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Origins as Evolution of Catalysts

Life is an energy releasing chemical reaction. At the heart of all chemical processes in cells there resides a main chemical reaction that allows environmentally available compounds to react in such a way that the free energy released during their reaction is not lost as heat but is harnessed instead, typically in the form of ATP, which can be hydrolyzed in a way that allows thermodynamically sluggish reactions of metabolism to go forward. Looking at life as a heavily interconnected energy releasing reaction that requires catalysts for rate acceleration can impact the way we view its origin.

Among microbiologists there is a long tradition of thought that the first cells were anaerobes, that they could endure the harsh conditions on the early Earth and that they were autotrophs, that is, that they satisfied their requirements for carbon from CO₂ alone [1]. In 1910, the Russian biologist Constantin Mereschkowsky concluded that the first cells were probably anaerobes that had the ability to survive temperatures near the boiling point of water and that were able to synthesize proteins and carbohydrates from inorganic compounds without the help of chlorophyll [2]. That was a surprisingly very modern view, in particular given what was known at the time about the chemistry of life, namely almost nothing. In 1910 there was Darwin's excellent and increasingly accepted theory about how organisms change over time, but the issue of what actually goes on in cells, what those reactions are and how they are catalyzed was completely obscure. For comparison, in 1910, physicists had full blown relativity, chemists had good working models for atoms and bonds, huge successes with synthetic dyes and new materials like bakelit, while biologists were still seriously discussing the concept of protoplasm, the notion that the matter of living beings is organized differently from inanimate matter and is furthermore bestowed with a special power, a *vis vitalis*, that distinguishes it from other kinds of matter and that makes it alive. Why were biologists so far behind? Biology is really hard. That is mainly because cells are very small and so diverse in nature. And if we give them substrate, they grow. For example, if we give an *Escherichia coli* cell with a doubling time of 20 minutes enough substrate, the culture will grow so as to outweigh the Earth in two days. No other form of matter does anything vaguely similar. What is life [3]? It is unique in its properties but it is still a chemical reaction.

Today we know that cells are made of the same matter as everything else, they operate because they have a network of roughly 1000 reactions that supply the building blocks of life and that organize those building blocks into polymers that make up the cell. Life is an exergonic chemical reaction and life is organized as cells. By dry weight, a typical cell (a unit of life) is made of about 50 % protein, 20 % RNA, 3 % DNA, ca. 10 % saccharides and cell wall, ca. 10 % lipids and some metabolites. The cell contains about 10,000 ribosomes, which make the proteins, and the process of protein synthesis consumes about 75 % of the biosynthetic ATP budget [4] with the proteins mainly serving as enzymes that catalyze the reactions that make more of the ribosomes that make more of the proteins that make more of the cell, etc., in what sometimes seems like an endless chicken and egg problem designed to frustrate scientists trying to understand life's origin. Cells self-organize matter into likenesses of themselves. The self-organization property of cells is not obvious. For Schrödinger, the self-organization aspect seemed to counter the concept of entropy [3]. But that is not the case, as Hansen et al. [5], who measured the entropy change in cells during growth for decades, succinctly explained. The measured entropy changes during cellular growth are always zero or close to zero because "cells are organized in a spontaneous process" [5, p. 1843]. That is, if a cell has what it needs to grow, it organizes environmentally available components into more of itself as an effortless byproduct of the exergonic growth process.

That only works, however, because the reactions in a cell are catalyzed by enzymes, which, once synthesized, spontaneously fold by themselves into the right conformation [6]. Without enzymes as catalysts, many of the reactions in a cell would be more than 10 orders of magnitude slower than without the enzyme. If only one essential reaction of a cell is 10 orders of magnitude slower than the others, the doubling time for growth changes from about 20 minutes to about 150,000 years. In a general sense, the function of enzymes is to catalyze reactions in the cell so that they all take place at about the same rate [7], a rate that is fast enough that the metabolic intermediates do not diffuse into the environment or spontaneously decay. As emphasized by Wolfenden though [7], some biological reactions, if left uncatalyzed, are 10⁵ times faster at 100 °C than at 25 °C, which is a new line of support for the old idea [2] of an origin of life at high temperatures.

In cells, the catalysts are enzymes. What are enzymes? Enzymes are of course proteins, and as Höxtermann [8] explained, the word 'enzyme' comes from Kühne (1876), whose original passage is this: "*Hr. W. Kühne berichtet über das Verhalten verschiedener organisirter und sog. ungeformter Fermente. Um Missverständnissen vorzubeugen und lästige Umschreibungen zu vermeiden schlägt Vortragender vor die ungeform-*

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ten oder nicht organisierten Fermente, deren Wirkung ohne Anwesenheit von Organismen und ausserhalb derselben erfolgen kann, als Enzyme zu bezeichnen. — Genauer untersucht wurde besonders das Eiweiss verdauende Enzym des Pankreas, für welches, da es zugleich Spaltung der Albuminkörper veranlasst, der Name Trypsin gewählt wurde.“ [9, p. 190]

It is fair to say that catalysts were essential at origins, because without catalysts too many chemical reactions of life are just too slow. How many enzymes were essential at origins? That seems like a difficult question, but we can get an estimate by looking at life itself. If we look at metabolism across prokaryotic cells using a comparative (top-down) approach, we can see that, starting from H_2 , CO_2 and NH_3 , there are only about 400 reactions that lead to the synthesis of the 20 main amino acids, the 4 main nucleotides of DNA and RNA and the ca. 18 cofactors that are common to all cells [10]. By estimating the change in Gibbs free energy across those reactions in the biosynthetic direction, we found that 97 % of those reactions are exergonic in the direction of cell synthesis. That is, the core metabolic network of chemolithoautotrophs comprises a set of exergonic reactions [11]. Fine, the microbiologist might say, it cannot be any other way, really, because otherwise life would not work to begin with. But that set of 400 reactions gives us insight into primordial metabolism because it was present in the last universal ancestor of all cells, LUCA (Figure 1). Note that the reactions are conserved but not all of the enzymes are conserved, as some of them have arisen multiple times in evolution. Ah, the reader might think, could it be some of the reactions are older than the enzymes that catalyze the reactions? Yes, exactly [1].

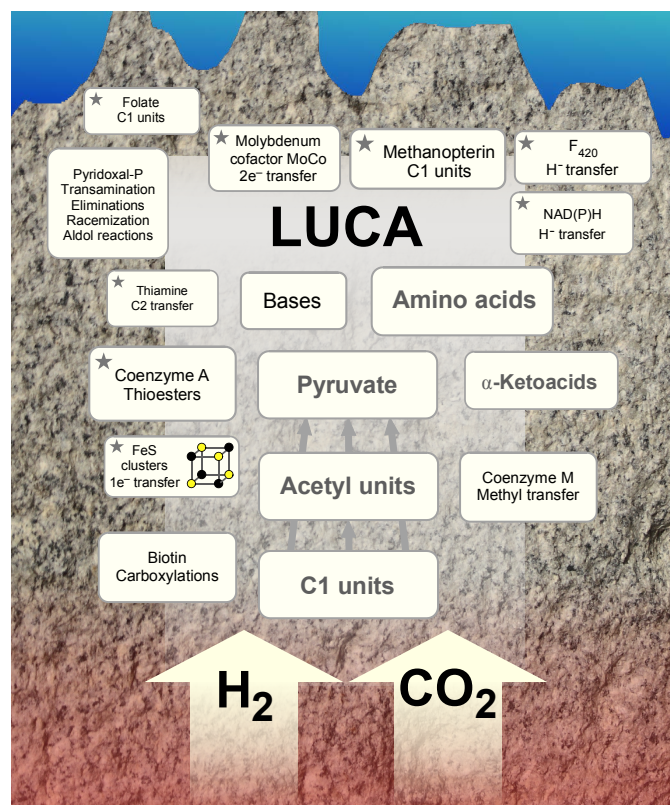


Fig. 1: Scheme representing the hypothetical evolution of life's building blocks starting from H_2 and CO_2 in serpentinizing hydrothermal vents (see [10]).

What are the oldest reactions in metabolism? Arguably, the oldest are those that led to the incorporation of CO_2 into organic compounds. Together with the team of Harun Tüysüz at the Max Planck Institute for Coal Research in Mülheim, an expert for heterogeneous catalysis, and Prof. Karl Kleinermanns from Düsseldorf, an expert for the physical chemistry of surface reactions, Dr. Martina Preiner recently set out to test the idea that the central backbone of microbial metabolism is simultaneously the evolutionary starting point of metabolism [12], and that its primordial reaction sequence could have arisen from purely mineral-catalyzed reactions of H_2 and CO_2 under conditions of a hydrothermal vent. We found that using either awaruite (Ni_3Fe) or magnetite (Fe_3O_4) as the sole catalyst, both of which are minerals that naturally occur and are naturally formed in H_2 producing hydrothermal vents, H_2 and CO_2 in water are converted into large amounts of formate, acetate and pyruvate [13] overnight at 100 °C in glass vials placed in a small laboratory reactor. The reactions also worked with greigite as the catalyst (Fe_3S_4) and in some experiments we obtained methane. Without the catalysts no products were obtained. Trivial, the physical chemist might say, simple surface chemistry. For the biologist, though, what we see is that the ancient core of microbial carbon and energy metabolism spontaneously unfolds in front of our eyes, overnight at 100 °C in a simulated hydrothermal vent on the laboratory bench (Figure 2). Maybe the reactions of central metabolism are more natural than one might think. Recent work by other groups has shown that an increasingly large number of reactions germane to central metabolism can be catalyzed by simple metals, either in the solid phase or in solution [14].



Fig. 2: Image of a stainless-steel high-pressure bioreactor used for origin of life experiments at the Institute for Molecular Evolution at HHU Duesseldorf. Image credit: Jessica Wimmer.

Was there a massive transition in early chemical evolution from metals to proteins? Nobody will ever know for sure because nobody was there. But the data as they are coming in at present suggest that catalyzed chemical reactions with natural similarity to metabolism are the starting point for the evolution of everything else in biological systems because metabolism supplies the parts (chemical precursors) that make up the machine (the cell).

This increasingly directs our attention to the evolution of catalysts. As one series of events we can imagine a sequence in catalytic evolution starting with inorganic mineral catalysts in the solid phase first [13], followed by inorganic catalysts like Fe(II) in solution [14], followed by the simplest kinds of organocatalysts in solution such as amino acids [15], with an additional step in complexity coming in the form of cofactors like NADH [16] and finally peptides capable of interacting with these catalysts coming last. With each step, increases in rate and specificity emerged that could have contributed to the formation of autocatalytic networks [17]. Life arose within about 500 million years of the Earth's formation whereby the first cells had ribosomes and enzymes [18]. Starting from such simple chemical reactions, and those recently reported by the groups of Oliver Trapp [19] or Thomas Carell [20] in Munich, life eventually learned to make enzymes. In the 4 billion years since life's origin, nature has apparently been unable to improve upon the basic principle of the enzyme as the currency of catalytic function (Figure 3). But that does not mean that fundamental improvement is not possible. With the help of modern mining and chemical technologies, humans have learned to access the catalytic activity of elements that nature does not use. Elements such as ruthenium, palladium, iridium, and platinum allow chemists to explore catalytic properties that nature never harnessed.

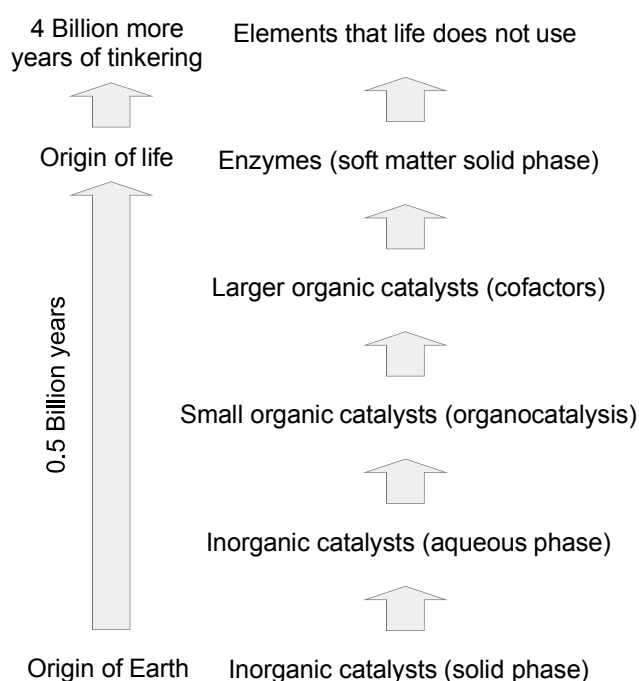


Fig. 3: Schematic evolution of catalysts over from the origin of Earth to date. At Earth's origin, inorganic catalysts existed in solid phase, changing to aqueous over time. Successively, small organic catalysts formed, developing into larger cofactors. At the origin of life, enzymes were on hand in a soft matter solid phase. While this process took 0.5 billion years, after another 4 billion years until now catalysts being utilized by nature did not evolve further.

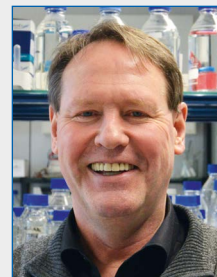
It is noteworthy that modern cofactors have no chiral atoms at their catalytically active moieties [21], suggesting that chirality might have been introduced into metabolism relatively late, by randomly arisen, handed catalysts. How so? There are about $1.8 \cdot 10^{48}$ tonnes of carbon on Earth, or about 10^{47} carbon atoms. That is roughly enough carbon to make all possible random peptide sequences 30 amino acids long using 20 D and L amino acids of avg. 5 carbon atoms each, if all the carbon was all available at one single place on earth. There is not enough carbon on Earth to keep handed catalysts from arising. If peptides could arise in evolution, chiral catalysts very quickly became an unavoidable product, by pure chance. Handed catalysts naturally lead to the accumulation of more handed catalysts, so that we do not strictly need to resort to enantiomeric excesses in space in order to account for the chirality of life. Catalysis need not have evolved in the sequence of steps outlined in Figure 3, but had it done so, we would be able to recognize that imprint in the metabolism and enzymes of modern cells.

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