemistry that matches the physical non-equilibra of early Earth? Lab experiments between chemistry, physics and geoscience make fast progress.

low salt cycling. Under the low Mg concentrations that also enhance RNA polymerization, strands were found to separate at temperatures 20-30 °C lower than the melting temperature of RNA under physiological conditions [14]. We showed that these combined effects lead to the replication and selection of up to 1300mers within 8 hours using a biological Taq polymerase as a proxy for replication [15–17].

The CO_2-water interface leads to cooperative accumulation of molecules at the warm side of the interface, triggering the formation of RNA gels [11, 18], RNA coacervates [19], the sedimenting RNA agglomerates [20] and last but not the least the encapsulation of the accumulated RNA into lipid vesicles [11], which are likely important for protection against environmental fluctuations and setting the stage for the evolution of self-sustained cellular life once the directed feeding through membranes has evolved. Additionally, thermal gradients can also create pH gradients [21], accumulate (or deplete) salts [22] or nucleotides [12, 23] using convection and thermophoresis [24]. All of these experiments are made possible with state of the art HPLC-ESI-TOF mass spectrometry, Illumina sequencing and rapid prototyping of microfluidic settings.

We are optimistic that the interplay of the shown mechanisms will generate Darwinian evolution from scratch. We think that this is not so much a matter of long timespans, but the tuned combination of physical non-equilibria with a mild and recyclable RNA chemistry. Our goal currently is to see sufficient polymerization in 1-2 days, sufficient templated ligation in 2-3 days and then trigger the emergence of catalytic sequences within a week. These reactions will be optimized in separated short term tests with varying initial conditions to be then later assembled into week long experiments.

Crucial will be to understand the evolution dynamics by starting either with fully random conditions to see what sequence patterns emerge or to probe how the same system can maintain a catalytic sequence that is found in the initial pool. With this, we can estimate how long it will take the system to find these target sequences by natural selection. By doing multiple parallel experiments and using pools of synthetic oligomers, we will efficiently screen for optimal geological geometries and probe suitable reaction conditions with high throughput. At this point, week long microfluidic experiments under a thermal gradient are already routinely done in our lab.

All of these experiments are embedded in a scientifically inspiring atmosphere provided by highly cross-disciplinary network of the first SFB on the Emergence of Life in Munich and the worldwide collaboration funded by the Simons foundation. We think lab experiments will be crucial to validate upcoming data from extrasolar atmospheres by JWST and the precious samples that will be returned from Mars and meteorites - not to forget the wealth of knowledge about the early phase of our solar system that we get from the analysis of meteorites fallen on the Earth.

We think that we live in a highly inspiring and very productive time for Origins of Life research. We hope that cross-disciplinary professorships for the topic will be possible in the near future to maintain the dynamics and provide the basis for the many young scientists that have made the recent progress of the field possible.

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Prof. Dr. Dieter Braun

Dieter Braun earned his first money in the 1980s by publishing computer programs or driving tractor. Recently, he is learning the Cello to complete the family quartet. He is running a W2 group at LMU in downtown Munich with minimal university resources, but great funding from Emmy Noether, ERC Starting and Advances, Simons and VW foundation and has initiated the Emergence of Life SFB (www.emergence-of-life.de/). Two of his PhD students turned Origin of Life research into a Biotech and founded NanoTemper whose more than 100 employees develop equipment that screen biomolecule binding and perform protein quality control, paying way more taxes than his lab ever spent. The Braun lab is located in the hidden cellars of downtown LMU. Thermophoresis was devised in the same rooms during WWII to separate U235 from U238 - luckily without success. So in a turn of events, these rooms now host experiments to accumulate the molecules for life, not death.