Probing the origin of microbial cells in a geochemical context

Inaugural-Dissertation

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Eidesstattliche Erklärung

Ich versichere an Eides Statt, dass die Dissertation von mir selbstständig und ohne unzulässige fremde Hilfe unter Beachtung der „Grundsätze zur Sicherung guter wissenschaftlicher Praxis an der Heinrich-Heine-Universität Düsseldorf“ erstellt worden ist. Die Arbeit wurde bisher keiner Prüfungsbehörde vorgelegt und auch noch nicht veröffentlicht. Ich habe bisher weder erfolgreiche noch erfolglose Promotionsversuche unternommen.

Loraine Schwander

Düsseldorf, den 29. September 2025 Loraine Schwander

To my amazing wife Maria, who brings happiness to my life every day and kept me sane throughout all this.
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1. Abstract

How life originated is still a highly controversial topic. Today, hypotheses about the origin of life can be roughly divided into two hypotheses: genetics-first and metabolism-first. Genetics-first theories assume life began with self-replicating RNA that evolved on the early Earth with reducing conditions from chemistry containing cyanide and nitrile moieties. Metabolism-first theories propose that carbon and nitrogen enter prebiotic chemistry through reactions that are still found in biochemistry today. The central assumption in metabolism-first is that non-enzymatic, metal-catalyzed reactions starting from carbon dioxide (CO_2) were sufficient to create and obtain protometabolic networks. The two theories have fundamental disagreements. However, they agree that life began with CO_2, which is essential for all life on Earth today as the main source of carbon. Water and CO_2 were made readily available about 4.5 billion years ago by the Moon-forming impact. During this event, part of the young Earth's mantle evaporated, leaving behind a steam atmosphere. Water rained out and formed the oceans. CO_2 dissolved into the oceans over a few million years and made available for geochemical processes such as serpentinization.

Serpentinization is a process that occurs in alkaline hydrothermal vents like those in the Lost City hydrothermal field. These vents were probably more abundant on early Earth and are a promising place for the origin of life in metabolism-first theories. They provided the necessary conditions: energy and electrons from molecular hydrogen (H_2), organics derived from CO_2, metal-catalysts, nutrients, temperature and pH gradients, and compartmentation. During serpentinization, water reacts with ultramafic rock to form minerals and H_2. In the process, native metals (Fe^0, Ni^0, Co^0) are deposited in the vent. In the presence of native metals, the H_2 generated can reduce CO_2 into organic molecules, such as pyruvate, acetate and formate. These molecules are the backbone of the most ancient metabolic pathway: the acetyl-CoA pathway.

The oldest living microbes, methanogens and acetogens, obtain their energy and carbon from H_2 and CO_2 via the acetyl-CoA pathway. It is the only metabolic pathway that combines energy production and carbon fixation into one process. This process can be abiotically recreated in the lab using metal-catalysts, CO_2, H_2 and H_2O. Getting from these simple reactions to free-living cells via the last universal common ancestor (LUCA) is incredibly complex.

2. Zusammenfassung

Wie das Leben entstanden ist, ist nach wie vor ein höchst umstrittenes Thema. Heutige Hypothesen zur Entstehung des Lebens lassen sich grob in zwei Gruppen unterteilen: Genetik-zuerst und Stoffwechsel-zuerst. Genetik-zuerst-Theorien gehen davon aus, dass das Leben mit selbstreplizierender RNA begann, die sich auf der jungen Erde unter reduzierenden Bedingungen aus Cyanid- und Nitrilchemie entwickelte. Stoffwechsel-zuerst-Theorien schlagen vor, dass Kohlenstoff und Stickstoff durch Reaktionen in die präbiotische Chemie eintreten, die auch heute noch in der Biochemie vorkommen. Die zentrale Annahme der Stoffwechsel-zuerst-Theorien ist, dass nicht-enzymatische, metallkatalysierte Reaktionen ausgehend von Kohlenstoffdioxid (CO_2) ausreichten, um protometabolische Netzwerke zu bilden und zu erhalten. Die beiden Theorien haben grundlegende Uneinigkeiten. Sie stimmen jedoch darin überein, dass das Leben mit CO_2 begann, das als Hauptkohlenstoffquelle für alles heutige Leben auf der Erde essenziell ist. Wasser und CO_2 wurden vor etwa 4,5 Milliarden Jahren durch den Mondformenden Einschlag leicht verfügbar. Bei diesem Ereignis vaporisierte ein Teil des Erdmantels und hinterließ eine Dampfatmosphäre. Wasser regnete aus und bildete die Ozeane. CO_2 löste sich über einige Millionen Jahre in den Ozeanen und wurde für geochemische Prozesse wie die Serpentinisierung verfügbar.

Serpentinisierung ist ein Prozess, der in alkalischen hydrothermalen Quellen wie denen im Lost City Hydrothermalfeld auftritt. Diese Quellen waren auf der frühen Erde wahrscheinlich häufiger und sind ein vielversprechender Ort für die Entstehung des Lebens in Stoffwechsel-zuerst-Theorien. Sie boten die notwendigen Bedingungen: Energie und Elektronen aus molekularem Wasserstoff (H_2), aus CO_2 gewonnene organische Stoffe, Metallkatalysatoren, Nährstoffe, Temperatur- und pH-Gradienten sowie Kompartimentierung. Bei der Serpentinisierung reagiert Wasser mit ultramafischem Gestein zu Mineralen und H_2. Dabei lagern sich native Metalle (Fe^0, Ni^0, Co^0) in der Quelle ab. In Gegenwart nativer Metalle kann das entstehende H_2 CO_2 zu organischen Molekülen wie Pyruvat, Acetat und Formiat reduzieren. Diese Moleküle bilden das Rückgrat des ältesten Stoffwechselwegs: des Acetyl-CoA-Stoffwechselwegs.

Die ältesten lebenden Mikroben, Methanogene und Acetogene, gewinnen ihre Energie und ihren Kohlenstoff aus H_2 und CO_2 über den Acetyl-CoA-Stoffwechselweg. Dies ist der einzige Stoffwechselweg, der Energieproduktion und Kohlenstofffixierung in einem Prozess kombiniert. Dieser Prozess kann im Labor mithilfe von Metallkatalysatoren, CO_2, H_2 und H_2O abiotisch nachgebildet werden. Der Weg von diesen einfachen Reaktionen über den letzten universellen gemeinsamen Vorfahren (LUCA) zu freilebenden Zellen ist unglaublich komplex.

3. The Moon-forming impact and timeline of the origin of life

The biochemistry of the most ancient prokaryotic lineages (methanogens among Archaea and acetogens among Bacteria) offers clues about potential environments for microbial origins (Weiss *et al.*, 2016). Notably, some early Earth environments are chemically aligned with the biochemistry of these organisms (Mrnjavac *et al.*, 2024b). Modern microbial physiology suggests that certain environmental catalysts may have accelerated the chemical reactions that led to metabolism and life. Microbial physiology can also help identify environments in which past microbes lived and what reactions these organisms used. Cells on Earth today need three basic requirements to grow and reproduce (Lipmann, 1965; Eck and Dayhoff, 1966; Decker *et al.*, 1970; Mrnjavac *et al.*, 2024b). They need carbon either in the form of carbon dioxide (CO_2), used by autotrophic organisms as a carbon source, or in the form of organic compounds, used by heterotrophic organisms. For their energy supply, they need either photons from sunlight, used by phototrophs, or chemical reactions, used by chemotrophs. Finally, they need electron donors in the form of inorganic or organic compounds, used by lithotrophs and organotrophs, respectively (Mrnjavac *et al.*, 2024b). Every ecosystem on this planet ultimately depends on autotrophs, which reduce CO_2 to organic carbon compounds that heterotrophs need (Fuchs, 2011).

The Moon-forming impact is essential to understanding how the origin of life could have occurred because without it, neither water (H_2O) nor CO_2 would have been available for the origin of life. Life on Earth today is water-based and starts and ends with CO_2 as its main carbon source (Fuchs, 2011; Mrnjavac *et al.*, 2023). The Moon was formed “shortly” after the Earth’s accretion period 4.55 billion years ago (4.55 Ga), between 4.5 and 4.35 Ga (Zahnle *et al.*, 2007; Borg and Carlson, 2023), when the young Earth collided with a Mars-sized planet called Theia (Goyal and Sahijpal, 2022; Hartmann and Davis, 1975; Cameron and Ward, 1976). This event vaporized 20% of the young Earth’s mantle (Zahnle *et al.*, 2007). In addition to rock particles, volatiles were also degassed during this impact, mainly CO_2 and H_2O, as well as some molecular nitrogen (N_2). These volatiles were poorly soluble in the magma ocean and, therefore, formed a new steam atmosphere (Abe and Matsui, 1985; Sleep *et al.*, 2014; Lammer *et al.*, 2018). The mantle cooled and solidified over 2–10 million years (Zahnle *et al.*, 2007; Sleep *et al.*, 2014). Most of the water in the newly formed steam atmosphere rained out as the Earth cooled, forming oceans around 4.4–4.3 Ga (Mojzsis *et al.*, 2001; Wilde *et al.*, 2001). After the water had rained out, a ~100 bar CO_2 atmosphere with a low N_2 content remained, i.e. conditions were like those on Venus today (Sossi *et al.*, 2020). Since the young Sun was much less “powerful” than today, the high CO_2 concentrations in the atmosphere of the young Earth most likely contributed to keeping the temperature at about 500 K and the water in liquid form by a strong greenhouse effect (Kasting, 2014; Sleep *et al.*, 2014; Charnay *et al.*, 2020; Mrnjavac *et al.*, 2023). While the oceans only formed after the Moon-forming impact, most of the water on Earth was already present beforehand, as it had accumulated during the accretion period (Zahnle *et al.*, 2007; Fischer-Gödde and Kleine, 2017; Greenwood *et al.*, 2018). The same is true for carbon on the early Earth. However, before the Moon-forming impact, this carbon was not present as CO_2, but as polyaromatic hydrocarbons (PAHs). These PAHs arrived on Earth during the accretion period in the form of carbonaceous chondrites (Sephton, 2002). During the Moon-forming impact, this inert carbon was converted to CO_2, which is much more reactive than PAHs and, therefore, an accessible carbon source for organic synthesis and life (Mrnjavac *et al.*, 2023).

CO_2 is the starting and ending point of all life on Earth. It is the carbon source for primary production and consequently the carbon source for all ecosystems on Earth (Fuchs, 2011; Mrnjavac *et al.*, 2023). Most of the CO_2 in the atmosphere had probably dissolved into the oceans by 3.8 Ga (Sleep *et al.*, 2014), leaving an N_2 atmosphere that still lacked the molecular oxygen (O_2) component that appeared around 2.4 Ga with the onset of cyanobacterial photosynthesis (Mrnjavac *et al.*, 2024a).

Based on fossil evidence, it is known that the first microbes on Earth must have existed by 3.8 Ga (Mojzsis *et al.*, 1996) in environments similar to today’s hydrothermal vents, as carbon of biogenic origin was isotopically detected (Baross and Hoffman, 1985; Mojzsis *et al.*, 1996; Ueno *et al.*, 2006; Arndt and Nisbet, 2012). Biotically generated carbon is lighter than abiotically generated carbon, and once at least –40 to –80 permille of ^{13}C is present, it is generally assumed to be of biogenic origin (Arndt and Nisbet, 2012; Blaser *et al.*, 2013). Life on Earth (and thus the last universal common ancestor (LUCA)) are water-based organisms, and the first fossils of microbes are already free-living cells and not an intermediate stage as LUCA was (Schwander *et al.*, 2023; Mrnjavac *et al.*, 2026). Given the constraints of when the Moon-forming impact occurred, when liquid water was present, and that isotopic evidence suggests that the first microbes were present on Earth around 3.8 Ga, the origin of life most likely occurred between 4.2 and 3.8 Ga. The timeframe for the origin of life is narrowed down by these events, but an even more controversial question is how and where the first organisms originated in the first place.

4. Origin of life theories

Today, the hypotheses about the origin of life can be roughly divided into two camps: Genetics-first (RNA-world theories, surface-reduction models, and heterotrophic origin theories include roughly the same ideas) (Gilbert, 1986; Benner *et al.*, 2020) and metabolism-first (subsurface reduction models and autotrophic origin theories include roughly the same ideas) (Mereschkowsky *et al.*, 1910; Eakin, 1963; Wächtershäuser, 1990, 1992; Martin and Russell, 2003, 2007; Mrnjavac *et al.*, 2023; Schwander *et al.*, 2023).

Genetics-first theories state that the first nucleic acid bases (Oró, 1961; Patel *et al.*, 2015), amino acids (Matthews and Moser, 1967; Powner *et al.*, 2009) and cofactors (Fairchild *et al.*, 2024) probably originated from cyanide chemistry created by meteorite impacts (Benner *et al.*, 2020), with carbon (C) and nitrogen (N) entering the prebiotic chemistry via nitrile bonds. However, a study by Wimmer *et al.* (2021a) has shown that there are no nitrile moieties among the 400 reactions that synthesize bases, amino acids and cofactors in cells.

In metabolism-first theories, carbon and nitrogen enter prebiotic chemistry through reactions that are still found in the biochemistry of life today (Martin and Russell, 2007). Before enzymes existed, inorganic surfaces and cofactors were the catalysts at the origin of life (Eakin, 1963). The catalysts were initially solid metals and metal alloys, which were then replaced by soluble catalysts and cofactors until they were finally replaced or supplemented by enzymes, which still catalyze reactions in cells today (Eakin, 1963; Mrnjavac *et al.*, 2023; Schwander *et al.*, 2023). In metabolism-first theories, the first organisms were chemolithoautotrophs: they obtain energy from exergonic reactions involving inorganic compounds (chemolithotrophy) and use CO_2 as their carbon source (autotrophy) (Mereschkowsky *et al.*, 1910; Fuchs and Stupperich, 1985; Wächtershäuser, 1992; Martin and Russell, 2003, 2007). The central assumption of autotrophic theories is that non-enzymatic, metal-catalyzed reactions originating from CO_2 possessed sufficient specificity and flux rates to sustain protometabolic networks. These networks produced life's core compounds that enabled further chemical evolution: nucleobases, amino acids, and cofactors (Xavier *et al.*, 2020; Wimmer *et al.*, 2021a). Evidence for biochemical reactions that do not require enzymes was found no later than 1967 by Degani and Halmann, who reported non-enzymatic glycolytic reactions (Degani and Halmann, 1967). Reactions catalyzed only by cofactors go back even further to the 1950s (Metzler and Snell, 1952a, 1952b; Nakada and Weinhouse, 1953; Martin and Russell, 2007). This concept also helps to explain something else that is confusing when considering the ancient metabolic pathways in the two prokaryotic domains Archaea and Bacteria: the enzymes that Archaea and

Bacteria use in the acetyl-CoA pathway (probably the oldest metabolic pathway in existence) are not the same, although the underlying chemistry and cofactors are very similar (Sousa and Martin, 2014).

Autotrophic theories are among the oldest origin theories of all. They are older than the heterotrophic origin theories put forward by Oparin in the 1920s (Oparin, 1957; Orgel, 2008). They are also older than the metabolism-first theories put forward by Eakin in 1963 (Eakin, 1963). Konstantin Mereschkowsky published a hypothesis on autotrophic origin as early as 1910 (Mereschkowsky *et al.*, 1910). At that time, carbon metabolism had not yet been discovered (Mrnjavac *et al.*, 2023). Mereschkowsky claimed that the first organisms had to be anaerobic thermophiles that were able to produce organic compounds from inorganic ones without photosynthesis (Mereschkowsky *et al.*, 1910). This is the definition of a chemolithoautotroph. Autotrophic theories have the limitation that there is yet no way to synthesize nucleobases in the laboratory from CO_2 and ammonia (NH_3/NH_4^+). They lack the link to replication in the origin of life. A caveat with genetics-first theories is that the components they use to synthesize nucleobases in the laboratory, namely nitrile moieties, are not present in either substrates or products of core microbial metabolism (Mrnjavac *et al.*, 2023; Wimmer *et al.*, 2021a).

The various theories about the origin of life contradict each other in many aspects, but they all agree on one thing: life began with CO_2 (Martin and Russell, 2003, 2007; Fuchs, 2011; Martin and Kleinermanns, 2024). CO_2 needs reducing conditions to react to form more complex organic molecules, and this is where one of the biggest arguments against genetics-first models comes into play, because they assume that the atmosphere of the early Earth was reducing. However, the Moon-forming impact left behind a reducing atmosphere and mantle. Isotopic evidence (Pahlevan *et al.*, 2019; Stüeken *et al.*, 2020) and oxygen fugacities from Hadean zircon crystals (Trail *et al.*, 2011) suggest that the early Earth's mantle was oxidizing. This led to the release of compounds such as CO_2, N_2 and H_2O into the atmosphere, which also became oxidizing (Pahlevan *et al.*, 2019; Sossi *et al.*, 2020; Zahnle *et al.*, 2020). Reduced gasses such as methane (CH_4) and NH_3 could only have been present in the atmosphere for a short time, as they would have dissociated due to photolysis (Kasting, 2014; Lammer *et al.*, 2018; Pahlevan *et al.*, 2019; Sossi *et al.*, 2020). To circumvent this, genetics-first models propose that reducing conditions briefly prevailed in Earth's atmosphere (Benner *et al.*, 2020; Zahnle *et al.*, 2020). This could have led to a stockpile of cyanide (Sasselov *et al.*, 2020), which could be reduced with sunlight to the compounds needed for the beginning of life (Benner *et al.*, 2020). Genetics-first models attribute transiently reducing conditions to the late heavy bombardment, a late veneer, or an additional post-Moon-forming impact event (Chou, 1978; Albarede *et al.*, 2013; Genda *et al.*, 2017; Benner *et al.*, 2020). However, recent evidence suggests that the late heavy bombardment never occurred (Sleep, 2016; Grewal *et al.*, 2019; Li, 2022). The Moon-forming impact alone can explain the presence of volatiles on the early Earth (Grewal *et al.*, 2019). However, cyanides and nitriles were not actually present in the atmosphere in current models after the Moon-forming impact (Trail *et al.*, 2011; Pahlevan *et al.*, 2019; Stüeken *et al.*, 2020). Even today, cyanide does not occur in volcanic emissions in a concentration that can be distinguished from the normal atmosphere (Rose *et al.*, 2006). Furthermore, these additional impactors would have had to be of a certain size and chemical composition to produce the NH_3 and CH_4 concentrations needed in this theoretical reducing atmosphere (Bottke *et al.*, 2010; Benner *et al.*, 2020; Li, 2022), and carbon from space is too reduced and structurally diverse to support the fermentations needed at the origin of life (Schönheit *et al.*, 2016). Additionally, genetics-first models based on the reduction of CO_2 and N_2 to cyanide and further reactions to form nucleotides and RNA do not overlap with microbial metabolism (Wimmer *et al.*, 2021a). No microbe requires cyanide as a growth substrate and no cyanides or nitriles are found in the biosynthetic pathways to bases, amino acids and cofactors (Wimmer *et al.*, 2021a). Metabolism-first models do not have the problem of "needing" a transient reducing state provided by something like the late heavy bombardment, because the geochemistry and environmental conditions that these models propose for the origin of life have provided reducing conditions in abundance since there have been oceans on Earth, and also fit well with the biochemistry of life (Russell, 1997; Martin and Russell 2003).

5. Serpentinizing hydrothermal vents and life's origin

Hydrothermal vents, specifically black smokers, were discovered in 1977 in the Pacific Ocean near the Galapagos Islands and immediately became of interest for theories about the origin of life (Corliss *et al.*, 1979, 1981; Baross and Hoffman, 1985). Criticism quickly arose that the origin of life could not have taken place in these vents because they were too hot for it to have ever occurred there (Miller and Bada, 1988). The upper temperature limit for the existence of life is 122 °C (Takai *et al.*, 2008), but black and white smokers have temperatures that start at around 200 °C, and black smokers can be hotter than 400 °C (Schwander *et al.*, 2023) and are usually located directly above magma chambers (Schrenk *et al.*, 2013). Soon after the criticism of black smokers as a possible site for the origin of life arose, arguments and evidence for the origin of life at another type of hydrothermal vent emerged: Previously undiscovered alkaline hydrothermal vents that may have vented at lower temperatures (Russell and Hall, 1997). Around the same time, in the late 1980s and early 1990s, compelling arguments for a metabolism catalyzed by metal-catalysts came up again (Wächtershäuser, 1988, 1990, 1992). In 2000, the Lost City hydrothermal field was discovered off the Mid-Atlantic Ridge in the Atlantic (Kelley *et al.*, 2001), which perfectly matched the previously described low-temperature alkaline hydrothermal vents. Lost City is not directly over a magma chamber, so the outflow is much cooler, and it does not emit smoke like the white and black smokers (Rona *et al.*, 1986). The vents at Lost City have a pH of 9–11, with effluent temperatures of 28–116 °C (Kelley *et al.*, 2005; Proskurowski *et al.*, 2008; Seyfried *et al.*, 2015). It quickly became clear that the Lost City-type vents could have been an ideal place for life to have originated not only because of their low temperatures, but also because of a process that takes place in them and provides molecular hydrogen (H_2) as an energy source: serpentinization.

Serpentinization is a geochemical process in which iron (Fe(II)) together with H_2O transforms into iron (Fe(III)) minerals and molecular hydrogen (H_2). Sleep *et al.* (2011) have simplified the reaction as follows:

$$3FeO \text{ (in rock)} + H_2O \text{ (liquid)} \Rightarrow Fe_3O_4 \text{ (magnetite)} + H_2 \text{ (in solution)}$$

This hydrogen production can lead to the production of methane and other hydrocarbons in Fischer-Tropsch reactions (FTT) at low temperatures through the reduction of CO_2 (Etiope and Sherwood Lollar, 2013; Etiope and Schoell, 2014). Serpentinization must have taken place since there was water on Earth. When ultramafic rocks encounter circulating liquid water at temperatures below 350 °C, serpentinization occurs (McCollom and Seewald, 2013). Ultramafic rocks are defined by high iron and magnesium content and <45 wt% silicate (SiO_2) (Holm *et al.*, 2015). On early Earth, the lithosphere was not yet differentiated, and ultramafic lava may have erupted at the surface and been exposed to the oceans. This means that serpentinization was not only present on early Earth, but most likely occurred much more frequently than today (Sleep *et al.*, 2004, 2011; McCollom and Seewald, 2013; Schrenk *et al.*, 2013; Preiner *et al.*, 2018). Serpentinization and FTT reactions are considered important for the origin of life because they generate C–C bonds from CO_2 and carbon monoxide (CO) (Russell *et al.*, 2010). They are also thought to produce hydrocarbons on other planets (Etiope and Schoell, 2014). Even today, H_2 serves as an energy and electron source for microbes in serpentinizing systems and may have played the same role in the origin of life (Martin and Russell, 2003, 2007; Schwander *et al.*, 2023). At lower temperatures, serpentinization leads to alkaline fluids because the protons are consumed and free OH^- is released, resulting in the production of H_2 (Mottl *et al.*, 2003; Schrenk *et al.*, 2013). The process of serpentinization has been known longer than the discovery of Lost City. Continental serpentinization sites have been studied since at least the 1960s (Barnes *et al.*, 1967).

Of the potential reducing agents on Earth that could directly reduce CO_2, only H_2 has a sufficiently negative midpoint potential, but it requires an alkaline pH for the midpoint potential to become negative enough. The redox potential of the serpentinizing effluent can be in the range of –800mV (Boyd *et al.*, 2020), which is sufficient for most biologically relevant reductions, except for the phosphate/phosphite couple, where the midpoint potentials become more negative with increasing pH (Bernhard Schink, pers. comm.). In addition, the reaction of H_2 and CO_2 to simple carbon compounds such as pyruvate requires solid-state catalysts such as magnetite and awaruite, which connect the origin of metabolism to serpentinizing vent systems (Chamberlain *et al.*, 1965; Preiner *et al.*, 2018). The reaction is very simple, needing no other components like RNA, sulfur, or nitrogen. It also operates without ion gradients, even though they are present in serpentinizing systems (Mrnjavac *et al.*, 2024b). Given all this information, for the origin of life low-temperature, serpentinizing hydrothermal vents such as Lost City are the best to study. Such vents would have provided several key requirements: energy and reducing agents (H_2), organic molecules (CH_4, acetate, formate, pyruvate), compartmentation for concentrating compounds, and essential gradients (temperature, pH, and ions) (Martin and Russell, 2003, 2007).

Lost City-type vents are also called carbonate chimneys. CO_2 is only detectable to a small extent or not at all because it precipitates as magnesium or calcium carbonate at high pH values (Tivey, 2016). The CO_2 in the 100 bar atmosphere on early Earth right after the Moon-forming impact dissolved into the oceans, making them slightly acidic with a pH of ~6.6, while modern oceans have a pH of 7.8–8.2. (Krissansen-Totton *et al.*, 2018; Schwander *et al.*, 2023). The CO_2 in the early Earth's oceans continued to precipitate as carbonates, which then entered the crust and mantle through subduction, where they were available for geochemical processes (Mrnjavac *et al.*, 2023).

Since the discovery of Lost City, very few vents similar to it have been discovered: Prony Bay in New Caledonia (Monnin *et al.*, 2014), the Shinkai Seep field off the coast of Japan (Okumura *et al.*, 2016), Old City in the Indian Ocean (Lecoeuvre *et al.*, 2021), and more recently the Kunlun hydrothermal field near New Guinea (Li *et al.*, 2025). None of these systems are exactly like Lost City. Prony Bay, for example, is in shallow seawater (16–47 m below the surface) and Kunlun appears, at least from initial observations, to have hydrothermal pipes rather than carbonate chimneys (Li *et al.*, 2025). These systems are similar in that they have alkaline effluent with low temperature and mostly high H_2 and CH_4 concentrations (Schwander *et al.*, 2023). Since not many Lost City-type vents have been discovered yet and they are difficult to reach, terrestrial serpentinizing sites can serve as analog sites as they also have low temperatures, high pH and large amounts of H_2 and CH_4. Unlike Lost City, they are fed by freshwater and are not submerged, so they are often exposed to sunlight. Serpentinizing white and black smokers are even more different from Lost City: Lower pH (3–4), higher temperatures, and higher concentrations of metals and CO_2 in the effluent. Broadly speaking, three types of serpentinizing systems can be distinguished (Schwander *et al.*, 2023):

i. Black and white smokers have high temperatures (200 °C – >400 °C), high efflux of H_2, CH_4 and CO_2 and acidic pH. These conditions are largely considered unfavorable for life.
ii. Terrestrial sites such as ophiolites, which are obducted ultramafic oceanic crust, orogenic peridotite massifs and igneous intrusions. They are alkaline, have high H_2 and CH_4 outgassing and moderate temperatures.
iii. Lost City-type vents are alkaline (pH 9–11), have moderate temperatures (40–116 °C), and have low content of CO_2 but high H_2 and CH_4 content (Etiope and Schoell, 2014).

When comparing different serpentinizing systems, Schwander et al. (2023) showed that the CH_4 concentrations in particular were well documented, especially in terrestrial sites. This focus exists because serpentinizing sites are a source of abiogenic CH_4, but CH_4 is widely discussed as a potential biomarker in the search for extraterrestrial life. Since the serpentinization process can produce it abiotically, it is a problematic biomarker (Etiope, 2017). On the other hand, if the origin of life was dependent on serpentinizing conditions, the presence of CH_4 could serve as an indicator of geochemical processes favoring the origin of life: exergonic reactions from H_2 and CO_2 (Schwander *et al.*, 2023).

Isotope fractionation is the usual method to distinguish between biotic and abiotic methane (Etiope and Sherwood Lollar, 2013; Etiope and Schoell, 2014). Abiotic methane has a higher ^{13}C content than biotic methane because biotic methane prefers ^{12}C in the acetyl-CoA pathway (Blaser *et al.*, 2013). This test is not infallible (Horita and Berndt, 1999), as biotic and abiotic methane can mix, as shown in the Samail ophiolite (Nothaft *et al.*, 2021). Therefore, deuterium fractionation is often used in combination with C-fractionation (Etiope *et al.*, 2011; Miller *et al.*, 2018). Radiocarbon methods (^{14}C) can also be used to determine the carbon source in CH_4 in serpentinizing systems. Lost City, for example, is “radiocarbon dead”, i.e. the carbon source cannot come from seawater, so the CO_2 source for the methane in Lost City most likely comes from the Earth's mantle (Proskurowski *et al.*, 2008). Another indicator to look for in serpentinizing sites is olivine, a type of ultramafic rock in which serpentinization can easily take place as soon as water is present. It is the most abundant silicate mineral in the solar system. Therefore, it is probably ubiquitous in all rocky bodies in the solar system (McCollom and Seewald, 2013).

Given these indicators, there are two other bodies in the solar system where the evidence is very promising that serpentinization once occurred or even still occurs: Mars and a moon of Saturn, Enceladus (Oze and Sharma, 2005; Glein *et al.*, 2015). Serpentinization most likely occurred on Mars in the past, when liquid water still existed. The olivine on Mars has an even higher iron content than olivine on Earth (Hoefen *et al.*, 2003; Christensen *et al.*, 2004; Ehlmann *et al.*, 2010). In addition, organic synthesis has been detected in Martian meteorites dating back 4 Ga, which could have been caused by serpentinization (McCollom and Seewald, 2013; Steele *et al.*, 2022). The data from the Cassini probe strongly suggests that serpentinization on Enceladus continues to this day. The ocean of Enceladus is also strongly alkaline with a pH of 11 (Zolotov, 2007; Glein *et al.*, 2015). H_2, CH_4 and CO_2 as well as organic substances were detected in the plumes of Enceladus (Glein *et al.*, 2015; Waite *et al.*, 2017). Serpentinization most likely also occurs on other icy moons such as Enceladus, where the rocky core is in contact with the global oceans that lie beneath thick ice crusts. However, no direct measurements have yet been made for other icy moons. There are only theoretical models, for example for Jupiter's moon Europa (Vance *et al.*, 2007, 2016).

One aspect that all serpentinizing systems have in common is the existence of significant concentrations of molecular hydrogen and methane (Schwander *et al.*, 2023). In ophiolites these compounds were sometimes not measured, because the studies focused on something else. There is a difference between the measurement methods in hydrothermal fields on the seafloor and ophiolites on continental surfaces. In hydrothermal fields, the effluent is investigated, but the water content inside the vents is rarely measured. In contrast, the water content in ophiolite springs is usually observed. In the few cases where ophiolite outgassing was measured, hydrogen and methane concentrations were substantial (Schwander *et al.*, 2023).

Microbial communities vary between serpentinizing sites, but microbes, methanogens (Archaea) and acetogens (Bacteria) that use H_2 as a major energy source and the Wood-Ljungdahl pathway for CO_2 fixation are abundant at many sites such as Lost City, Prony Bay and even at hyperalkaline sites that do not serpentinize, such as the Strytan site in Iceland (Fones *et al.*, 2021; Frouin *et al.*, 2022; Twing *et al.*, 2022; Quéméneur *et al.*, 2023). Formate reducers are also abundant and often even the primary producers in several serpentinizing systems (Lost City, Oman ophiolite, Cedars, Hakuba Happo, Prony Bay) (Schwander *et al.*, 2023) because in alkaline sources CO_2 is often precipitated as carbonate (Palandri and Reed, 2004) and formate is the most abundant carbon species available for metabolism and produced abiotically (Lang *et al.*, 2018). Frouin *et al.* (2022) compared the microbial communities in serpentinizing systems and found that they are inhabited by diverse microbial groups, with acetogens (Firmicutes) and methanogens being particularly abundant. For example, in Prony Bay and the Oman ophiolite the acetogenic phylum Candidatus Bipolaricaulota dominates (Colman *et al.*, 2022; Frouin *et al.*, 2022). The abundance of the phylum Firmicutes (also called Bacillota) correlates with the dissolved H_2 present (Quéméneur *et al.*, 2023). Enzymes for the acetyl-CoA pathway are ubiquitous in serpentinizing systems (Frouin *et al.*, 2022; Schwander *et al.*, 2023).

Considering all this, serpentinizing systems are widespread on Earth and possibly even on extraterrestrial bodies and are environments in which a large variety of organisms can thrive. But how well do the geochemical environments they provide match biochemistry?

6. Metabolism first and serpentinizing conditions in the lab

Metabolism-first theories and serpentinizing hydrothermal vents as a place where the origin of microbes may have occurred have received much support in recent years from laboratory results. The backbone of microbial metabolism in acetogens and methanogens, the acetyl-CoA pathway, can be simulated abiotically under serpentinizing conditions, where CO_2 reacts with H_2 via metal catalysts to form acetate, formate and pyruvate (Varma *et al.*, 2018; Preiner *et al.*, 2020; Beyazay *et al.*, 2023a, 2023b; Belthle *et al.*, 2024). A source of nitrogen is also essential for the origin of life as the basis for amino acids and nucleobases (Schwander *et al.*, 2023). While ammonia has been detected in serpentinizing systems (Rempfert *et al.*, 2017), abiotic ammonia synthesis has not yet been observed in any of these systems. In experiments simulating serpentinizing conditions, ammonia could be synthesized from N_2 with H_2 as the reductant and peridotite rock as the catalyst at 300 °C and 50 bar N_2, with the presence of CO_2 accelerating ammonia formation (Shang *et al.*, 2023). Although abiogenic ammonia has not yet been detected in serpentinizing systems, abiogenic amino acids such as glycine have been reported in Hakuba Happo (Nobu *et al.*, 2023) and in the Atlantis Massif (Ménez *et al.*, 2018), which harbors many serpentinizing systems (Schwander *et al.*, 2023). It has also been synthesized in the laboratory under simulated serpentinizing conditions (Mayer *et al.*, 2021; Mayer and Moran, 2022; Dherbassy *et al.*, 2023; Kaur *et al.*, 2024; Schlikker *et al.*, 2025) and traces of glycine have been detected in Hakuba Happo (Nobu *et al.*, 2023). The synthesis of amino acids is easy under serpentinizing conditions when nickel is used as a catalyst (Kaur *et al.*, 2024), especially when the cofactor pyridoxal is involved, which increases the yield of alanine by 4.4-fold (Schlikker *et al.*, 2025).

Schlikker *et al.* (2025) investigated how the cofactor pyridoxal affects the H_2-dependent amination of pyruvate to alanine and whether Ni^0 can catalyze this reaction under serpentinizing conditions (Kelley *et al.*, 2001, 2005; Lang and Brazelton, 2020). They also examined whether pyridoxal is reductively aminated to pyridoxamine under these conditions, thereby enabling it to serve as an active amino-donor in transamination reactions for amino acid synthesis. It was found that pyridoxal "favors" the synthesis of pyruvate to alanine with a nitrogen source, because without pyridoxal the reduction of pyruvate to lactate is preferred (Schlikker *et al.*, 2025). This only occurs at higher temperatures of 80–100 °C. At 40 °C pyridoxal even appears to inhibit alanine accumulation (Schlikker *et al.*, 2025).

Another important compound that had to be present at the origin of life is phosphate, but the low bioavailability of phosphate has always been a problem for the origin of life. The reduction of phosphate to the more soluble and biologically available phosphite has also been demonstrated under simulated serpentinizing conditions, although the redox potential of phosphite and phosphate becomes more negative at higher pH values (Pasek *et al.*, 2013, 2022). Other genes for the oxidation of phosphite were found in serpentinizing systems (Frouin *et al.*, 2022).

In addition to the basic compounds essential for carbon, amino acid and phosphate metabolism, other compounds react similarly under serpentinizing conditions to how they do in biochemical systems. Ferredoxin is an ancient redox carrier in cells and acts as an electron donor. Under serpentinizing conditions in alkaline vents, ferredoxin can be reduced with metallic iron and H_2 without enzymes (Brabender *et al.*, 2024). In addition, the cofactor NAD^+ can be reduced to NADH with H_2 via metal catalysts under serpentinizing conditions (Henriques Pereira *et al.*, 2022). Therefore, except for nucleobases, all other important components that had to be present at the origin of life can be synthesized in the laboratory under serpentinizing conditions (Martin *et al.*, 2016; Wimmer *et al.*, 2021a). The chemical backbone provided by serpentinization opens the door from simple, disconnected chemical reactions to a networked primordial metabolism in the last universal common ancestor, and at its core is an ancient biochemical pathway: the acetyl-CoA pathway.

7. The acetyl-CoA pathway

Carbon, energy, and electrons are key components that are essential for all life. Only one pathway combines all three: the acetyl-CoA pathway, also called the Wood-Ljungdahl pathway. It is the oldest CO_2 fixation pathway (Fuchs and Stupperich, 1985, 1986; Berg, 2011; Fuchs, 2011). In this pathway, electrons from H_2 reduce CO_2 to pyruvate for carbon supply. Furthermore, methane synthesis in methanogens and acetate synthesis in acetogens are both coupled to energy conservation by ATP (Thauer *et al.*, 2008; Schoelmerich and Müller, 2019; Mrnjavac *et al.*, 2024b). Of the seven known CO_2 fixation pathways (Fuchs, 2011; Sánchez-Andrea *et al.*, 2020), the acetyl-CoA pathway is the only CO_2 fixation pathway that is exergonic and does not require a net ATP input (Fuchs, 1994, 2011), the only one that combines carbon and energy metabolism (CO_2 reduction and ATP synthesis) in the same pathway, and also the only one that occurs in both Archaea and Bacteria (Martin and Russell, 2007; Berg, 2011). The oldest archaeal and bacterial lineages, methanogens and acetogens, use the acetyl-CoA pathway (Weiss *et al.*, 2016). Most other autotrophs have separate pathways for carbon and energy metabolism (Fuchs and Stupperich, 1985; Fuchs, 2011; Martin, 2020).

The acetyl-CoA pathway comprises three major synthesis steps: methyl, acetyl and pyruvate synthesis. So far, this is the only CO_2 fixation pathway that can be abiotically replicated in the lab (Martin and Kleinermanns, 2024). The enzymes for the acetyl-CoA pathway are ubiquitous in serpentinizing systems (Frouin *et al.*, 2022; Schwander *et al.*, 2023). Formate and pyruvate are intermediates of the acetyl-CoA pathway in acetogens and methanogens, with pyruvate being the major carbon source for biosynthesis in both. Acetate is the end-product of the acetyl-CoA pathway in acetogens, and methane is the end-product in methanogens (Martin and Russell, 2007). Archaea and Bacteria use different enzymes for the acetyl-CoA pathway, but the underlying chemistry is the same, a strong indication that the chemistry came before the enzymes for this pathway. Furthermore, ancestral Archaea likely had hydrogen-dependent methanogenesis (Williams *et al.*, 2017; Mei *et al.*, 2023), while the Bacteria had hydrogen-dependent acetogenesis (Xavier *et al.*, 2020). Genetic manipulation could convert a methanogen to an acetogen, suggesting a physiological link between the two (Schöne *et al.*, 2022).

In the laboratory, it has been shown that iron (Fe), cobalt (Co) and nickel (Ni) and their alloys (e.g. magnetite (Fe_3O_4) or awaruite (Ni_3Fe)) can replace 127 enzymes required for the acetyl-CoA pathway to reduce H_2 to CO_2. Approximately 20 of these enzymes are required for the acetyl-CoA pathway and more than 100 enzymes are needed for the synthesis of cofactors

(Mrnjavac *et al.*, 2023, Mrnjavac et al., 2024b). The acetyl-CoA pathway has almost all the universal cofactors that were likely present in LUCA, except for pyridoxal phosphate, which is required for transaminations, and biotin, which is needed for carboxylations (Mrnjavac *et al.*, 2024b). This suggests that the acetyl-CoA pathway is a biochemical fossil linking acetogens and methanogens, where the combination of carbon and energy metabolism is unique to them (Martin, 2020; Schöne *et al.*, 2022).

In the evolution of microbes, CO_2 was the most important electron acceptor because the synthesis of organic compounds, which the acetyl-CoA pathway produces with the simultaneous release of energy, was required to initiate the metabolic pathways. Many organisms today use nitrogen and sulfur compounds as terminal electron acceptors. However, they require cytochromes and quinones, which methanogens and acetogens lack. Therefore, some methanogens and acetogens have a simpler system in which they use CO_2 as a terminal acceptor (Schwander *et al.*, 2023). Chemolithoautotrophic prokaryotes which lack cytochromes obtain their ATP from ion gradients that they generate during the exergonic reduction of CO_2 with electrons from H_2 using the Mtr complex in methanogens (Thauer *et al.*, 2008) and the Rnf complex in acetogens (Müller *et al.*, 2018). Both acetogens and some methanogens can synthesize ATP via the acetyl-CoA pathway by substrate-level phosphorylation with acetyl-phosphate (Schöne *et al.*, 2022).

The coupled carbon and energy metabolism of the acetyl-CoA pathway probably preceded a differentiation into a dedicated carbon and energy metabolism that uses cytochromes, nitrogen and sulfur compounds as terminal acceptors (Schwander *et al.*, 2023). Cytochromes and quinones provide free-living microbes with access to electron donor-acceptor pairs that can generate ion gradients with ion pumps (Martin and Kleinermanns, 2024). In acetogens and methanogens that use H_2 as an electron donor, flavin-based electron bifurcation is needed because the midpoint potential of H_2 (–414mV) is insufficient to reduce ferredoxin (–450mV) or CO_2 (–430mV) under standard conditions (25 °C, pH 7, 1 bar) (Brabender *et al.*, 2024; Mrnjavac *et al.*, 2024b). This process provides the necessary reduced ferredoxin, an ancient protein with iron-sulfur clusters (4Fe4S). This means H_2 must flow energetically uphill under standard conditions. Flavin-based electron bifurcation avoids this problem by splitting the electron pair of H_2 and sending one electron energetically uphill to ferredoxin and the other downhill to a more energetically favorable acceptor such as NAD^+ (Mrnjavac *et al.*, 2024b). In this way, the overall reaction of H_2-dependent CO_2 reduction in the acetyl-CoA pathway in acetogens and methanogens is exergonic (Buckel and Thauer, 2018). Before enzymes existed, electron bifurcation was not necessary. H_2 readily reduced CO_2 to formate, acetate, pyruvate and methane if catalysts made of native Fe, Co or Ni or their alloys were present at an alkaline pH, as found in low-temperature serpentinizing vents (Schwander *et al.*, 2023). H_2 can also reduce 4Fe4S clusters of ferredoxin in the presence of native iron (Brabender *et al.*, 2024).

Since serpentinizing systems do all this without enzymes and metabolic pathways, why have they been replaced by over 100 different enzymes? The acetyl-CoA pathway emerged despite the efficiency of single metal catalysts because, as metabolism evolved from solid-state metal catalysts to enzymes with complex cofactors, its exergonic reactions had to be broken down into a series of linked reactions for specificity and rate control. To achieve this, more complex catalysts (enzymes) were needed (Martin and Kleinermanns, 2024). The metal clusters in the active sites of the enzymes of the acetyl-CoA pathway today are likely relics of a protometabolism catalyzed by metals in serpentinizing vents (Mrnjavac *et al.*, 2024b). This shows that serpentinizing environments have accompanied the process of the origin of life from its beginning and remain thriving ecosystems to this day. A wide variety of organisms thrive on the products of these environments, with the oldest bacterial and archaeal lineages forming the backbone of these ecosystems (Martin, 2020). Therefore, it stands to reason that serpentinization also formed the backbone of metabolism for LUCA and its later "descendants", the last archaeal common ancestor (LACA) and the last bacterial common ancestor (LBCA). The transition from these simple compounds and reactions to something as complex as LUCA, LBCA, and LACA (not to mention free-living cells) is a complex process, but serpentinization provided all the necessary components to support it (Weiss *et al.*, 2016; Schwander *et al.*, 2023).

8. From serpentinization to LUCA, LBCA and LACA

To think of the origin of life as a collection of reactions, with life at its core being an exergonic chemical reaction (Martin, 2011), makes it seem easy to move from chemical reactions occurring in serpentinizing systems to LUCA and then to the first free-living cell. But life is very complex. There is a huge gap between some simple reactions and compounds and this level of complexity. Many theories exist about the nature of LUCA. There is the case of a bacterial root from which Archaea later branched off (Cavalier-Smith, 2006). The reverse case with an archaeal root and Bacteria branching off later (Caetano-Anollés *et al.*, 2014). Then, there was the suggestion that LUCA was very complex, i.e., that there were different types of lipids that only differentiated when Bacteria and Archaea branched off (Wächtershäuser, 2003; Mrnjavac *et al.*, 2026). Given the previously discussed serpentinizing conditions and hydrothermal environments, it is more likely that LUCA was much simpler than modern organisms. Rather than being truly alive, it existed in a semi-living intermediate state as a chemolithoautotroph, living off geochemical reactions provided by serpentinization (Weiss *et al.*, 2016, 2018). LUCA, LBCA, and LACA, and later the first free-living cells, must have been chemolithoautotrophs, deriving their energy from chemical reactions. In most cases, the energy is released through a series of electron transfer reactions (redox reactions). In redox reactions, electrons move from an electron donor (reductant) to an electron acceptor (oxidant) (Martin and Kleinermanns, 2024). In the past, glycolysis was thought to be the first pathway of carbon metabolism (Degani and Halmann, 1967). Therefore, LUCA would have been a heterotroph. But the early Earth lacked substrates from which microbes could have derived energy and carbon for heterotrophic growth (Schönheit *et al.*, 2016).

LUCA already had a lot of the properties that modern cells have, but many of them were not yet complete. For example, the enzymes for DNA replication are not homologous in all living organisms, suggesting that LUCA could not replicate DNA the way modern cells do (Mrnjavac *et al.*, 2026). Wimmer *et al.* (2021a) identified around 400 core reactions required for the synthesis of nucleotides, amino acids and cofactors. Not all organisms on Earth use these 400 core reactions, because sometimes they obtain the required compounds from the environment, which was probably the case with LUCA (Mrnjavac *et al.*, 2026). These 400 reactions are far from what free-living cells need. Metabolism in prokaryotes today consists of at least 1000 catalyzed and interconnected reactions (Sousa *et al.*, 2013). The smallest genome size of H_2-dependent chemolithoautotrophs today is 1500 genes (Martin and Kleinermanns, 2024). The last universal common ancestor did not yet require 1000 reactions (Weiss *et al.*, 2016; 2018).

The 400 reactions of the autotrophic core (or metabolic core) reactions most likely already existed in LUCA, LBCA, and LACA (Wimmer *et al.*, 2021a). These reactions start from H_2, CO_2, NH_3, hydrogen sulfide (H_2S), inorganic phosphate (P(i)), H_2O and salt, and the reactions in this metabolic core synthesize the 20 canonical amino acids, the eight nucleobases of DNA and RNA (without modifications) and the 18 main cofactors used by modern cells. These reactions are 97% exergonic under the reducing conditions of serpentinizing hydrothermal vents (Wimmer *et al.*, 2021b). The remaining 3% are endergonic and involve the participation of a phosphoryl donor (Wimmer *et al.*, 2021b; Mrnjavac *et al.*, 2023). When polarizing in the biosynthetic direction, most reactions in the metabolic core are exergonic. Since most of the 400 core reactions are exergonic it stands to reason that the first reactions at the origin of life also had to be exergonic, otherwise the reactions would not have gone forward to form the first cells (Mrnjavac *et al.*, 2024b). It is important to remember that enzymes merely accelerate reactions that occur anyway, a further indication that the reactions at the origin of life are older than enzymes (Mrnjavac *et al.*, 2024b). This autotrophic core comprises only monomeric components. The synthesis of ribosomes, tRNA modifications, and the handling of nucleic acids are not included (Wimmer *et al.*, 2021a, 2021b; Mrnjavac *et al.*, 2024b).

So how could all these reactions and components be combined to create even a halfway living cell? Autocatalytic networks have been proposed as systems to fill this gap. They are systems of self-organization and likely the link between these initial reactions and LUCA (Xavier *et al.*, 2020). Autocatalytic networks, called RAFs (reflexively autocatalytic food-generated networks), are networks in which the products within the network can also act as catalysts, accelerating reactions and generating more products and subsequently reactions (Hordijk and Steel, 2017). RAFs can be detected in the metabolic maps of modern microbes (Sousa *et al.*, 2015). Interestingly, in the RAF of LUCA, nucleobases arise from metabolic networks and not the other way around, again consistent with the view that metabolism comes first and not the RNA world. This RAF of LUCA also did not generate all amino acids or cofactors (Xavier *et al.*, 2020). If the RAFs closed the gap between the geochemical origins and LUCA, what else had to happen for LUCA to become free-living? DNA had to be developed to serve as a genetic storage medium. LUCA probably already had DNA, but the genome had to be large enough to perform all the syntheses that a free-living cell needs, which was probably not the case with LUCA. In addition, lipids and cell walls had to have been developed so that the reactions could be isolated from the metal catalysts (Martin and Kleinermanns, 2024).

Archaea and Bacteria differ in their cell membrane lipids. Bacteria have fatty acid esters in their cell membranes, Archaea have isoprene ethers (Koga *et al.*, 1998). LUCA probably used hydrophobic compounds as lipids provided by the environment (Martin and Russell, 2003). What else distinguishes LBCA and LACA from LUCA? The best-studied example is the ribosomal proteins. There are 33 ribosomal proteins that are shared by Archaea and Bacteria, but there are 21 Bacteria-specific and 30 Archaea-specific ribosomal proteins (Fox, 2010). The most strongly conserved part is a 70-nucleotide long section containing the peptidyl-transferase site, called the protoribosome, which makes up only about 1% of the mass of modern ribosomes but undoubtedly has a crucial function. It makes the peptide bonds during translation and can bind aminoacyl-tRNAs as well as minihelices, which are 7–11 nucleotide long RNA molecules that may be the precursors of tRNA (Yonath, 2009; Bose *et al.*, 2022). Other examples are the ion pumps Mtr and Rnf. Mtr is a methyltransferase and an archaeal invention. Rnf is a NADH:ferredoxin oxidoreductase and a bacterial invention. They both fulfill analogous functions in bacteria and archaea (Thauer *et al.*, 2008; Schuchmann and Müller, 2014; Martin and Sousa, 2016).

This indicates increasing complexity from LUCA to free-living cells. Apart from the above examples, the metal catalysts that trigger these reactions also had to be incorporated in proteins within cells. In addition, the energy from a geochemical ion gradient had to have been converted into chemical energy via ATP-synthase and the natural ion gradient between the alkaline vent and the acidic ocean must have been replaced by ion pumps so that the cell could become energy self-sufficient (Martin and Kleinermanns, 2024).

9. Aim of the thesis

The aim of the present thesis is to obtain a more comprehensive picture of the early chemical reactions that led to the origin of microbial cells, through bioinformatic investigations, literature studies and experimental work. These investigations are presented in the publications in the following chapters.

- Publication 1 compares different serpentinizing sites on Earth. It shows why these sites might have been the ideal environment for the origin of microbial cells, with particular attention to their H_2, CH_4, and CO_2 concentrations and to the microbial communities that live there today.
- Publication 2 presents the Moon-forming impact as a key event in Earth's history. It explains why life on Earth might never have arisen without this event.
- Publication 3 deals with the chemical antiquity of the acetyl-CoA pathway. It presents how the underlying biochemistry of this pathway aligns with the geochemistry of serpentinizing hydrothermal vents and with an earlier reconstruction of the physiology of LUCA.
- Publication 4 explains the nature of LUCA. It compares the various hypotheses surrounding LUCA and explains why it was most likely a chemolithoautotroph.
- Publication 5 is an experimental study that replicates serpentinizing conditions in the laboratory. It shows how serpentinization creates a favorable environment for transamination reactions with the co-factor pyridoxal, converting pyruvate to alanine.

10. Publications

10.1. Publication 1

Title: Serpentinization as the source of energy, electrons, organics, catalysts, nutrients and pH gradients for the origin of LUCA and life

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Serpentinization as the source of energy, electrons, organics, catalysts, nutrients and pH gradients for the origin of LUCA and life

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Serpentinization in hydrothermal vents is central to some autotrophic theories for the origin of life because it generates compartments, reductants, catalysts and gradients. During the process of serpentinization, water circulates through hydrothermal systems in the crust where it oxidizes Fe (II) in ultramafic minerals to generate Fe (III) minerals and H_2. Molecular hydrogen can, in turn, serve as a freely diffusible source of electrons for the reduction of CO_2 to organic compounds, provided that suitable catalysts are present. Using catalysts that are naturally synthesized in hydrothermal vents during serpentinization H_2 reduces CO_2 to formate, acetate, pyruvate, and methane. These compounds represent the backbone of microbial carbon and energy metabolism in acetogens and methanogens, strictly anaerobic chemolithoautotrophs that use the acetyl-CoA pathway of CO_2 fixation and that inhabit serpentinizing environments today. Serpentinization generates reduced carbon, nitrogen and — as newer findings suggest — reduced phosphorous compounds that were likely conducive to the origins process. In addition, it gives rise to inorganic microcompartments and proton gradients of the right polarity and of sufficient magnitude to support chemiosmotic ATP synthesis by the rotor-stator ATP synthase. This would help to explain why the principle of chemiosmotic energy harnessing is more conserved (older) than the machinery to generate ion gradients via pumping coupled to exergonic chemical reactions, which in the case of acetogens and methanogens involve H_2-dependent CO_2 reduction. Serpentinizing systems exist in terrestrial and deep ocean environments. On the early Earth they were probably more abundant than today. There is evidence that serpentinization once occurred on Mars and is likely still occurring on Saturn's icy moon Enceladus, providing a perspective on serpentinization as a source of reductants, catalysts and chemical disequilibrium for life on other worlds.

KEYWORDS
serpentinization, hydrothermal vents, origin of life, microbial metabolism, astrobiology, hydrogen, methane, Lost City

1. Introduction

The question of how life arose is sometimes called, "the biggest question in science," which is possibly an exaggeration. What is true is that it is perhaps the only scientific question to which everyone would like to know the answer. The origin of living things has concerned humans since antiquity. Modern scientific approaches to the problem build on Pasteur's 19th century demonstration that life cannot be created spontaneously (Bulloch, 1938), except of course, at the origin of life from the elements of the early Earth, and Darwin's inference that we all originate from a single common ancestor (Darwin, 1859). Mereschkowsky et al. (1910) proposed that the first organisms on Earth were probably thermophilic chemolithoautotrophs, a concept that — for good reasons — is still current among microbiologists, while Oparin and Haldane proposed a theory involving a primordial soup (Haldane, 1929; Oparin, 1957) of the type that Miller synthesized in the famous 1953 experiment powered by electric discharge (Miller, 1953).

Oparin and Haldane as well as Miller worked under the assumption that the early Earth had a reducing atmosphere. More recent studies indicate that early Earth's mantle was oxidized, which in turn released oxidized compounds such as CO_2, N_2 and H_2O into the atmosphere such that the early Earth's atmosphere must have also been oxidizing (Sossi et al., 2020). However, reducing conditions are needed for the origin of life because, despite the coexistence of contradictory theories on origins, they all tend to concur that the carbon source for the first complex molecules was ultimately CO_2, which needs reducing conditions to react to more complex organic molecules. There are currently two general categories of solutions afloat in the literature as to where to obtain reductants at origins: the surface (the atmosphere) and the subsurface (the crust via serpentinization).

In surface reduction models, additional impactors subsequent to the Moon-forming impact are evoked as a source of native metals at the surface. These additional impactors generate (theoretical) transient phases of reducing conditions in the atmosphere, phases that are argued to reduce CO_2 and N_2 to cyanide that is, in turn, central to some models for the origin of life (Benner et al., 2020; Zahnle et al., 2020; Itcovitz et al., 2022). Notably, the additional impactors are not required to explain the composition of the early Earth or the early atmosphere, they are solely "required" as a source of transient supplies of gas phase ammonia, cyanide and nitriles as starting materials for the laboratory-style organic synthesis of RNA bases, under the dual assumption that i) RNA arose from cyanide and nitriles on the Earth's surface and that ii) something like an RNA world ever existed (Benner et al., 1989; Zahnle et al., 2007; Righter et al., 2008; Sleep, 2016; Sasselov et al., 2020; Grewal et al., 2023). Surface reduction models do not interface well with microbial metabolism, because there are neither cyanides nor nitriles in the biosynthetic pathways to bases, amino acids and cofactors and no microbe is known to require cyanide as a growth substrate. Geochemical sources of cyanide are not known, not even from volcanic exhalates (Rose et al., 2006).

In subsurface reduction models, the source of reductant for origins is geochemical and continuous: H_2 supplied by serpentinization. The electrons released by serpentinization reduce H_2O to H_2 which in turn reduces CO_2 to CH_4 and organics (Sleep et al., 2004; Martin et al., 2008; Sleep et al., 2011; McCollom and Seewald, 2013). The diffusible reductant, H_2, is generated within the crust, where suitable catalysts for CO_2 reduction such as awaruite (Ni_3Fe) and magnetite (Fe_3O_4) are synthesized (Preiner et al., 2018). If life originated in serpentinizing systems, it would not have needed anything from the Earth's early atmosphere except N_2 and CO_2, because serpentinizing systems create the reducing conditions essential for the origin of life (Russell et al., 2010). The initial products of H_2 dependent CO_2 reduction under serpentinizing conditions in the laboratory are formate, acetate, pyruvate and methane, which comprise the backbone of microbial carbon and energy metabolism in organisms that use the acetyl-CoA pathway (Preiner et al., 2020). Subsurface reduction models have good congruence with microbial carbon and energy metabolism. Nucleic acid synthesis that might underpin an RNA world is more challenging under hydrothermal vent conditions (Muchowska et al., 2020; Yi et al., 2022; Harrison et al., 2023) but at the same time, there is no clear evidence that an RNA world ever existed (Baross and Martin, 2015) and there are no self-replicating RNA molecules in any life form as far as we know. Here we will focus on subsurface reduction as it occurs in modern serpentinizing systems as a starting point for origins.

Isotope evidence suggests that the first forms of life existed at least 3.8 billion years ago because carbon with an isotope signature lighter than that of abiogenic reduced carbon appears in sediments of that age (Mojzsis et al., 1996). Such light isotope signatures ($\delta^{13}C$) in the range of –40‰ to –80‰ are generally interpreted to indicate the presence of methanogens (archaea) (Arndt and Nisbet, 2012), but acetogens (bacteria) have a similarly light isotopic signature (Blaser et al., 2013). Ultralight isotopes indicate the presence of the acetyl-CoA pathway of CO_2 fixation in primordial bacteria and archaea (Tashiro et al., 2017), in line with its exergonic nature (Berg, 2011), ancient physiology (Rühlemann et al., 1985; Fuchs, 2011), abundance of metal cofactors (Martin and Russell, 2003; Ragsdale, 2006) and carbon-metal bonds (Martin, 2020), its dual role as a pathway of carbon and energy metabolism in acetogens and methanogens (Martin and Russell, 2007) and in line with metabolic and phylogenetic reconstructions of LUCA (Weiss et al., 2016).

Methanogens and acetogens are chemolithoautotrophs and therefore grow anywhere where sufficient H_2 and CO_2 exists and where temperatures are biocompatible (Thauer et al., 2008; Schuchmann and Müller, 2014), from rumen to termite guts to anaerobic environments such as hydrothermal vents on the sea floor. Deep-sea hydrothermal vents were first discovered in 1977 near the Galapagos islands (Corliss et al., 1979) and since then they have been of interest for theories on the origin of life (Corliss et al., 1981; Baross and Hoffman, 1985). Criticism of vents as sites for the origin of life arose very quickly, however, because of the high temperature of black smokers (*ca.* 400°C) (Bada and Lazcano, 2002): the upper limit for life is currently 122°C (Takai et al., 2008). This is one reason why it was proposed that if life originated at a hydrothermal vent, it must have happened at a low-temperature, alkaline hydrothermal vent that undergoes serpentinization (Russell et al., 1994, 1997), a proposal that predated the discovery of the Lost City hydrothermal field (Kelley et al., 2001, 2005; Proskurowski et al., 2008) which contains multiple serpentinizing deep-sea alkaline hydrothermal vents. The Lost City hydrothermal field offers a window in time for the study of the origin of life (Martin and Russell, 2007) and fosters ideas for laboratory experiments that simulate the reducing and catalyst rich conditions of hydrothermal vents (McCollom, 2013; McCollom and Seewald, 2013;

Schrenk et al., 2013; Möller et al., 2017; Muchowska et al., 2017; Boyd et al., 2020; Preiner et al., 2020).

Serpentinization releases energy, generates reductants, and provides small organic compounds that directly interface with microbial metabolism. It occurs both in terrestrial systems (continental, on land) and in submarine systems on the sea floor, usually close to the borders of tectonic plates (Schrenk et al., 2013; Wang et al., 2014; Preiner et al., 2018). Continental serpentinizing systems, for example those hosted by ophiolites, are a valuable source of information about the process, as deep-sea hydrothermal vents are much harder to access and few Lost City type systems have been discovered so far (Lecoeuvre et al., 2021). Even more remote are, of course, environments outside Earth, but they are relevant in an astrobiology context, where serpentinization has come into focus as a source of energy and reductant. There is accumulating evidence to suggest that serpentinization has occurred on Mars and might still be occurring on Mars and Enceladus (a moon of Saturn). It is suspected to be an important process on other icy moons of the gas giants in our solar system, especially where their oceans have contact with the rocky core, such as Europa and Titan (Schulte et al., 2006; Zolotov, 2007; Ehlmann et al., 2010; Glein et al., 2015; Vance et al., 2016; Waite et al., 2017; Johnson et al., 2019; Steele et al., 2022). It is useful to collate and compare data from different serpentinizing systems, which will be shown in section 3.

2. The importance of gradients and catalysts

When the effluent of submarine hydrothermal vents interfaces with seawater at the ocean floor, two fluids with different physicochemical properties continuously mix, generating far from equilibrium conditions over the lifespan of the vent, which in the case of Lost City can be over 30,000 years (Früh-Green et al., 2003). Some very hot hydrothermal fields can also reach ages in excess of 30,000 years (Kuznetsov et al., 2006) but this does not necessarily describe the age of individual active chimneys. The continuous far from equilibrium state of serpentinizing systems produces pH-, temperature- and redox-gradients, which create a steady supply of chemical energy sources that are similar to those used by modern microbes to run metabolic reactions and synthesize ATP. The gradients at hydrothermal vents were recognized early on as harboring similarity to energy releasing processes of cells, providing links between chemical processes in the early Earth and early forms of life and hence conducive to origin of life processes (Corliss et al., 1981; Baross and Hoffman, 1985; Holm, 1992; Russell and Hall, 1997). Gradients are interesting in an origins context because all living cells generate gradients during life and growth.

Temperature gradients at hydrothermal vents have been extensively studied by Braun and coworkers in the context of thermophoresis, a physicochemical process that leads to chemical concentration gradients in physically confined compartments (Baaske et al., 2007; Möller et al., 2017; Hudson et al., 2020; Matreux et al., 2023). Thermophoresis can lead to orders of magnitude accumulation of organic compounds in compartments on scales the size of µm to cm. All theories for origins require sufficient concentrations of reactants in order to react at a significant rate, generating enough products to react further and attain higher complexity. Naturally occurring networks of inorganic microcompartments that form at the vent-ocean interface of serpentinizing hydrothermal systems can serve as sites of natural chemical concentration processes (Russell and Hall, 1997; Martin and Russell, 2003) thermophoresis amplifies that effect by orders of magnitude (Baaske et al., 2007).

The pH gradients of serpentinizing systems are important in an origins context for two reasons. First, the alkaline nature of effluent in serpentinizing systems, in the range of pH 9–11, stemming from continuous Mg $(OH)_2$ synthesis during the serpentinization process (Chavagnac et al., 2013), together with high H_2 concentrations of 1–10 mM or more, generates extremely negative redox potentials, on the order of −435 to −830 mV which where both measured and calculated at and for serpentinizing systems (Suzuki et al., 2017; Boyd et al., 2020; Nobu et al., 2023; Quéméneur et al., 2023). Provided that enough catalysts are present (Preiner et al., 2018) these conditions are sufficient to abiotically reduce CO_2 (or solid phase inorganic carbon) to formate, with the result that formate of abiotic origin is a very common solute in the effluent of serpentinizing systems (Lang et al., 2010, 2018), continuously present in micromolar concentrations. Formate can serve as a substrate for growth of methanogens (Dolfing et al., 2008; Stams and Plugge, 2009) and acetogens (Moon et al., 2021), microbial groups that inhabit serpentinizing systems (Fones et al., 2021; Kraus et al., 2021; Lecoeuvre et al., 2021; Brazelton et al., 2022; Colman et al., 2022). The synthesis of formate in high mM amounts from H_2 and CO_2 is readily catalyzed by minerals such as awaruite (Ni_3Fe), magnetite (Fe_3O_4) and greigite (Fe_3S_4) under hydrothermal conditions in the laboratory (Preiner et al., 2020).

The second role of pH gradients in serpentinizing systems is that they generate a continuous and natural chemiosmotic gradient with the same polarity as cells. Modern oceans have a pH of 7.8–8.2 [...] with a calculated pH of 6.6 because of higher CO_2 concentrations (Schrenk et al., 2013; Krissansen-Totton et al., 2018). At the vent-ocean interface, this generated a natural proton gradient of roughly three pH units, a far greater gradient than the roughly one pH unit or less that modern cells require to power their rotor-stator ATP synthetase (Tran and Unden, 1998; Silverstein, 2014). Geochemically formed pH gradients could have been harnessed by cells before the origin of proton pumping proteins, which would explain why the ATP synthase and the principle of chemiosmotic energy conservation is universally conserved across all prokaryotic cells while the proteins that generate ion gradients are not (Martin and Russell, 2007; Lane et al., 2010; Lane and Martin, 2010).

It has been suggested that pH gradients might themselves be a source of chemical energy for CO_2 reduction (Sojo et al., 2019; Hudson et al., 2020). Hudson et al. (2020) reported *ca.* 1 µM formate in the presence of gradients, Sojo et al. (2019) obtained no gradient dependent CO_2 reduction. A number of recent studies of H_2-dependent CO_2 fixation under simulated hydrothermal vent conditions clearly show that no gradient or compartmentation is required for CO_2 reduction. Preiner et al. (2020) obtained 0.3 M formate, and up to 560 µM acetate and 10 µM pyruvate (C3 synthesis) from H_2 and CO_2 using alkaline vent conditions in free solution with no gradients or compartments. The key to organic synthesis in serpentinizing systems are not gradients but catalysts, for example hydrothermally formed minerals like awaruite, magnetite and greigite in the case of Preiner et al. (2020), or Ni or Fe metals (Muchowska et al., 2017). Recent work by the group of Harun Tüysüz in Mülheim has characterized in considerable detail the properties that influence the efficiency of CO_2 reduction reactions with nanoparticular and silicate-supported solid state Fe, Ni and Co catalysts (Belthle et al., 2022; Beyazay et al., 2023a,b). For organic synthesis under simulated hydrothermal vent conditions, the catalytic properties of the solid phase and the right reactants are key, not the existence of gradients *per se*.

3. Serpentinizing systems and their similarities and differences

Serpentinization involves reactions of ultramafic rocks with water. Ultramafic rocks have low silicate (SiO_2) content (<45 %) and have a relatively high magnesium and iron content (the term *mafic* stems from *ma*gnesium and *f*errum rich). Olivine and orthopyroxene are the main minerals of ultramafic rocks (Proskurowski et al., 2008). Serpentinization produces hydrogen gas (H_2), which in Fischer-Tropsch type reactions (FTT) can lead to the production of methane (CH_4) and other hydrocarbons at low temperatures (<150°C) through the reduction of carbon dioxide (CO_2) (Etiope and Sherwood Lollar, 2013; Etiope and Schoell, 2014). The serpentinization reaction of olivine, an iron-magnesium silicate, and water to serpentinite and iron-rich brucite, with further reaction of the latter with silica to serpentinite, magnetite and hydrogen, can be written in several ways. Bach et al. (2006) summarize the reaction as:

$$2Mg_{1.8}Fe_{0.2}SiO_4 + 3H_2O \rightarrow Mg_{2.85}Fe_{0.15}Si_2O_5(OH)_4 + Mg_{0.75}Fe_{0.25}(OH)_2$$

$$57Mg_{0.75}Fe_{0.25}(OH)_2 + 30SiO_2(aq) \rightarrow 15Mg_{2.85}Fe_{0.15}Si_2O_5(OH)_4 + 23H_2O + 4Fe_3O_4 + 4H_2$$

while Chamberlain et al. (1965) summarize the reaction as follows:

$$6Mg_{1.5}Fe_{0.5}SiO_4\,(\text{olivine}) + 7H_2O \rightarrow 3Mg_3Si_2O_5(OH)_4\,(\text{serpentine}) + Fe_3O_4\,(\text{magnetite}) + H_2$$

whereby Sleep et al. (2011) simplify the reaction to the essential H_2-production process:

$$3FeO\,(\text{in rock}) + H_2O\,(\text{liquid}) \rightarrow H_2\,(\text{in solution}) + Fe_3O_4\,(\text{magnetite})$$

The H_2 produced by serpentinization can reduce CO_2 to organic compounds and methane, N_2 to ammonia, and divalent Fe and Ni ions to mineral alloys of native metals. Serpentinization is an abundant source of geological reducing power. Paul Sabatier first discovered the abiotic formation of methane in 1913 by letting CO_2 and H_2 react with metal catalysts. Franz Fischer and Hans Tropsch generated more complex hydrocarbons with CO and H_2 in 1925. Both are labeled Fischer-Tropsch-type (FTT) reactions (Etiope and Schoell, 2014). Large quantities of abiotic gas are generated on Earth by such reactions. As soon as a CO_2 source is available to a serpentinizing system, FTT reactions produce CH_4 by serpentinization (Etiope and Sherwood Lollar, 2013; Etiope and Schoell, 2014). Transition metals including Ni and Fe as well as transition metal minerals including magnetite (Fe_3O_4) and awaruite (Ni_3Fe) catalyze FTT reactions. The conversion of the gas occurs after chemisorption to the metal surface (Sleep et al., 2004; Etiope and Schoell, 2014; Holm et al., 2015; Preiner et al., 2018). Ni, Fe and Cr are the most abundant metals in ultramafic rock. Both serpentinization and FTT reactions are considered to be important for origin of life because they forge C–C bonds from CO_2 and CO as starting materials (Russell et al., 2010). In an astrobiological context, they are suspected to be involved in the production of hydrocarbons on other planets (Etiope and Schoell, 2014).

H_2 is a source of energy and an electron donor for microorganisms in modern serpentinizing systems, and it could have carried out the same role in prebiotic chemistry (Etiope, 2017). The generation of H_2 from serpentinization is itself exergonic and has been taking place on Earth ever since there was water present (Sleep et al., 2004; Preiner et al., 2018). On the early Earth the lithosphere was not yet differentiated and higher mantle temperatures allowed ultramafic lava to erupt on Earth's surface, such that ultramafic rocks were probably much more exposed to the surface oceans than today, meaning that serpentinization was almost certainly much more prevalent on early Earth than now (Sleep et al., 2004, 2011; McCollom and Seewald, 2013; Schrenk et al., 2013). Serpentinization can occur at temperatures lower than <100°C (Holm et al., 2015) and usually generates an alkaline pH, especially at lower temperatures (usually > pH 9) because protons are consumed and free OH^- is released during the process that leads to the production of H_2 (Mottl et al., 2003; Schrenk et al., 2013).

Products of serpentinization generate [...] formate and CH_4 as the main products of CO_2 reduction measured in modern vents. Methane is always present in continental serpentinization sites and usually in considerable amounts. Lang et al. (2010) reported acetate in the effluent of Lost City but suggested that it stemmed from microbial metabolism. Abiotically formed acetate has not been detected in the effluent of serpentinizing hydrothermal systems to our knowledge. A recent report detected 1.2 mM abiotic acetate in host rocks of the Canadian Shield (Sherwood Lollar et al., 2021), although there is no direct evidence of it being synthesized by serpentinization so far. In effluent samples, CH_4 concentrations range from 0.01 to 14 mg/L, while normal water in equilibrium with the atmosphere has a CH_4 concentration of roughly 0.00003 mg/L. Longer-chain hydrocarbons (ethane to pentane) are also abiotically produced in serpentinizing systems, both submarine (Proskurowski et al., 2008) and on the surface (Etiope and Schoell, 2014), however in much lower quantities than methane.

Continental serpentinization sites have been studied since at least the 1960s (Barnes et al., 1967) and some of them, such as Chimera in Turkey, have been known to humans for thousands of years (Etiope, 2015). There are three types of mafic host rocks that support continental serpentinizing systems: ophiolites (ultramafic mantle rocks obducted to the surface), orogenic peridotite massifs and igneous intrusions. Some continental serpentinizing systems have been well-studied with respect to microbiology, gas composition and geology. In studies of gas composition, the isotope compositions of CH_4 are particularly important to determine whether it has a biogenic or abiogenic origin (Etiope and Schoell, 2014). Differentiating the two is difficult because the gases in these systems can be a mix of both abiotic and biotic gas, as for example, in The Cedars spring in California, where abiotic gas is present, but microbes also produce CH_4 through CO_2 reduction because of the abundant H_2 present as their energy source (Nealson, 2005; Morrill et al., 2013; Etiope and Schoell, 2014; Suzuki et al., 2017).

3.1. Hydrothermal vent types

Hydrothermal vents are often divided into two types — black and white smokers — but off ridge systems like Lost City belong to neither category because they do not emit "smoke." Both the smoke of black and white smokers comes from metal sulfide and calcium and barium precipitation, but white smokers vent slower and at slightly lower temperatures (220–330°C) than black smokers, which is why a greater percentage of the metals precipitates in the chimneys and is not vented out causing the white color of the smoke, Lost City type vents have carbonate chimneys but no smoke (Rona et al., 1986; Kelley et al., 2005). A map showing the geographical distribution of some vents discussed here is shown in Figure 1.

Black smokers are hydrothermal fumaroles with abundant sulfides usually located close to or directly on top of the spreading zones of mid-ocean ridges. Because of their proximity to magma, the water in them can be up to over 400°C hot. They are also characterized by a low pH, even though black smokers that are hosted in ultramafic rocks can host serpentinization, such as the Rainbow and Logatchev hydrothermal fields on the mid Atlantic ridge (MAR) (Schrenk et al., 2013). These serpentinizing black smokers, in contrast to Lost City type vents, have higher temperatures, higher metal concentrations and an acidic pH (3–4), but unlike hydrothermal vents hosted in basaltic rock, they have high hydrogen and methane concentrations like other serpentinizing systems. The Rainbow and Logatchev systems are the best studied vents of this type. They also vent C2–C5 hydrocarbons in trace amounts (Charlou et al., 2002; Schrenk et al., 2013). Serpentinization does not happen in basalt-hosted vents because of the higher silica content of the basaltic rock (McCollom and Seewald, 2013).

Off ridge vents, or Lost City type vents, are characterized by carbonate chimneys, a high effluent pH (9 to 11) and moderate temperatures (between 28 to 116°C) compared to black or white smokers (Proskurowski et al., 2008; Seyfried et al., 2015). They are mainly represented by the first system of its kind discovered: the Lost City hydrothermal field. Lost City is located 750 m below sea level, but 4 km above the sea floor on the Atlantis massif (Kelley et al., 2005). Submarine systems that are comparable to Lost City are Prony Bay in New Caledonia (Monnin et al., 2014), the Old City hydrothermal field in the Indian ocean (Lecoeuvre et al., 2021) and the Shinkai Seep Field on the Mariana arc (Okumura et al., 2016).

Prony Bay is in much shallower seawater than Lost City (only 16 to 47 m below the surface) and its water source is fresh water, not crustal circulated seawater as in Lost City (Kelley et al., 2001; Quéméneur et al., 2014; Postec et al., 2015; Twing et al., 2022). Other important differences between shallow seawater vents and deep-sea vents are the access to sunlight, the influence of tidal forces and the different pressures (Price and Giovannelli, 2017). However, in terms of effluent parameters, Prony Bay is more similar to Lost City than the black smokers or the continental serpentinizing systems. It has elevated concentrations of H_2 and CH_4, a high pH and low venting temperatures (see Table 1) (Monnin et al., 2014).

The main difference in effluent chemical composition between the high temperature black smokers that serpentinize like the Rainbow and Logatchev hydrothermal fields and the low temperature Lost City hydrothermal field, is that Rainbow and Logatchev have higher concentrations of CO_2 and metals and acidic pH in their effluent whereas Lost City has a high pH and is depleted in metals and CO_2 (Proskurowski et al., 2008).

FIGURE 1
Locations of the serpentinizing and basalt hosted sites listed in Table 1. White circles indicate serpentinizing sites, black circles indicate non-serpentinizing sites. The sample of studies shown is not comprehensive and makes no statement about the relative frequencies of serpentinizing vs. non-serpentinizing sites. References: See Table 1.

TABLE 1 Parameters of different serpentinizing and basalt-hosted systems covered in this review.

Name	Loc/Water/Serp	Temp [°C]	Depth [m]	pH	H_2	CH_4	CO_2
Aqua de Ney[1]	USA / f / Y	11.6	sur.	13	n.d.	1.92 mM	n.d.
Ashadze[2,3]	MAR / s / Y	296–372	–	3–4	19–26.5 mM	0.8–1.2 mM	n.d.–3.7 mM
Broken spur[4]	MAR / s / N	356–364	3,200	–	0.43–1.03 mM	65–130 µM	6.0–7.1 mM
Cedars[1]	USA / f / Y	17.4	sur.	12	157 µM	22 µM	44 µM
Chimaera[5]	Turkey / f / Y	–	sur.	–	9.82 vol%	85.33 vol%	trace
Conical[2]	MF / s / Y	–	3,083	12.5	–	2 mM	–
Hakuba Happo[6,7]	Japan / f / Y	50–60	500 (terr.)	>10	201–664 µM	124–201 µM	n.d.
Kairei Field[2]	CIR / s / Y	–	2,400	–	8 mM	500 µM	–
Voltri massif[2]	Italy / f / Y	10.5–23	sur.	10–12	–	6–867 µM	12–3,636 µM
Logatchev[2-4,8]	MAR / s / Y	320–352	3,000	3–4	11.1–12.5 mM	1.2–2.6 mM	4.4–10.1 mM
Lost City[2,3,9-11]	MAR / s / Y	40–116	7–800	9–12	0.5–16 mM	1–2 mM	trace
Lucky Strike[4]	MAR / s / N	170–364	1700	3.5	20–730 µM	0.5–0.97 mM	13–28 mM
Mark–1/2[4]	MAR / s / N	335–350	3,460	4	190–480 µM	23–62 µM	5.2–6.7 mM
Menez Gwen[4]	MAR / s / N	275–284	850	4.3	24–48 µM	1.35–2.63 mM	17–20 mM
Nibelungen[2]	MAR / s / Y	192–372	3,000	3	11.4 mM	1.4 mM	–
Old City[12,13]	CIR / s / Y	–	3,100	8	–	–	–
Prony Bay[2,14-16,22]	N.C. / f+s / Y	22–43	16–47	8–11	21.1–731.3 µM	153–376.6 µM	trace
Rainbow[4]	MAR / s / Y	365	2,300	3	12.9–16 mM	1.65–2.5 mM	16–17 mM
Samail[2,17]	Oman / f / Y	25–36.3	sur.	9–12	253 µM	0.1–483 µM	n.d.
Shinkai[18]	MF / s / Y	–	5,555	–	–	–	–
S. Chamorro[2]	MF / s / Y	–	2,960	12.5	–	2 mM	–
Strytan[13,19]	Iceland / f / N	70	16–70	<10	0.1–5.2 µM	0.5–1.4 µM	–
Tablelands[1,2]	Canada / f / Y	9.6	sur.	12–13	30–600 µM	23.7 µM	25–619 µM
TAG[4]	MAR / s / N	290–321	3,670	3	150–370 µM	124–147 µM	2.9–3.4 mM
Tekirova[2,5,20]	Turkey / f / Y	–	–	–	7.5–11.3 vol%	65–93 vol%	trace
Zambales[2,20,21]	Phillip. / f / Y	110–125	sur.	–	8.4–45.6 vol%	13–55 vol%	trace

Loc = Location; Water = Water source; f = freshwater source; s = seawater source; Serp = Serpentinizing system; Y = Yes (serpentinizing system); N = No (non-serpentinizing system, basalt hosted); MAR = Mid Atlantic Ridge = Atlantic Ocean; MF = Mariana Forearc, Pacific Ocean; CIR, Central Indian Ridge, Indian Ocean; N.C. = New Caledonia; Phillip = Phillippines; sur, surface = continental serpentinizing site; terr = terrestrial site, a hole was drilled 500 m into the ground; n.d. = not detected; trace = trace amounts detected; –, not reported.

[1](Cook et al., 2021), [2](Schrenk et al., 2013), [3](Konn et al., 2015), [4](Charlou et al., 2002), [5](Etiope et al., 2011b), [6](Suda et al., 2017), [7](Suda et al., 2014), [8](Proskurowski et al., 2008), [9](Kelley et al., 2001), [10](Kelley et al., 2005), [11](Seyfried et al., 2015), [12](Lecoeuvre et al., 2021), [13](Twing et al., 2022), [14](Monnin et al., 2014), [15](Postec et al., 2015), [16](Quéméneur et al., 2014), [17](Kraus et al., 2021), [18](Okumura et al., 2016), [19](Price and Giovannelli, 2017), [20](McCollom and Seewald, 2013), [21](Abrajano et al., 1988), [22](Quéméneur et al., 2023).

3.2. Conditions in hydrothermal vents

Continental serpentinizing systems have comparatively cool effluent under 100°C, and they also have hyperalkaline waters, although they are fed by fresh water (usually called meteoritic water, which is a complicated name for rainwater or snow) rather than altered sea water. Numerous continental serpentinization sites are known (Etiope et al., 2011b; Etiope and Schoell, 2014; Etiope, 2015). They are important analog sites to deep-sea serpentinizing sites because of their chemical similarities, and because they are easier to access. Like submarine serpentinizing systems, they also produce large amounts of H_2 and CH_4. They are the main source for abiotic gas on Earth (Etiope, 2017). Abiotic volatile hydrocarbons are still poorly understood but they can be generated by various inorganic mechanisms, including FTT. At some sites the amount of abiogenic methane can reach up to 90 vol% of the gas content (Etiope and Schoell, 2014), most of the remainder being H_2.

Abiogenic methane is widely discussed in the search for extraterrestrial life because methane is considered a potential biomarker, the sign of methanogenesis. But if Earth can generate large amounts of methane through abiotic processes, other wet, rocky planets and moons probably can as well, making methane itself problematic as a sign of life (Etiope, 2017). However, if biochemistry and life arose from exergonic H_2-dependent CO_2 reducing reactions, then abiotic methane serves as a proxy for the existence of geochemical conditions that are conducive to life's origin: exergonic reactions of H_2 and CO_2 under continuously far from equilibrium conditions.

The distinction between biotic and abiotic methane in usually made by isotope fractionation (Etiope et al., 2013; Etiope and Schoell, 2014). Abiotic methane is enriched in ^{13}C relative to biotic methane, which is isotopically light because of isotope discrimination in favor of ^{12}C in the acetyl-CoA pathway (Blaser et al., 2013) of methyl synthesis from H_2 and CO_2 underpinning methanogenesis. However distinguishing biotic and abiotic methane by carbon isotope fractionation is not always straightforward (Horita and Berndt, 1999), which is why deuterium fractionation is also often taken into account (Etiope et al., 2011a) and often even combined (Miller et al., 2018) as shown in a study conducted in the Samail Ophiolite where it could be determined that biotic and abiotic methane mix (Nothaft et al., 2021).

Radiocarbon methods (^{14}C) can also be used to determine the source of C in CH_4 from serpentinizing systems. In the upper atmosphere, ^{14}C is constantly produced by neutron capture of ^{14}N to generate ^{14}C and a proton. The neutrons stem from cosmic radiation, the resulting ^{14}C has a half-life of 5,700 years. There is no radiocarbon in CH_4 of Lost City effluent. That is, CH_4 from Lost City is "radiocarbon dead," meaning that the carbon source cannot be bicarbonate from seawater, unless the circulation time exceeds 57,000 years (ten ^{14}C half-lives) because seawater contains ^{14}C that is constantly synthesized in the upper atmosphere. It is estimated that the entire volume of the ocean circulates through hydrothermal vents every 100,000–500,000 years (Fisher, 2005). The low CO_2 concentrations at Lost City together with its "radio-carbon-dead" CH_4 suggest that CH_4 in Lost City is likely derived from CO_2 of mantle origin that was reduced during serpentinization (Proskurowski et al., 2008).

3.3. CO_2 and H_2

Most of the carbon emitted from Lost City comes from the Earth's mantle in the form of formate (Lang et al., 2012, 2018). Formate can be used as a substrate for methanogenesis in environments where CO_2 is limiting. Methanogens can readily convert formate into CO_2 and H_2 or use it to reduce coenzyme F_{420} to $F_{420}H_2$, a flavin similar to FAD (Holden and Sistu, 2023). This allows them to access formate as a source of carbon and electrons for methanogenesis. This has been observed not only in Lost City but also in the Samail ophiolite (Fones et al., 2021). Formate forms abiotically under the high pH, reducing conditions of serpentinizing fluids. This can also be replicated in the laboratory (Preiner et al., 2020), where formate is typically the most abundant reduced carbon species under a variety of conditions (Belthle et al., 2022; Beyazay et al., 2023a,b). In Lost City, formate is the second most abundant carbon species after CH_4 and the second most available reductant after H_2 (Lang and Brazelton, 2020; Brazelton et al., 2022).

High concentrations of effluent H_2 are characteristic for serpentinizing systems, as summarized in Table 1. In terms of H_2 concentration, the non-serpentinizing Strytan hydrothermal field has the lowest out of all the systems where H_2 was detected. It is in the range of normal seawater. At first glance it might seem as if continental serpentinizing systems do not have high concentrations of H_2, however it should be mentioned that sometimes only the liquid phase was measured, not the actual gas phase emitted from the systems. In the Tekovira and Zambales ophiolite, where the gas phase composition was measured, H_2 can comprise 45 vol% of the emitted gas. High H_2 tends to correlate with high CH_4, but not strictly (see Supplementary Figure S1). Lost City and other serpentinizing systems of the mid Atlantic ridge (MAR) have high concentrations of CH_4 and H_2. The effluent at Prony Bay, a shallow sea vent with a fresh water source and similar microbial communities as continental ophiolites, has lower H_2 and CH_4 concentrations than Lost City of about a magnitude but still in significant amounts (Quéméneur et al., 2023). The Ashadze system on the MAR has very high H_2 concentration in its venting fluids of up to 26.5 mM. For CH_4 concentrations, many deep-sea hydrothermal vents, with a few exceptions, have a concentration of 1–3 mM. In continental serpentinizing sites CH_4 can reach between 20 vol% in New Zealand to 93 vol% of the gas phase in the Tekovira ophiolite (Etiope and Schoell, 2014). CH_4 is also extensively studied in continental serpentinization sites because they are the main contributor to abiotic gas (Etiope, 2017).

Alkaline serpentinizing systems are low in CO_2 because at high pH dissolved CO_2 turns into carbonate (CO_3^{2-}), which precipitate within the vent as Ca^{2+} and Mg^{2+} carbonates. This is the reason why hot and acidic hydrothermal vents such as Rainbow or Lucky Strike have very high CO_2 concentrations (up to 28 mM), while alkaline systems such as Lost City and Prony Bay harbor only have trace amounts of dissolved inorganic carbon. For continental serpentinization sites CO_2 was only detected in trace amounts in the gas phase. In the liquid phase CO_2 [often in the form of bicarbonate (HCO_3^-)] could sometimes be detected up to the mM range, but trace amounts are typical (Table 1). More complex organics than CH_4, when reported, were only detected in trace amounts both in ocean and continental serpentinization systems. Formate and acetate, when reported, were detected in Lost City in µM amounts and in the Conical Seamount of the Mariana Forearc in mM (formate) and µM (acetate) amounts, whereby the acetate at Lost City is likely biogenic (Lang et al., 2010).

Comparing different serpentinizing systems across studies is not straightforward because the sampling methods are often different and because of local heterogeneity: even in the same system values can differ greatly depending on where in the system the samples were taken. Cook et al. (2021) showed that the values measured in different samples, be it the temperature, the pH or the concentrations of the different compounds, can be vastly different depending on the sampling site. For example, if the groundwater, the well water or the surface water were sampled at three different sites — Tablelands, the Cedars, Aqua de Ney — the pH values could range between pH 8–9 in the surface water to pH 12–13 in the well water.

4. Microbiota of hydrothermal vents: a window into the past

Serpentinizing hydrothermal vents yield insights into primordial geochemical conditions, thereby providing a window in time into the nature of the environments that the first prokaryotic communities on the early Earth inhabited and the role of inorganic catalysts in the chemical setting of life's origin (Corliss et al., 1981; Baross and Hoffman, 1985). Serpentinizing systems also link the geochemistry of primitive environments to autotrophic theories for origins. It is estimated that about 60 % of all microbes inhabit Earth's crust: ~35 % in the submarine crust and ~25 % in the continental crust (Flemming and Wuertz, 2019). There is no sunlight, so primary production in the crust has to start with chemolithoautotrophs. The most prevalent (and obvious) source of energy for autotrophy is the H_2-CO_2 couple that fuels acetogen and methanogen metabolism. Serpentinizing hydrothermal vents constantly generate H_2 and reducing conditions that mobilize nutrients.

Many reports have investigated the chemistry and microbial communities of hydrothermal vents, earlier reviews are provided by (Kelley et al., 2002; Martin et al., 2008; Lang and Brazelton, 2020). Serpentinizing hydrothermal systems were the topic of a recent special dedicated issue of the Philosophical Transactions of the Royal Society with contributions covering new insights from the serpentinite reactions at the Marianas trench (Fryer et al., 2020), the effect of pH on H_2 production (McCollom et al., 2020), hydrocarbons in fluid inclusions within olivine rich rocks (Grozeva et al., 2020), the role of serpentinite in the search for life beyond Earth (Vance and Daswani, 2020), the thermodynamics of the H_2-rich environments of serpentinizing systems and how that effects microbial CO_2 fixation (Boyd et al., 2020), a thorough review of microbial investigations at Lost City (Lang and Brazelton, 2020). Another worthy read is an in-depth study focusing on Prony Bay showing the relationship of microbial communities in hyperalkaline waters with high concentrations of H_2 (Quéméneur et al., 2023). A number of new reports shed light on the relationship between geochemical reactions at hydrothermal vents and autotrophic origins of microbial metabolism and life. In an origins context we can focus on anaerobic processes.

4.1. Carbon

Although CO_2 was very abundant on the early Earth (Sossi et al., 2020), modern serpentinizing systems are generally low in CO_2 because of magnesium carbonate and calcium carbonate precipitation at high pH. The effluent of serpentinizing systems can however be rich in formate ($HCOO^-$). Formate is readily converted by simple inorganic catalysts (Preiner et al., 2020; Belthle et al., 2022; Beyazay et al., 2023a,b) and by microbes into H_2 and CO_2 via the near-equilibrium reaction:

$$HCOO^- + H+ \rightarrow H_2 + CO_2$$

with $\Delta G_o' = -3.5\,kJ\cdot mol^{-1}$ (Maden, 2000). Microbes have a number of different enzymes for that reaction. The formate to CO_2 converting reaction is catalyzed by the enzyme hydrogen-dependent CO_2 reductase, HDCR (Schuchmann and Müller, 2013), or by NADH-dependent formate dehydrogenase (FDH), which is the typical enzyme of the acetyl-CoA pathway in acetogens (Schuchmann and Müller, 2013). In methanogens the reaction is catalyzed via hydrogenases that convert H_2 into reduced ferredoxin and the activity of formyl-methanofuran dehydrogenase, which contains a FDH domain and 46 [4Fe4S] clusters (Wagner et al., 2016). A third kind of formate dehydrogenase, HylABC-Fdh2, performs a reaction called electron bifurcation in which an electron pair is split, with the individual electrons being transferred to different acceptors with different redox potentials (Buckel and Thauer, 2013; Buckel and Thauer, 2018). HylABC-Fdh2 converts formate into CO_2 with one of the electrons being transferred to NAD^+ and the other being transferred to ferredoxin (Wang et al., 2013). Oxidation of two formate molecules via this reaction generates two CO_2, one NADH and two reduced ferredoxins. This bifurcating form of FDH is found in *Bipolaricaulota*, a deeply branching acetogen identified by metagenomics in the effluent of the hyperalkaline water of the Oman ophiolite (Colman et al., 2022). There are also cytochrome- dependent formate dehydrogenases (Sebban-Kreuzer et al., 1998). All formate dehydrogenases known so far, including HDCR, use Mo (in molybdopterin) or W (in tungstopterin) as a cofactor. The enzymatic mechanism of FDH is thought to involve binding of formate to Mo (or W) via its oxygen atoms (Hartmann et al., 2016), the abiotic mechanism is thought to involve bonding of the carbon atom in formate to Ni or Fe (Preiner et al., 2020).

The use of formate as a carbon source is a common theme in the effluent of serpentinizing systems. Although organisms have not yet been cultivated from hydrothermal vents that exhibit growth on formate, acetogens (Moon et al., 2021) and methanogens (Ferry et al., 1974) are known that use formate as their sole carbon and electron source, and some methanogens use formate as their sole carbon source (Costa et al., 2010; Lie et al., 2012). All indications are that formate based primary production is taking place at the submarine system of Lost City (Lang et al., 2018) and in some terrestrial serpentinizing systems including the Oman ophiolite (Colman et al., 2022), the Cedars (Suzuki et al., 2018), at Hakuba Happo (Nobu et al., 2023) and in the Prony Bay system, a freshwater fed submarine serpentinizing system that vents in seawater at a depth of about 50 m (Frouin et al., 2022), because there is no other carbon source reported to fuel primary production in these systems. For acetogens and methanogens living in these systems, formate is apparently the source of CO_2 and a source of electrons for primary production via the acetyl-CoA pathway, which generates reduced carbon to support the growth of fermenters that live from microbial cell mass (Schönheit et al., 2016) and other heterotrophs in the system.

The results of microbial sampling studies from different sites are not simple to compare directly because community composition within sites can vary, and at very small scales, depending on the specific position of the sample within the vent, effluent seawater mixing in the case of submarine vents, presence or absence of O_2 in the specific sample, the methods of analysis and other factors. For example, in the hyper-alkaline Strytan site, which has alkaline effluent but is not serpentinizing, anaerobes and the Wood Ljungdahl pathway of CO_2 fixation dominate in inner regions of vents while O_2-tolerant pathways dominate at the vent surfaces in contact with sea water (Twing et al., 2022). In the basement of Lost City alone, a broad diversity of phylotypes across sites sampled is observed (Brazelton et al., 2022). Methanogens are common in serpentinizing systems and can access carbon as formate (Fones et al., 2021). Quéméneur et al. (2023) showed that the abundance of certain prokaryotes is positively correlated to the H_2/CH_4 ratio in hyperalkaline springs and that bacteria that use H_2 as their main energy source are the most abundant among the microbial communities of Prony Bay.

A recent study by Frouin et al. (2022) stands out among surveys of microbial communities from different serpentinizing systems because the authors aimed to identify community and physiological properties that i) are shared among different serpentinizing systems and that ii) distinguish serpentinizing systems from hydrothermal vents in non-serpentinizing systems. They found that serpentinizing systems tend to be colonized by diverse communities, and that bacterial groups encountered often include acetogens (Firmicutes) and methanogens like Methanosarcinales (Archaea), as originally discovered at Lost City. Frouin et al. (2022) found that Prony Bay was dominated by the acetogen *Bipolaricaulota*, which was also the main inhabitant of the Oman ophiolite community recently characterized by Colman et al. (2022) on the other side of the globe. Abundance of Firmicutes, which includes many acetogens, has been shown to be proportional to dissolved H_2, however why that is still has to be determined (Quéméneur et al., 2023).

A common physiological theme in serpentinizing systems is the ubiquitous presence of enzymes for the acetyl-CoA pathway (or Wood-Ljungdahl pathway) of CO_2 fixation. The Wood-Ljungdahl pathway is considered to be the most ancient pathway of carbon fixation (Fuchs and Stupperich, 1985; Fuchs and Stupperich, 1986; Berg, 2011; Fuchs, 2011). It is still the only exergonic pathway of CO_2 fixation known. The reaction to the level of the energy-rich thioester

$$2CO_2 + 8[H] + CoASH \rightarrow CH_3COSCoA + 3H_2O$$

is exergonic with $\Delta G_o' = -59.2$ kJ/mol if $2[H] = H_2$ and slightly endergonic with $\Delta G_o' = +13.2$ kJ/mol if $2[H] = NADH$ (Fuchs, 1994). The NADH forming reaction is slightly exergonic in the direction of CO_2, which is one reason that the pathway is so versatile among microbes (Fuchs, 1994; Zinder, 1994). It is the only pathway of CO_2 fixation that does not require net ATP input (Fuchs, 2011) and that occurs in both bacteria and archaea (Berg et al., 2010). It is a joined pathway of carbon and energy metabolism in which acetogens and methanogens obtain their ATP from ion gradients that they generate in the process of the exergonic reduction of CO_2 with electrons from H_2 at MtrA-H in methanogens (Thauer et al., 2008) and at Rnf in acetogens that lack cytochromes (Müller et al., 2018). In addition, acetogens and some methanogens can generate ATP in the acetyl-CoA pathway using substrate-level phosphorylation via acetyl-phosphate (Schöne et al., 2022). This kind of combined carbon and energy metabolism is so far unique to acetogens and methanogens. The acetyl-CoA pathway can be a dedicated pathway of CO_2 fixation (carbon metabolism) with no role in energy metabolism (ATP synthesis), for example in sulfate reducers that grow autotrophically, or in bacteria with phosphite-dependent energy metabolism (Mao et al., 2021). The acetyl-CoA pathway is a biochemical fossil that links acetogens and methanogens via functional, ecological and ancient evolutionary aspects (Martin, 2020; Schöne et al., 2022).

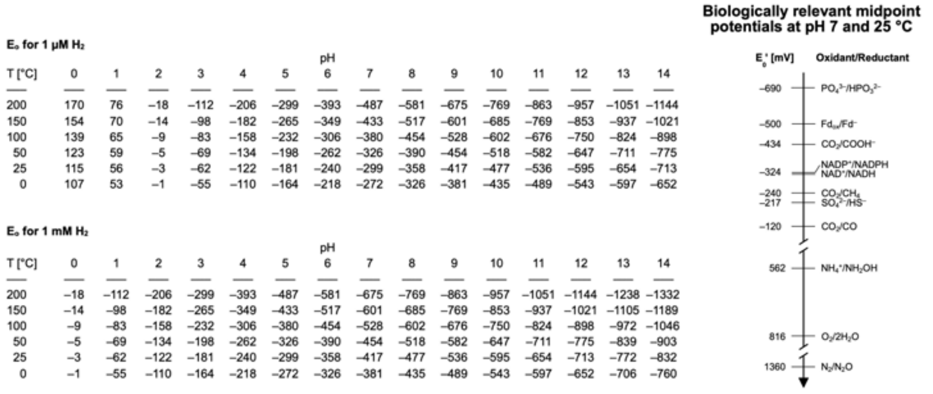
4.2. Electrons

The diffusible carrier electrons for reduction reactions in serpentinizing systems, H_2, is present in overabundance. At Lost City the effluent H_2 concentration is on the order of 10 mmol/kg [0.5 to 14 mmol/kg: (Proskurowski et al., 2008)], which is about 5–6 orders of magnitude more H_2 than is required to support methanogenesis (Thauer et al., 2008). Because of the high alkalinity and high H_2 concentration, the calculated redox potential of serpentinizing effluent can be in the range of −800 mV (Boyd et al., 2020), sufficient for most biologically relevant reduction as shown in Figure 2, with the exception of the phosphate/phosphite couple, where the midpoint potentials become more negative with increasing pH (Bernhard Schink, pers. comm.). Alkalinity favors the dissociation of H_2 into protons and electrons by consuming protons from the reaction $H_2 \rightarrow 2e^- + 2H^+$. Electrons from H_2 enter metabolism via hydrogenase. All hydrogenases except the Fe hydrogenase of methanogens (Thauer et al., 2008; Shima et al., 2011), reduce 4Fe4S clusters and most of them generate reduced ferredoxin as the reaction product. The redox potential needed to reduce ferredoxin as the source of electrons in metabolism is on the order of −500 mV. Suzuki et al. (2018) found no hydrogenases in metagenomes from The Cedars, raising the possibility that other routes of ferredoxin reduction might be possible under such reducing conditions. Studies of microbes that grow on native iron and on electrodes suggest that side activities of extracellular enzymes might be able to substitute for hydrogenases (Deutzmann et al., 2015). Early studies by Menon and Ragsdale (1996) indicated that acetyl-CoA synthase and pyruvate synthase have latent hydrogenase side activity, and the H_2-producing side reaction of nitrogenase (Hu and Ribbe, 2016) is well known. All of those side activity hydrogenases have FeS or NiFeS clusters that are likely candidates for the moonlighting active site. Metals (Fe, Ni, Co) and metal oxides can serve as hydrogenases, activating H_2 by splitting it into two metal bound hydrogen atoms for CO_2 reduction (Preiner et al., 2020; Belthle et al., 2022; Beyazay et al., 2023a,b) or for NAD^+ reduction (Henriques Pereira et al., 2022).

The highly reducing conditions of serpentinizing hydrothermal vents are key to origins because CO_2 has to be converted to organic carbon. There are, however, also alternative views about the source of energy at origins in serpentinizing hydrothermal systems. Duval et al. (2021), for example, argue that the energy required for the origin of metabolism was not exergonic H_2-dependent CO_2 reduction catalyzed by reduced transition metals as it occurs in the laboratory (Weiss et al., 2016; Preiner et al., 2020; Wimmer et al., 2021b; Beyazay et al., 2023a) and in acetogen and methanogen metabolism, but that instead the energy at origins stemmed from nitrate-dependent methane oxidation with the mixed valence iron oxide fougerite (green rust), acting as the catalyst (Duval et al., 2021) in alkaline hydrothermal systems. How energy release from methane oxidation is coupled to either prebiotic organic synthesis or carbon metabolism is not explained by Duval et al. (2021), changes in Gibbs free energy for the fougerite dependent reactions have not been presented, and abiotic laboratory versions of the fougerite dependent carbon assimilation reactions have not been reported. In laboratory versions of the acetyl-CoA pathway (Preiner et al., 2020; Beyazay et al., 2023a) and in acetogens and methanogens *in vivo* (Figure 3), energy for ATP synthesis is released in the exergonic H_2 dependent synthesis of acetate, pyruvate and methane from CO_2.

4.3. Nitrogen and sulfur

Frouin et al. (2022) looked carefully at sources and pathways for nitrogen but found no consistent trend. The source of nitrogen is still not well resolved in serpentinizing systems and might differ across systems. In very recent work, Shang et al. (2023a,b) found that experimental serpentinization reactions in the laboratory readily reduce N_2 to NH_3 in very large amounts and on time scales approaching 30 days. Cells are about 50% carbon and about 10% nitrogen by dry weight (Heldal et al., 1985), hence there has to be access to a nitrogen supply for primary production in serpentinizing systems. The simplest solution is that N_2 present in the water circulating through serpentinizing systems is reduced by nitrogenase. Most studies have detected nitrogenase genes among the microbiota



E_o for 1 μM H_2

T [°C]	pH 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
200	170	76	−18	−112	−206	−299	−393	−487	−581	−675	−769	−863	−957	−1051	−1144
150	154	70	−14	−98	−182	−265	−349	−433	−517	−601	−685	−769	−853	−937	−1021
100	139	65	−9	−83	−158	−232	−306	−380	−454	−528	−602	−676	−750	−824	−898
50	123	59	−5	−69	−134	−198	−262	−326	−390	−454	−518	−582	−647	−711	−775
25	115	56	−3	−62	−122	−181	−240	−299	−358	−417	−477	−536	−595	−654	−713
0	107	53	−1	−55	−110	−164	−218	−272	−326	−381	−435	−489	−543	−597	−652

E_o for 1 mM H_2

T [°C]	pH 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
200	−18	−112	−206	−299	−393	−487	−581	−675	−769	−863	−957	−1051	−1144	−1238	−1332
150	−14	−98	−182	−265	−349	−433	−517	−601	−685	−769	−853	−937	−1021	−1105	−1189
100	−9	−83	−158	−232	−306	−380	−454	−528	−602	−676	−750	−824	−898	−972	−1046
50	−5	−69	−134	−198	−262	−326	−390	−454	−518	−582	−647	−711	−775	−839	−903
25	−3	−62	−122	−181	−240	−299	−358	−417	−477	−536	−595	−654	−713	−772	−832
0	−1	−55	−110	−164	−218	−272	−326	−381	−435	−489	−543	−597	−652	−706	−760

FIGURE 2
Range of redox potentials for two different concentrations of hydrogen (1 μM and 1 mM) over different pH ranges and temperatures. The pH has the biggest influence on the redox potential, followed by temperature and then hydrogen. Biologically relevant midpoint potentials are shown on the left, all of them measured at pH 7 (Vanysek, 2000; Schink et al., 2002; Lundblad and Macdonald, 2018; Martin et al., 2018; Madigan et al., 2021; Nelson and Cox, 2021).

of serpentinizing systems, though nitrate reduction can also be detected (Frouin et al., 2022). Trutschel et al. (2022) reported high concentrations of ammonia at Ney Springs. Ammonia has also been reported in the Oman ophiolite system (Rempfert et al., 2017), but in both cases the source of the ammonia is not resolved, with decaying organic matter within the system being the most widely discussed source. With the new report by Shang et al. (2023a), abiotic sources of NH_3 need to be considered in natural systems (Shang et al., 2023a).

The state of nitrogen in the primordial atmosphere was N_2 (Sossi et al., 2020). In an origins context, before the existence of biological N_2 fixation via nitrogenase, the source of nitrogen would have to be geochemical. Three sources are discussed: High pressure high temperature N_2 reduction by deep geochemical Haber-Bosch like processes (Brandes et al., 1998), low pressure low temperature FeS-mediated N_2 reduction processes (Dörr et al., 2003) and serpentinization-related geochemical processes. Ménez et al. (2018) reported the presence of abiotic tryptophan and other amino acids in the Atlantis massif that hosts the Lost City hydrothermal field. The exact route of synthesis is not yet known but might involve iron-dependent Friedel-Crafts like syntheses. The significance of amino acid synthesis in the crust for theories that life arose at hydrothermal vents is evident (Baross, 2018).

Lost City does not appear to emit abiotic amino acids in its effluent, but the terrestrial hydrothermal system Hakuba Happo does. Nobu et al. (2023) recently reported the presence of glycine (5 nM) in the effluent of Hakuba Happo which is likely of abiotic origin. Notably, there were no other amino acids in the effluent. Were glycine of biotic origin, the 19 other amino acids should be present. Some of the microbes that Nobu et al. characterized from Hakuba Happo, *ca.* Lithacetigenota, possessed genes for glycine reductase, an unusual selenoenzyme that synthesizes acetyl phosphate from glycine and P_i (Andreesen, 2004). The acetyl phosphate can be used for acetyl-CoA (carbon metabolism) or ATP synthesis via substrate level phosphorylation. Although the concentration of glycine in the Hakuba Happo effluent was low, it was sufficient to render the glycine reductase pathway thermodynamically favorable under the conditions of the vent. No archaea were detected in Hakuba Happo, but glycine reductase utilizing Lithacetigenota were detected at the Cedars (Nobu et al., 2023).

The glycine reductase variant of carbon assimilation and energy metabolism employed by Lithacetigenota starts with environmental glycine. It is distinct from the H_2-dependent glycine reductase pathway of autotrophic CO_2 fixation used by *Desulfovibrio desulfuricans* (Sánchez-Andrea et al., 2020). The findings of Ménez et al. (2018) indicate that geochemical nitrogen fixation to amino acids is possible. The findings of Nobu et al. (2023) suggest that it is ongoing today, continuously in the hyperalkaline serpentinizing environment of Hakuba Happo.

Sulfur is a trace element in cells. It is highly volatile in various oxidation states and readily eluted from rocks as H_2S or HS^- during serpentinization, but sulfate is also a common constituent of effluent and sulfate reducers are very common in serpentinizing systems (Reveillaud et al., 2016; Lang et al., 2018). Sulfur is an ancient substrate of energy metabolism (Rabus et al., 2006; Liu et al., 2012) and fulfills important catalytic functions in many cofactors (Kirschning, 2021). It is a component of ancient metabolism. Note however that the synthesis of formate, acetate and pyruvate from H_2 and CO_2 under simulated hydrothermal conditions using Ni_3Fe or Fe_3O_4 as catalysts requires no participation of sulfur (Preiner et al., 2020; Belthle et al., 2022; Beyazay et al., 2023a,b).

Today, nitrogen and sulfur compounds that occur in hydrothermal systems serve as terminal electron acceptors in anaerobic respiratory chains (Calisto and Pereira, 2021). At the origin of metabolism, CO_2 was arguably the most important electron acceptor because the synthesis of organic compounds was required in order to get metabolic pathways established. This underscores the evolutionary significance of the acetyl CoA pathway in acetogens and methanogens: it generates the starting compounds of metabolism while releasing energy that can

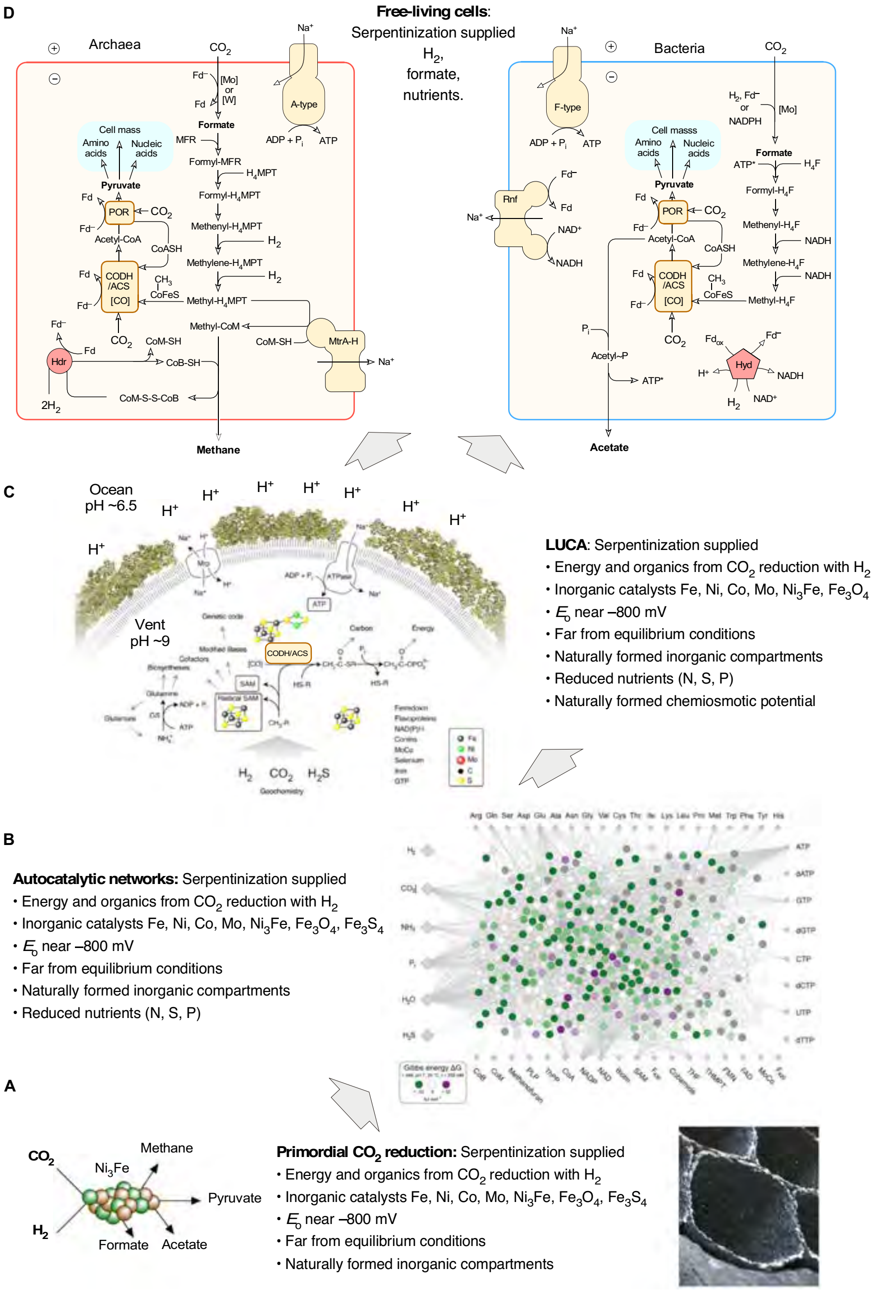


FIGURE 3
Contributions of serpentinization to the origin of free-living cells. **(A)** The H_2 produced in serpentinization synthesizes awaruite, Ni_3Fe (Preiner et al., 2018). Awaruite is a highly efficient catalyst for the conversion of H_2 and CO_2 into formate, acetate and pyruvate — the backbone of microbial

(Continued)

FIGURE 3 (Continued)

metabolism in anaerobic autotrophs that obtain their carbon and energy via the acetyl-CoA pathway — the reaction takes place overnight in water at pH 8-10 and 70-100°C in the dark using aqueous H_2 concentrations of 5 mM, similar to 10 millimolar H_2 observed at Lost City (Preiner et al., 2020). At that H_2 concentration and pH, the midpoint potential of the H_2/H^+ couple is on the order of −600 mV (see Figure 2), in Lost City the redox potential has been calculated to be in the order of −800 mV (Boyd et al., 2020), sufficient to reduce both CO_2 to formate ($E_o' = -450$ mV) and to reduce ferredoxin (E_o = *ca.* −500 mV in cells). These low values of E_o are the reason why electron bifurcation is not required for CO_2 reduction with H_2 ($E_o' = -414$ mV) in the reaction shown in the panel (Martin, 2022), but is strictly required in enzyme based reduction in cells (Buckel and Thauer, 2018; Müller et al., 2018). Naturally formed inorganic microcompartments (an artist's impression is shown) can concentrate the organic products around their site of synthesis. **(B)** Autocatalytic networks are objects of molecular (self) organization. Because of the specificity and efficiency observed for their formation from H_2 and CO_2 using Ni_3Fe or Fe_3O_4, formate, acetate and pyruvate serve as elements of the food set (F) in food- (F-) generated reflexively autocatalytic networks, abbreviated as RAFs. RAFs can be identified in the metabolism of modern cells (Sousa et al., 2015; Xavier and Kauffman, 2022) and in acetogens and methanogens, cells that obtain their carbon and energy from H_2 dependent CO_2 reduction via the acetyl CoA pathway (Xavier et al., 2021). The reactions that are required to synthesize 20 amino acids, the (unmodified) bases of DNA and RNA, and 18 cofactors are called the autotrophic core (Wimmer et al., 2021a). Of the 400 reactions in the autotrophic core, 97 % reactions are exergonic under the reducing and far-from-equilibrium conditions of serpentinizing hydrothermal vents, as indicated by green dots on reactions of the network [image from Wimmer et al. (2021b)]. Serpentinization also provided reduced N, S and possibly P species as nutrients (see corresponding sections in the text). Natural inorganic compartments could have promoted the formation of autocatalytic networks by generating high reactant and product concentrations from specific food sets (Martin and Russell, 2007). Autocatalytic networks help to bridge the gap between reactions catalyzed by simple inorganic compounds and reactions catalyzed by peptides synthesized on ribosomes. **(C)** The last universal common ancestor, LUCA, possessed the universal genetic code and ribosomes was able to synthesize proteins as catalysts. Genomic reconstructions of LUCA (Weiss et al., 2016, 2018) indicate that the energy required for the synthesis of encoded peptide catalysts (protein synthesis cost 4 ATP per peptide bond) stemmed from substrate level phosphorylation, SLP (Weiss et al., 2016). Serpentinization provided H_2 for CO_2 reduction. The thermodynamics of laboratory scale bifurcation-independent acetate synthesis from H_2 and CO_2 are sufficient to enable SLP (Preiner et al., 2020). Serpentinization generates naturally occurring pH gradients between the alkaline effluent and the nearly neutral ocean water (see main text), the magnitude and polarity of the gradients are sufficient to drive an ATP synthase. The rotor stator ATP synthase is as universal among cells as the ribosome (Martin and Russell, 2003), it was present in LUCA (Weiss et al., 2016) and, inserted into a hydrophobic layer would provide the cytosol with a very high energy charge to support for protein synthesis. The origin of the genetic code is handled here as a given, not as an explanandum, it is not evident how serpentinization (or any other geochemical process) would specifically foster the origin of the genetic code, whose structure is determined by aminoacyl-tRNA synthetases (Carter and Wolfenden, 2015), bases in tRNA (Schimmel et al., 1993) and the peptidyl transferase site of the protoribosome (Bose et al., 2022). **(D)** By coupling H_2 dependent CO_2 reduction to the formation of ion gradients (pumping), and with the integration of flavin based electron bifurcation into the reduction of ferredoxin by H_2 in a stoichiometrically balanced energy metabolic reaction, the ancestors of acetogens (LBCA) and methanogens (LACA) could emerge as free-living cells. The lipids of bacteria and archaea are different in chemistry and biosynthesis, they evolved independently from LUCA en route to LACA and LBCA. See also Martin (2012, 2020) and Martin and Sousa (2016). Note the position of H_2, CO_2, formate, acetate, pyruvate and methane in panel A and panel D. In free living cells, various properties provided in different phases of biochemical evolution by serpentinization are replaced by biogenic molecules and catalysts. In all phases of the figure, the energy is supplied by serpentinization. In panel **(D)** the archaeal physiological map is for Methanothermobacter marburgensis, from Thauer et al. (2008), the bacterial map is for Acetobacterium woodii, from Schuchmann and Müller (2014).

be harnessed to synthesize ATP. In that sense, acetogenesis and methanogenesis are cytochrome-free and quinone-free anaerobic respirations that use CO_2 as the terminal acceptor in a reaction sequence that also generates a net gain of acetyl CoA. Anaerobic respirations that reduce sulfur and nitrogen compounds require siroheme, cytochromes and quinones, acetogenesis and methanogenesis do not, they are simpler in that regard. Coupled carbon and energy metabolism in the acetyl-CoA pathway using CO_2 as the electron acceptor probably preceded a differentiation into dedicated carbon metabolism and dedicated energy metabolism involving cytochrome dependent respiratory chains that use nitrogen and sulfur compounds as terminal acceptors.

4.4. Phosphorus: phosphate and phosphite

Phosphate has always presented problems in prebiotic chemistry because of its low solubility in contact with calcium to generate apatite, which is more or less insoluble, leading to various suggestions, that certain kinds of carbonate rich lakes existed on the primordial Earth that maintained phosphate in solution (Toner and Catling, 2020). The recent findings of Frouin et al. (2022) from Prony Bay place the phosphate problem in a fundamentally different light. They estimate that about half of the microbes that they sampled from Prony Bay possess the genes required for the metabolism of phosphonates and they found that phosphonate metabolism was fairly common in microbial communities from serpentinizing systems. What are phosphonates? Phosphonates are compounds that contain a C–P bond and are so far only known to be synthesized by microbes via a pathway that starts from phosphoenolpyruvate. Phosphonates can have concentrations in open ocean water that account for up to 25% of dissolved organic phosphorus (Acker et al., 2022). Their exact function is still not known, but they might represent a means of sequestering phosphorus among community members (Metcalf et al., 2012). That proposal has however recently been challenged because the phosphonate synthesis and utilization genes are so common: phosphonates might also mediate biological interactions at the cell surface (Acker et al., 2022). The question posed by Frouin et al. (2022) is: What are microbes from serpentinizing systems doing with a high frequency of phosphonate metabolizing enzymes?

Moreover, in some of the Prony Bay metagenomes, the gene for an additional enzyme, NAD^+-dependent phosphite oxidoreductase, *ptxD*, was often inserted into the phosphonate metabolizing operon (Frouin et al., 2022). The *ptxD* gene product converts phosphite and NAD^+ to phosphate and NADH (Vrtis et al., 2001). Frouin et al. (2022) noted the ability of Prony Bay microbes to utilize reduced phosphorous compounds. They were mainly concerned with the question of what the source of phosphonates in the Prony Bay fluid might be. While about half of the genomes from the Prony Bay vents contained genes related to phosphonate metabolism, metabolizing operons represent a frequency that is significantly higher than average for marine microbes (Frouin et al., 2022), it is not orders of magnitude higher: up to 30 % of marine microbial genomes have the potential for phosphonate degradation (Acker et al., 2022).

New findings by Pasek et al. (2022) suggest that the phosphite oxidase gene, *ptxD*, that Frouin et al. find inserted into the phosphonate (phn) operon might hold an important clue. Why is phosphite relevant? Two reasons: First, phosphite is much more soluble than phosphate. One solution to the "phosphate problem" of early evolution is that phosphorus in the ancient oceans was present in the form of the much more soluble phosphite ion (Schwartz, 2006). Studies by Pasek and colleagues have suggested that phosphite might have been more prevalent on the early Earth than it is today. Archaean oceans may have contained substantial amounts of phosphite in addition to phosphate (Pasek et al., 2013; Herschy et al., 2018). If phosphite was present in early environments but phosphate was needed, enzymatic phosphite oxidation would have been needed. Might the presence of phosphonates in the ocean, with P^{+3} oxidation state (like phosphite) and oxidation to phosphate (P^{+5}) in the cytosol, reflect a relic of ancestral marine environments? It is possible.

Second, where does phosphite in the environment come from such that so many microbes should have genes for phosphite metabolism (Schink et al., 2002)? In older papers, a source of ancient phosphite was originally suggested to be schreibersite from meteorite bombardments (Pasek et al., 2013). Schreibersite is a highly reactive phosphide mineral $(Ni, Fe)_3P$, that is indeed only known from meteorites (Pasek, 2008). But phosphite oxidation genes are commonplace today (Schink and Friedrich, 2000; Vrtis et al., 2001), being found in roughly 1.5 % of all genomes (Ewens et al., 2021), whereas huge schreibersite spreading impacts are rare at best. This clearly indicates that there are sources of phosphite in the environment that do not require bolide impacts.

The midpoint potential for the reduction of phosphate to phosphite is −690 mV at pH 7 and 25°C (Schink et al., 2002) (see Figure 2) but becomes more negative with higher pH (Bernhard Schink pers. comm.). Considering the measured and calculated reduction and midpoint potentials for serpentinizing systems is within the range of −435 to −830 mV, the reduction of phosphate to phosphite might not be within the range covered by H_2 in modern serpentinizing systems, [...] but might lie within the range of serpentinization processes that generate H_2 on early Earth. This brings us to a question relevant for modern microbial communities and for life's origins: do serpentinizing systems actually generate phosphite? The answer appears to be yes (Pasek et al., 2022), although phosphite has not been reported to be present directly in the effluent of serpentinizing systems. Pasek et al. (2022) measured phosphorus species in samples of rock from formations altered by serpentinization, they found that 20–50 % of the total P in some samples was phosphite (the remainder was phosphate). That corresponds to a substantial amount of phosphite stemming from serpentinization. The implications of i) genes for phosphite oxidation being present in microbes from serpentinizing systems (Frouin et al., 2022) and ii) phosphite being a component of rock in serpentinizing hydrothermal systems (Pasek et al., 2022) suggest that there is phosphite available in serpentinizing systems and that microbes that live there are using it. Could they be using this as a soluble source of P for enzymatic phosphate synthesis? If so, this would tie together some loose ends in phosphate metabolism and early evolution.

It is possible that genes for phosphite uptake and conversion into its biologically more useful form, phosphate, might reflect an ancient, possibly even ancestral state of phosphorus metabolism. As Buckel (2001) put it: "*At the time of the origin of life, about 3.8 billion years ago, phosphites could have been more important than today.*" This would mean that the highly exergonic conversion of phosphite into its biologically relevant form, phosphate, occurred inside cells. But in early chemical evolution, before there were enzymes, was there a role for phosphite? This has not yet been extensively studied. Incorporation of phosphate into the fabric of early protobiochemical synthesis still presents a few sticking points in early metabolic evolution (Martin, 2020), environmental phosphite offers some alternative entry routes (Buckel, 2001).

Finally, the relevance of phosphite is underscored by the circumstance that it is a growth substrate for microbes, specifically for anaerobic autotrophs (Schink and Friedrich, 2000). Schink et al. (2002) characterized *Desulfotignum phosphitoxidans*, that grew autotrophically with phosphite as the sole electron donor and CO_2 as the sole electron acceptor. The energy-yielding metabolic reaction was the synthesis of phosphate with some acetate being produced as well. *D. phosphitoxidans* can also grow as a sulfate reducer that uses the acetyl-CoA pathway. In the absence of sulfate, its metabolism was that of an acetogen with energy generated by CO_2 reduction to acetate but using phosphite instead of H_2 as a source of electrons (Schink et al., 2002). Another phosphite oxidizer was recently isolated, but it was an autotrophic phosphite specialist — the strict anaerobe grew as a phosphite-dependent autotroph, using only phosphite as the electron donor and only CO_2 as the electron acceptor (Mao et al., 2021).

Buckel (2001) suggested that the highly exergonic process of phosphite oxidation might be coupled to substrate level phosphorylation, which is thermodynamically possible, but has not yet been shown so far. Phosphite in the environment appears to be derived from serpentinization. It is the only reductant other than H_2 that is known to fuel chemolithoautotrophic growth using the acetyl-CoA pathway. This links phosphite to serpentinization and ancient acetogenic physiology. Phosphite has a sufficiently negative midpoint potential that electron bifurcation would not be needed to reduce ferredoxin in cells that use phosphite, but its enzymatic oxidation product is NADH, which would require electron bifurcation for ferredoxin reduction.

5. Serpentinization, biochemical networks, physiology, and autotrophic origins

Under theories for autotrophic origins, the first free-living cells were able to synthesize all of their components from CO_2, a reductant (H_2), a nitrogen source and inorganic salts. It is a long way from the $H_2 + CO_2$-dependent synthesis of formate, acetate and pyruvate to the synthesis of free-living autotrophic cells, which require on the order of 1,500 genes and proteins to survive. Chemical reaction systems called autocatalytic networks are typically seen as intermediates in that evolutionary transition. Autocatalytic networks are objects of molecular (self-)organization. They involve catalytic properties of compounds within the network that act as simple catalysts to accelerate reactions within the network such that more products within the network, hence more catalysts, arise from the starting compounds (typically called the food set for the network) (Hordijk et al., 2011). Autocatalytic sets called RAFs (reflexively autocatalytic food-generated networks) are of particular interest for origins because they can be easily modeled on the computer (Hordijk and Steel, 2017) and because they can be detected in the metabolic maps of modern microbes (Sousa et al., 2015). Xavier et al. (2020) detected RAFs in the metabolic maps of a well-curated acetogen and a well-curated methanogen. The sizes of those networks were 394 reactions (acetogen) and 209 reactions (methanogen) respectively. The acetogen and methanogen RAFs overlapped by 172 reactions that correspond to the RAF of their last common ancestor, which is effectively the RAF of the last universal common ancestor LUCA, which was highly enriched for transition metal catalysts and carbon metal bonds.

Note that the structure of RAFs (mathematical constructs) and the structure of real metabolic maps are not identical, such that a number of simplifying assumptions have to be made in order to apply RAF-detecting algorithms to real metabolic maps; Sousa et al. (2015) spelled out 11 simplifying assumptions that need to be taken into account when identifying RAFs in metabolic maps. The RAF of LUCA had the interesting property that RNA nucleobases arise from metabolic networks, but metabolic networks do not arise from bases, in line with a metabolism first view of origins as opposed to an RNA first view (Xavier et al., 2020). Xavier and Kauffman (2022) recently found that small-molecule autocatalytic networks are present in over 6,000 metabolic maps investigated, suggesting that autocatalytic networks are not only ancient, tracing to LUCA, but that they are also universal in metabolism.

The requirement for catalysis in RAFs means that cofactors are highly represented in RAFs detected computationally and that essential products that are required for life might not be included in a RAF. For example, the RAF of LUCA (Xavier et al., 2020) did not generate all amino acids or cofactors. How many reactions did primordial metabolism encompass? Autotrophs would need to synthesize all small molecules of metabolism themselves. Wimmer et al. (2021a) found that only 404 reactions are required to synthesize the 20 canonical amino acids, the bases of RNA and DNA (excluding modifications) and the 18 cofactors of ancient metabolism from H_2, CO_2, NH_3, H_2S, H_2O and P_i. Furthermore, 97 % of those reactions were exergonic under the conditions of a serpentinizing hydrothermal vent (alkaline, high pH, H_2 as a reductant), indicating that there is a natural tendency for reaction of metabolism to unfold under far from equilibrium hydrothermal conditions (Wimmer et al., 2021b). A theoretical study by Nunes Palmeira et al. (2022) involving autocatalysis in a simplified metabolic model concluded that various lines of evidence indicate that metabolism emerged from a geochemical protometabolism fueled by H_2 and CO_2.

Reconstructions of metabolism are also in line with an autotrophic origin of life at hydrothermal vents. Mei et al. (2023) and Williams et al. (2017) found that the ancestral physiology of archaea was likely hydrogen-dependent methanogenesis. Xavier et al. (2021) found that the ancestral physiology of bacteria was likely hydrogen-dependent acetogenesis. Coleman et al. (2021) found that the acetyl-CoA pathway reconstructs to the root of bacterial phylogeny although there is some discussion about the method they employed to infer the position of the root in the bacterial tree (Bremer et al., 2022). Schöne et al. (2022) found that with considerable genetic manipulation, a methanogen could be converted into acetogenic physiology, reflecting ancient physiological connections between the two groups and possibly uncovering an ancestral state of ATP synthesis via substrate level phosphorylation prior to the origin of chemiosmotic ATP synthesis. These findings that trace the exergonic reactions of H_2 with CO_2 in acetogenesis and methanogenesis to the first cells are consistent with genomic reconstructions of LUCA indicating that LUCA arose from similar reactions at a serpentinizing hydrothermal vent and that the most ancient lineages of anaerobes are acetogens and methanogens (Weiss et al., 2016). While there are numerous genomic reconstructions of LUCA present in the literature, they almost all focus on attributes and processes that are universal or nearly so among cells such as protein synthesis, ribosomal proteins, conserved pathways, and nucleic acids (Goldman et al., 2013) or genetics and lateral gene transfer (Crapitto et al., 2022). The studies of Weiss et al. (2016, 2018) took a different approach by looking carefully at physiology rather than universal gene distribution, in order to extract information about the environment in which LUCA arose and diversified and the presence of ancient physiological attributes such as transition metal catalysis, autotrophy, exergonic H_2-dependent CO_2 reduction, geochemical methyl groups, substrate level phosphorylation, and chemiosmotic ATP synthesis utilizing geochemical ion gradients. The studies of Weiss et al. (2016, 2018) are sometimes criticized for inferences about thermophily and use of the term "progenote "(Gogarten and Deamer, 2016), but no other study of LUCA makes a statement on the origin of ATP to drive any of the processes that are ascribed to LUCA by gene and genome-based inference. To drive ATP synthesis, exergonic chemical reactions of compounds in the environment have to be harnessed and the energy conserved as a biologically useful form such as thioesters, acyl anilides, acyl phosphates or ATP. In our model for LUCA and origins, LUCA's energy conservation and ATP synthesis comes from serpentinization.

5.1. Serpentinization: brimming with the energy of life

There are only two basic ways that cells conserve energy as high energy phosphate bonds: substrate level phosphorylation (Decker et al., 1970) and chemiosmotic energy harnessing using a rotor-stator ATP synthase (Walker et al., 1982; Walker, 2013). Serpentinizing systems provide a chemical environment that can support the origin of both forms of biological energy conservation.

Substrate level phosphorylation usually involves the generation of an acyl phosphate (or enol phosphate) bond during the oxidative breakdown of reduced carbon compounds. The classical example is the synthesis of the acyl phosphate bond in 1,3-bisphosphoglycerate at the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase by oxidizing the aldehyde group on C1 of glyceraldehyde-3-phosphate with NAD^+ to form a thioester bond between the enzyme and the substrate, which is cleaved via phosphorolysis, generating the acyl phosphate bond at C1 which can phosphorylate ADP to generate ATP. There are also reductive routes of SLP, for example the conversion of the thioester bond in acetyl-CoA from the (reductive) acetyl-CoA pathway to acetyl phosphate, which can also phosphorylate ADP. Although the direct synthesis of acyl phosphates from thioesters has so far not been reported, it has long been known that acyl phosphates can phosphorylate ADP without enzymes (Kitani et al., 1991). More recently, Whicher et al. (2018) have shown that thioacetate can readily react with P_i to form acyl phosphates without enzymes, although thioacids are not known in central carbon or energy metabolism. The synthesis of acetate and pyruvate from H_2 and CO_2 under serpentinizing vent conditions using catalysts synthesized at hydrothermal vents — awaruite and magnetite

— is facile whereby there is furthermore enough energy released in the synthesis of acetate to energetically support SLP (Preiner et al., 2020; Belthle et al., 2022; Beyazay et al., 2023a,b). Reductive SLP also takes place via acetyl phosphate synthesis at the glycine reductase reaction (Andreesen, 2004) and the subsequent phosphorylation of ADP. Otherwise, net ATP synthesis involves either SLP via oxidation reactions of organic compounds (Müller, 2003; Martin and Thauer, 2017), which are thermodynamically unfavorable under the reducing conditions of serpentinizing systems, or it involves chemiosmotic ATP synthesis using the rotor-stator ATP synthase.

The rotor-stator ATP synthase is universal among cells. It generates ATP by the passage of protons (or Na^+) from the outside of the cell through the ATP synthase to the inside of the cell. The passage of protons through the ion channel of the stator subunit *a* and the subsequent protonation and deprotonation of an acidic residue in each of the c subunits of the ring-shaped rotor sets the rotor of the ATP synthase in rotation, inducing conformational changes in the catalytic subunits of the "head," changes that forge a phosphoanhydride bond between ADP and P_i and lead to release of ATP from the enzyme. Three ATP molecules are synthesized per complete 360° rotation of the head, this requires roughly 12 protons traversing the enzyme. This mechanism of ATP synthesis is universal in bacteria and archaea, it is furthermore reversible such that cells can expend ATP that they obtain from SLP to generate ion gradients for the import of small molecules (Franklin, 1986) or to generate reduced ferredoxin (Buckel and Thauer, 2013). The ion gradients that modern cells use to power chemiosmotic ATP synthesis are generated by membrane integral ion pumping proteins, or coupling sites, that pump protons (or Na^+) from the inside of the cell to the outside. There are two ways that pumping can be achieved: i) with the help of conformational changes within membrane-integral domains, and ii) with the help of what Mitchell called vectorial chemistry (Mitchell, 1991). In the former, the general mechanism (conformational change) is the same as that for the ATP synthase, except that the energy for conformational change and pumping stems from exergonic redox reactions that are part of the overall bioenergetic reactions of the cell, for example methane synthesis from H_2 and CO_2 (in methanogens) or acetate synthesis from H_2 and CO_2 (in acetogens). The second, evolutionarily more advanced mechanism (vectorial chemistry) requires the existence of quinones or quinone analogs (Berry, 2002), membrane soluble redox factors that can accept an electron pair and two protons on the cytosolic side and transfer the electron pair to an acceptor on the outside of the cell, thereby transferring protons from the inside of the cell to the outside merely by the relative position of hydride donor and hydride acceptor across the membrane. Ancient lineages of methanogens and acetogens lack quinones. They generate their ion gradients solely via conformational changes in membrane proteins (pumping).

It is a very curious observation that the principle of harnessing ion gradients for ATP synthesis and the enzyme that does it, the rotor-stator ATP synthase, are as universally conserved across all bacteria and archaea as the ribosome and the genetic code itself. This indicates that ion gradient harnessing was present in LUCA. LUCA could *use* ion gradients. Yet there is no similar form of conservation to indicate that LUCA could *generate* ion gradients. But if LUCA arose and existed at a serpentinizing vent, as much other evidence summarized above indicates, there is also no need to assume that LUCA had to generate ion gradients by itself, because serpentinization generates geochemical ion gradients continuously and for free that remain stable for the duration of the serpentinization process. Recalling that Lost City has been actively serpentinizing for roughly 100,000 years (Ludwig et al., 2011; Denny et al., 2016), a stable geochemically generated pH gradient of the right polarity (alkaline, lower proton concentration on the inside) and sufficient magnitude (cells synthesize ATP with the help of a pH difference of about one pH unit) (Tran and Unden, 1998; Silverstein, 2014) would present a continuous environmental setting in which natural chemiosmotic potential existed and could be harnessed (Martin and Russell, 2007; Martin, 2012).

This natural chemiosmotic energy source would have allowed the ancestral ATP synthase to function but would have required the evolution of protein based pumping mechanisms in the ancestors of the archaea and the bacteria in order for LACA (Last Archaeal Common Ancestor), (a methanogen) and LBCA (Last Bacterial Common Ancestor) (an acetogen) to make the transition to the free-living state. Methanogens without cytochromes pump with energy derived from H_2-dependent CO_2 reduction. The pumping reaction takes place at the methyl transferase step catalyzed by the membrane protein MtrA-H, which in a cobalamine-dependent reaction transfers a methyl group from a nitrogen atom in methyl-H_4MPT to a sulfur atom in coenzyme M; the free energy of the reaction $\Delta G_o'$ is $-30\,kJ\cdot mol^{-1}$ and pumps two Na^+ ions from the inside of the cell to the outside (Thauer et al., 2008). This energy conserving reaction is universal among H_2-dependent methanogens and likely represents their ancestral state. In acetogens without cytochromes, the pumping reaction is energetically driven by H_2-dependent CO_2 reduction and is catalyzed by the membrane protein Rnf, which pumps Na^+ from the inside of the cell to the outside while transferring electrons from reduced ferredoxin to NAD^+ (Biegel et al., 2011) with a $\Delta G_o'$ of $-34.7\,kJ\cdot mol^{-1}$ (Kuhns et al., 2020).

By coupling H_2-dependent CO_2 reduction to ion gradient formation, cells were able to generate their own ion gradients, were no longer dependent on geochemical ion gradients for ATP supply and could make the transition to the free-living state. The machinery to generate ion gradients by coupling exergonic reactions of H_2-dependent CO_2 reduction to ion pumping arose independently in the lineages leading to LBCA and LACA, because different steps were harnessed and unrelated enzymes were used. Note that both acetogens and methanogens require flavin based electron bifurcation to generate reduced ferredoxin for CO_2 reduction (Buckel and Thauer, 2018; Müller et al., 2018), but this is not required in CO_2 reduction promoted by metal catalysts (e.g., Ni_3Fe, Fe_3O_4) because of the very low midpoint potentials for H_2 oxidation that are generated by very high pH (Martin, 2022). A summary of energy inputs from serpentinization into the origin of biochemistry is given in Figures 3,4.

6. Serpentinization in our solar system

Since olivine is the most abundant silicate mineral that condensed during the formation of the solar system, ultramafic rocks are not unique to Earth and probably ubiquitous in most, if not all, rocky bodies of the solar system (McCollom and Seewald, 2013). Besides Earth there are currently two other solar system bodies where there is evidence for serpentinization: Mars and the Saturn moon Enceladus (Oze and Sharma, 2005; Glein et al., 2015). On Mars ultramafic and

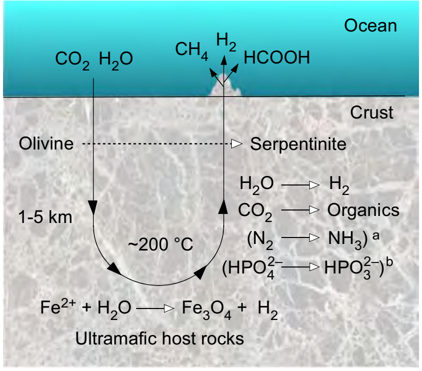


FIGURE 4
Serpentinization is a planetary scale reducing agent. The figure is modified from (Preiner et al., 2018). During serpentinization, surface water (seawater or freshwater) is drawn down into cracks in the crust where it reacts with ultramafic rocks at temperatures of about 200°C. During the reaction, Fe^{2+} in olivine is oxidized by water to Fe^{3+}, generating iron oxides in serpentinite and magnetite, Fe_3O_4, as well as H_2, diffusible reductant (Sleep et al., 2004; Bach et al., 2006; Lang et al., 2010). The reduction process converts water to H_2, CO_2 to formate and methane as well as other organics, and in newer studies of laboratory scale serpentinization, it converts N_2 to NH_3 (Shang et al., 2023b). Simulated geochemical systems using Co and Fe as catalysts can generate hydrocarbons from bicarbonate at high temperature and pressure (He et al., 2021). Although any kind of hydrophobic compounds could serve as insulators for the function of an ATP synthetase, long chain hydrocarbons are an option prior to the origin of genetically encoded enzymes for lipid synthesis. [a]The reduction of N_2 to NH_3 is indicated in parentheses because this has not been directly shown in modern serpentinizing systems, […] but it occurs in laboratory scale serpentinization and very likely occurred in the Hadean crust […] (Shang et al., 2023a,b). [b]Phosphate to phosphite is shown in parentheses because it is a recent discovery that serpentinzing systems might be able to generate it because naturally occurring serpentinized rocks can contain phosphite (Pasek et al., 2022); as with N_2 reduction to NH_3 the reaction has not been demonstrated in serpentinizing systems today.

serpentinized rocks have been found, with the olivine on Mars having an even higher iron content than that found on Earth (Hoefen et al., 2003; Christensen et al., 2004; Ehlmann et al., 2010; Holm et al., 2015; Tutolo and Tosca, 2023). Organic synthesis has been detected in Martian meteorites that date back 4 Ga which likely happened through serpentinization (McCollom and Seewald, 2013; Steele et al., 2022). Since water (in frozen form) has been detected on the surface of Mars, it is likely that it is also present in Mars' crust where it could interact with ultramafic material, which means that serpentinization could occur and H_2 and CH_4 could be produced (Etiope, 2017). Since both gases would disperse immediately in the Martian atmosphere [which mainly consists of CO_2 (Mahaffy et al., 2013)], direct monitoring is the only way these gases could be detected (Etiope and Sherwood Lollar, 2013; Holm et al., 2015). Such measurements have been conducted, for example with the Curiosity Rover (House et al., 2022) and on other missions (Lyons et al., 2005) and through observations from Earth (Mumma et al., 2009), however it is unclear if the detection of methane in some of these measurements originates from a contamination in the method employed by the rover (Schoell, 2022).

Using observational data from the Cassini spacecraft, Glein et al. (2015) constructed a model determining the pH and likely chemical composition of Enceladus. They found that Enceladus' ocean is a Na-Cl-CO_3 solution with a pH of 11–12. Zolotov (2007) also concluded in a different study that the ocean of Enceladus has a hyperalkaline pH. Enceladus shares the dominance of dissolved Na-Cl with Earth's oceans but the ubiquity of dissolved Na_2CO_3 is more comparable to soda lakes. The high pH is most likely a consequence of serpentinization and after the discovery of H_2 in the plume of Enceladus (Waite et al., 2017) it is even more likely that serpentinization is still actively occurring. CO_2 and CH_4 has also been detected in Enceladus plume and active serpentinization happening would also explain the presence of other organic species in Enceladus plume (Glein et al., 2015; Waite et al., 2017). In a recent study by Postberg et al. (2023) orthophosphates have been found in ice grains of Enceladus plume. The concentrations found indicates that Enceladus ocean has about a 100 times larger abundance of phosphates than Earth's oceans, with a phosphate: phosphite […] 10:1, since phosphates are more stable under the alkaline conditions of Enceladus ocean. Of the elements considered essential for life (CHNOPS), phosphorus is found the least in astronomical observations and Enceladus is the first ocean world in our Solar System where it has been detected. The availability of phosphorus has been considered a bottle-neck for bio-essential elements on Enceladus and other icy moons and with the discovery of phosphorus in its oceans, Enceladus satisfies yet another requirement for potential habitability.

Other solar system bodies where it is strongly suspected that serpentinization might be happening are other icy moons of the gas giants (Jupiter, Saturn, Neptune, Uranus) (Vance et al., 2007; Schrenk et al., 2013; Vance et al., 2016; Lunine, 2017). Basically, wherever ultramafic rocks get in contact with circulating liquid water with temperatures below 350°C, serpentinization will occur (McCollom and Seewald, 2013). However, unlike with Enceladus, where we are lucky enough to have direct data from the plume because of the Cassini Mission, no direct measurement could be taken yet at these other icy moons and only theoretical models exist (Vance et al., 2007, 2016). It is however likely that we will have data of Europa soon, an icy moon of Jupiter, because missions have been planned from both ESA and NASA, with ESA's JUICE mission having launched earlier this year (ESA, 2023).

7. Conclusion

We return to the passage in the introduction of this paper about meteorite impacts as surface reduction processes vs. reduction in the crust. Available data indicate that the crust can generate reduced nitrogen in serpentinizing systems (Nobu et al., 2023; Shang et al., 2023b) and phosphite during serpentinization (Pasek et al., 2022), with carbon and sulfur reduction reactions being facile under serpentinizing conditions. From that it follows that all of the essential reduction reactions underlying the conversion of the elements on the early Earth from their ancestrally oxidized states (CO_2, N_2, HPO_4^{2-}, SO_2) to their biologically relevant state — reduced C, NH_3, H_2S, and in the case of P, the biochemically accessible state — now appear to lie within the range that serpentinizing systems can generate naturally (Russell et al., 2010; Boyd et al., 2020). In traditional cyanide-based RNA-world theories, the reducing functions required at origins are attributed to meteorite impacts or UV-dependent reactions. In subsurface origins theories, serpentinization provides all the reducing power needed for organic synthesis and life. In the subsurface theory, the first organisms were anaerobic chemolithoautotrophs that arose, lived and diversified in complete darkness within the walls of serpentinizing, hence strongly reducing, hydrothermal vents. Such a scenario is much in line with what many microbiologists have thought for many decades. It would also be compatible with the environments presented by a growing number of moons and planets that lie beyond the confines of our atmosphere.

Serpentinization occurs at many sites on Earth and probably elsewhere in our solar system. It is relevant to the study of the origin of life and astrobiology for many reasons outlined here, but mainly because it is a source of a strong and diffusible reductant: H_2. It generates favorable conditions for organic synthesis and the emergence of life. It synthesizes catalysts that can act as the precursors to enzymes and electron donors that provide energy for metabolism and simple carbon compounds as a nutrient source. Microbial communities of serpentinizing systems, such as Lost City and Old City, provide what could be windows into the physiology of LUCA. The microbial communities of continental and shallow serpentinizing systems like Prony Bay might provide the same kind of window into the physiology of the first microbes as acetogens and methanogens serve as primary producers. Despite the differences between serpentinization sites on land and in the oceans, there is enough overlap between the geochemically produced compounds, the physiological conditions such as the pH and the temperature, as well as the microbial communities, that continental serpentinizing systems can serve as important proxies for the less easily accessible and less abundant deep-sea systems as well as the currently inaccessible serpentinizing systems beyond the confines of Earth.

Author contributions

LS: Conceptualization, Data curation, Visualization, Writing – original draft, Writing – review & editing. MB, Writing – review & editing. NM: Writing – review & editing. JW, Data curation, Visualization, Writing – review & editing. MP: Data curation, Visualization, Writing – review & editing. WM: Conceptualization, Data curation, Funding acquisition, Project administration, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1257597/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Linear regression between the H_2 and CH_4 concentrations in the serpentinizing systems in this study. Here we show a linear regression of hydrogen and methane concentrations of the serpentinizing systems in this study when both compounds were reported or detected. Since mostly ranges of concentrations are reported in Table 1, the maximum concentrations reported were used for the linear regression shown in this figure for simplicity. During the analysis two data points stood out. **(A)** At first there seems to be no relationship between the hydrogen and methane concentrations between the different serpentinizing systems in this study, with $r^2 = 0.0551$ and $p = 0.4626$. However, the Prony Bay data reported by (Twing et al., 2022) in Table 4 of their study stands out in contrast to the others and after checking the sources that (Twing et al., 2022) used for Table 4, it seems like they accidentally reported mM amounts of hydrogen and methane for Prony Bay even though the two sources they cite for that data ((Quéméneur et al., 2014) (Postec et al., 2015)) report hydrogen and methane in vol% in their supplementary material. It also contradicts the data reported by (Quéméneur et al., 2023) where the hydrogen and methane concentrations are a magnitude lower, and the ratio of the abundances is also different. **(B)** Linear regression of the serpentinizing systems in this study without the Prony Bay data from (Twing et al., 2022) with $r^2 = 0.0596$ and $p = 0.0136$. This already suggests a high significance of the relationship between the concentrations for hydrogen and methane, however Ashadze still seems to be a clear outlier. **(C)** Linear regression of the serpentinizing systems without the data from Prony Bay (Twing et al., 2022) and Ashadze (Schrenk et al., 2013) with $r^2 = 0.8419$ and $p = 0.0002$.

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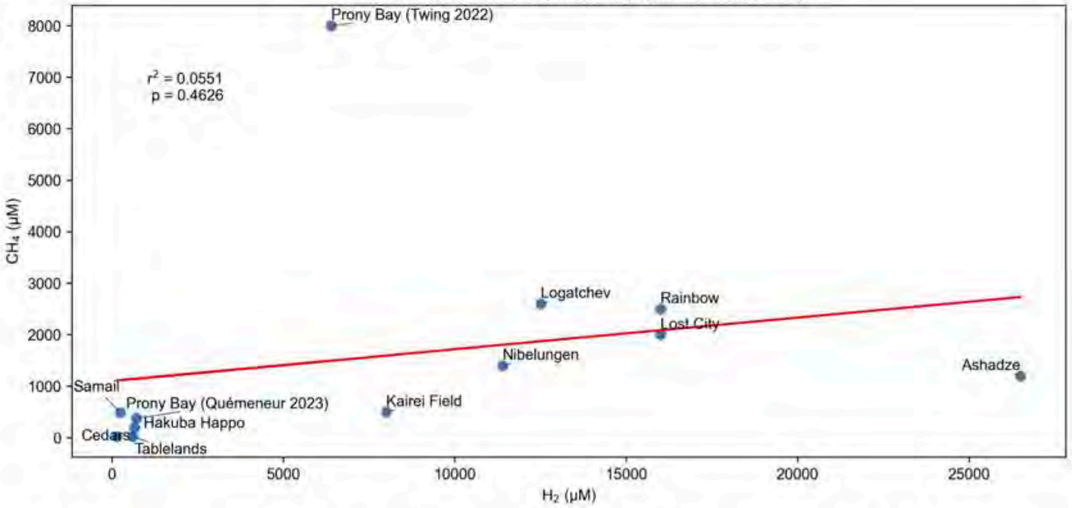
Supporting Information Publication 1

A



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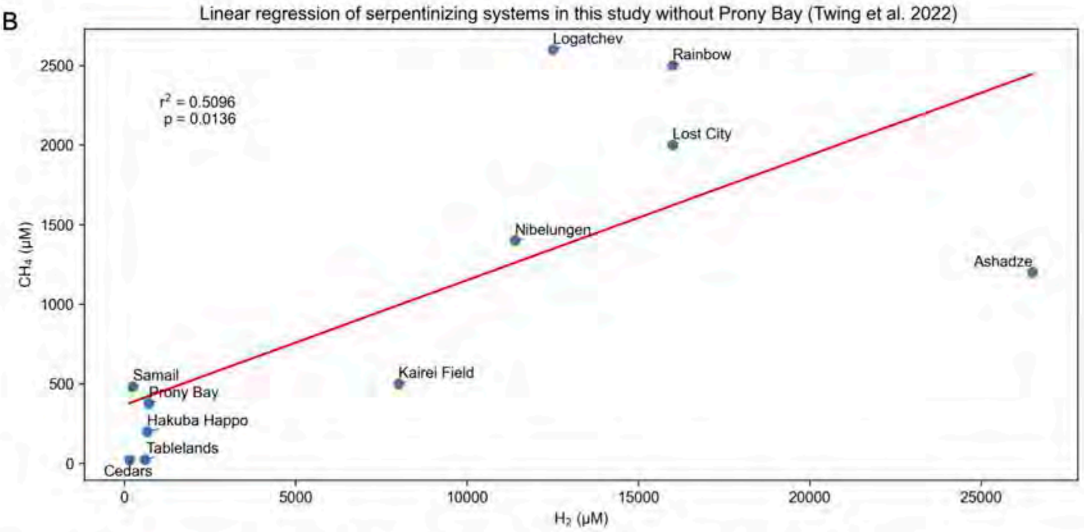

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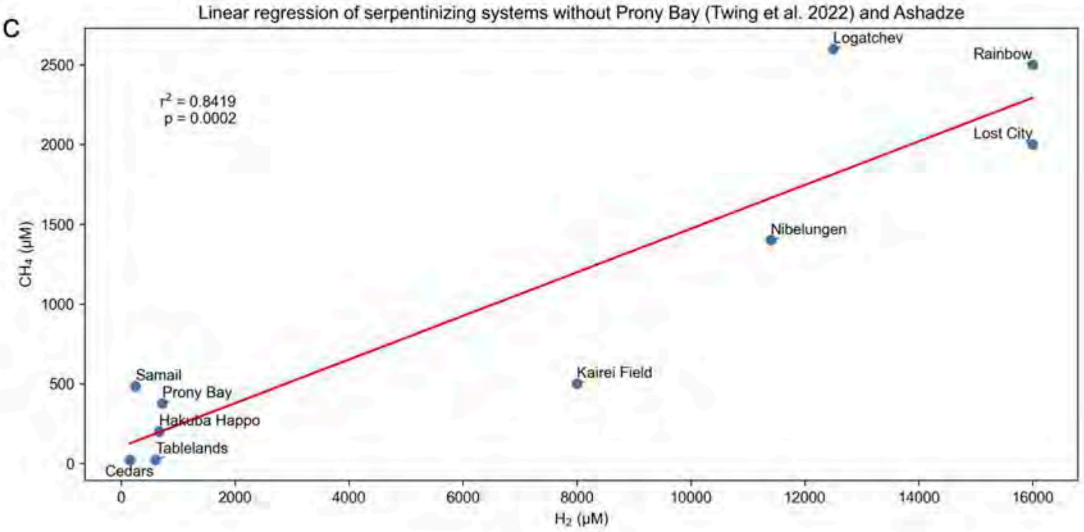

10.2 Publication 2

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The Moon-Forming Impact and the Autotrophic Origin of Life

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The Moon-forming impact vaporized part of Earth's mantle, and turned the rest into a magma ocean, from which carbon dioxide degassed into the atmosphere, where it stayed until water rained out to form the oceans. The rain dissolved CO_2 and made it available to react with transition metal catalysts in the Earth's crust so as to ultimately generate the organic compounds that form the backbone of microbial metabolism. The Moon-forming impact was key in building a planet with the capacity to generate life in that it converted carbon on Earth into a homogeneous and accessible substrate for organic synthesis. Today all ecosystems, without exception, depend upon primary producers, organisms that fix CO_2. According to theories of autotrophic origin, it has always been that way, because autotrophic theories posit that the first forms of life generated all the molecules needed to build a cell from CO_2, forging a direct line of continuity between Earth's initial CO_2-rich atmosphere and the first microorganisms. By modern accounts these were chemolithoautotrophic archaea and bacteria that initially colonized the crust and still inhabit that environment today.

1. Formation of the Earth-Moon system, early atmospheres and the origin of life

1.1. On the early Earth and the Moon-forming impact

Thoughts on the origin of life are tightly linked to inferences about the chemical environment of the early Earth in a young solar system. The modern Earth is a rocky planet in the habitable zone of the solar system with surface liquid water and an oxidizing atmosphere composed predominantly of N_2. It harbors tectonic activity and a magnetic field, both consequences of geophysical processes in the Earth's interior.[1–2] But what about the early Earth?

In the standard model, the Earth formed by accretion of smaller bodies and planetesimals over 4.5 billion years ago (Ga), during a relatively short period of time after the formation of the solar system, accompanied by differentiation: the partitioning of siderophile (iron-loving) elements into the core and lithophile (rock-loving) elements into the silicate mantle.[3–4] The most widely accepted hypothesis for Moon formation is the giant impact hypothesis (Figure 1), which posits that the Moon formed by the collision of a giant Mars-sized impactor named *Theia* with the proto-Earth.[5–7] This occurred shortly after the formation of the solar system, the impact event dating to roughly 4.50 Ga[8] or perhaps as late as 4.35 Ga according to newer findings.[9] The energy released at impact melted the Earth's mantle into a magma ocean. An estimated 20% of the mantle was vaporized.[8] The atmosphere of rock vapor formed this way was accompanied by degassing volatiles (some N_2 but mostly H_2O and CO_2) that were poorly soluble in the magma ocean, which underwent differentiation.[10–12] The degassing continued as the mantle cooled and solidified over 2–10 million years, giving rise to a secondary steam atmosphere.[8,11]

Isotopic evidence indicates that the Earth's silicate mantle was oxidized, such that its degassing resulted in a secondary atmosphere that was oxidizing, not reducing.[13–14] This is in agreement with evidence from Hadean oxygen fugacities from zircon crystals.[15] Highly reduced gases such as CH_4 and NH_3 could have been present transiently, at best, and with short lifetimes, dissociating due to photolysis.[12–13,16–17] Some models suggest the existence of a series of impactors that followed the Moon-forming impact in order to explain the higher-than-expected concentrations of siderophile elements in the Earth's mantle (the late veneer) and the concentration of Earth's volatiles as well.[18–19] Other models entail only one additional impactor after Moon formation.[20–21] In an origins context, the main role of these additional impactors is to chemically alter the atmosphere, making it transiently more reducing.[21–22] These additional impactors need to have specific sizes and specific compositions (different types of chondrites have been suggested) if they are to generate the observed veneer and atmospheric NH_3 and CH_4.[21,23–24] Alternatively, it has also been suggested that the source of the excess siderophile elements, whose existence gave rise to the idea of a late veneer in the first place, could have simply been the core of Theia – the

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Moon-forming impactor itself[25] – such that no additional impactors other than Theia are needed to explain the observed mantle composition of some siderophile elements.[26] In that case, there was no late veneer. Recent results by Grewal et al. indicate that the Moon-forming impactor could, on its own, account for the abundance of volatiles on Earth as well.[27] It is thus possible that the series of impactors corresponding to the late veneer never took place, as the evidence for their inferred existence can be explained by the Moon-forming impact itself.[24–25,27]

1.2. On the secondary atmosphere and the origin of life

The vast majority of water on Earth is thought to predate the Moon-forming impact, stemming from the accretion period.[8,28–29] Water that degassed from the Moon-forming impact generated an atmosphere rich with water vapor. As the Earth cooled, the water vapor condensed and rained to form the oceans. Isotopic evidence from detrital zircons date liquid water on Earth to 4.4–4.3 Ga.[30–31] This left behind a ~100 bar CO_2-rich atmosphere and a surface temperature of about 500 K.[11] The model by Sossi et al., in which an atmosphere equilibrated with the magma ocean was allowed to cool, indicates that the Earth's atmosphere following cooling was very similar to that of Venus: mostly CO_2 (~80 bars) and some N_2 (~2 bars).[17] Such high CO_2 concentrations are expected to mitigate the faint young Sun problem and help maintain liquid water on Earth's surface through a greenhouse effect.[11,16,32]

Eventually the CO_2 in the Earth's atmosphere dissolved in oceans of liquid water, rendering them slightly acidic. Dissolved CO_2 then precipitated as carbonates, which were sequestered in the mantle by subduction. Most of the atmospheric CO_2 was likely subducted by 3.8 Ga.[11] After that the atmosphere was mostly N_2, like today, but lacking the O_2 component, which did not come about until the origin of cyanobacterial photosynthesis some 2 billion years later. To summarize, as Zahnle et al. put it *"geological evidence suggests that Earth's mantle has always been relatively oxidized and its emissions dominated by CO_2, H_2O, and N_2"*[22] or as Sossi et al. put it, Earth likely had *"a prebiotic terrestrial atmosphere composed of CO_2-N_2, in proportions and at pressures akin to those observed on Venus."*[17]

How is this relevant for the origin of life? As a consequence of the Moon-forming impact, much, most, or virtually all of the carbon on the proto-Earth was converted to CO_2 and degassed into the atmosphere, likely going through a short-lived intermediate high temperature phase containing large amounts of CO.[17] The Moon-forming impact converted Earth's carbon into CO_2, which is a pure, clean, homogeneous gas that is available to react in the presence of suitable catalysts, providing an excellent starting point for organic synthesis. Today, CO_2 serves as the entry point of carbon into the carbon cycle, it is

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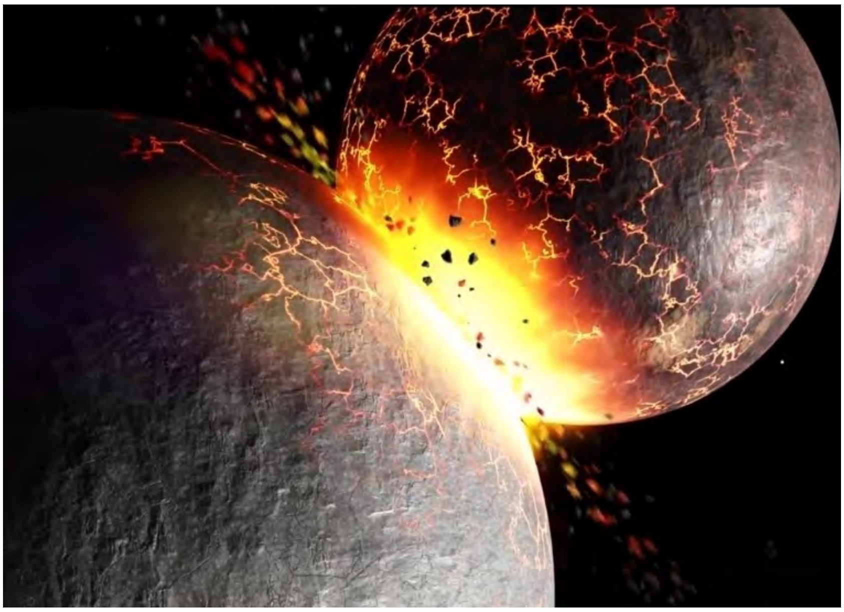


Figure 1. The Moon-forming impact. An artist's impression of the collision of Theia with Earth. Credit: STEP-ANI-MOTION Studio für Computertrick GmbH, Cologne, Germany.

the starting point of primary production in all of life's ecosystems. Under autotrophic theories for the origin of metabolism, the same was true at life's onset as well. CO_2 is of course inert by itself, but it is readily converted into organic compounds, provided that a sufficiently strong reductant (H_2) and suitable catalysts (transition metals) are present. It is the simplest carbon source for life, and the only carbon source required for autotrophic life.

2. Autotrophic origins, starting from CO_2

Life (cell mass) is ~50% carbon by dry weight. It is therefore not surprising that theories for life's origins are always tied to sources of carbon on the early Earth. At the most basic level, there are two main schools of thought about origins that differ with respect to the source of carbon and energy used by the first cells. In the literature, this dichotomy is sometimes cast in the terms of autotrophic vs. heterotrophic origins, although a division into metabolism-first vs. genetics-first theories generates roughly the same divide. Metabolism-first theories have been around in various guise for a long time. An early paper that is explicit on the issue, is Eakin[33] from 1963: "*hypotheses in which metabolism antedates enzymes and nucleoproteins have been set forth in broad generalities by several scientists including Anker, Bernal, Calvin, Gaffron, Oparin, and Pirie.*" Eakin was thus also not the first, but he does succinctly express the idea that surfaces predate cofactors, which in turn predate proteins: "*… these ancestral cofactors could and did function catalytically without proteins (just as it is possible to so demonstrate with most modern cofactors), and that these primitive organic cofactors (along with inorganic ions) acting on surfaces were the original "bio" catalysts, active long before specific polypeptides evolved.*"[33]

2.1. Chemolithoautotrophic origins with H_2 as electron source

Autotrophic theories are older than their heterotrophic counterparts. They are also older than metabolism-first theories. They start with Konstantin Mereschkowsky (1910), who like all of his contemporaries knew next to nothing about carbon metabolism (for lack of its discovery), yet still inferred that life arose when the Earth's surface was covered with boiling water, and that the first life forms had to be anaerobic thermophiles capable of synthesizing organics from inorganic compounds without photosynthesis ("*Fähigkeit, Eiweiße und Kohlenhydrate, letzteres ohne Vermittlung des Chlorophylls, aus unorganischen Stoffen zu bilden*").[34] In modern terms, that translates to a chemolithoautotrophic origin of life, even though in 1910 no one knew how cells fix CO_2. After the discovery of the Calvin cycle,[35] the reductive TCA cycle[36] and the acetyl-CoA pathway,[37–38] Georg Fuchs and his team worked out half of the known pathways of CO_2 fixation.[39] The distribution of the acetyl-CoA pathway in distant anaerobic lineages of both prokaryotic domains, its low energy requirements and its versatility for the assimilation of various one-carbon and two-carbon compounds prompted Fuchs and Stupperich to propose that the acetyl-CoA pathway of carbon fixation is the most ancient among the CO_2 fixation pathways known at the time.[40] This meshed well with the presence of the pathway in anaerobic autotrophs that lack cytochromes and that had previously been suggested to be primitive, notably clostridial acetogens[41] and methanogens.[42] It also meshed well with Wächtershäuser's later proposals for autotrophic origins,[43] although it should be mentioned that Wächtershäuser used CO as a starting material in his experiments, rather than CO_2.[44–46] The catalysts he used to test the theory of an iron-sulfur world[47] were Fe and Ni sulfides, which perform one electron reactions. Wächtershäuser argued that the electrons for the first organic syntheses stemmed from the formation of pyrite (FeS_2) and opposed the view that H_2 was the original reductant for CO_2,[43] because the redox potential of H_2 at pH 7 and 1 atm H_2 ($E_0' = -414$ mV) is not sufficiently negative to reduce CO_2.[48]

However, recent studies show that H_2 can readily reduce CO_2 to several intermediates and end-products of the acetyl-CoA pathway using only transition metal catalysts in the laboratory under the conditions of serpentinizing hydrothermal vents, which are realistic environments for prebiotic chemistry and the origin of life under autotrophic theories.[49–51] A curious question arises from those observations – how, from an energetic standpoint, can H_2-dependent reduction of CO_2 be facile in the laboratory if the midpoint potential of H_2 under standard conditions is insufficient?

The answer is that i) serpentinizing systems do not harbour standard physiological conditions (pH 7 and 1 atm of H_2) and ii) that laboratory simulations of CO_2 fixation under simulated hydrothermal vent conditions are typically performed under alkaline conditions in order to simulate the effluents of serpentinizing systems, which present a pH in the range of 9–11 or higher.[52–56] Because the effluent of serpentinizing systems is alkaline and H_2 rich, often 10 mM H_2 and more,[57] and the redox potential of the $2H^+/H_2$ pair is pH-dependent, the redox potential in serpentinizing systems (and laboratory simulations thereof) is on the order of -700 to -800 mV (Table 1).[57–59] This supplies the reducing power necessary for CO_2 reduction to proceed, provided that suitable metal or mineral surfaces such as Ni_3Fe as catalysts are present.[49] The first reduced carbon compounds relevant for the origin of life could have formed from H_2 and CO_2. Serpentinizing systems have immense reducing power within the range of biologically relevant reactions. They can convert CO_2 to organics, N_2 to NH_3, possibly phosphate to phosphite (recently reviewed by Schwander et al.[58]) and might have permitted the reduction of FeS clusters of ferredoxin (E' of ca. -500 mV under cytosolic conditions), before the evolution of hydrogenases.[48]

Another observation favours H_2 as the ancestral reductant. Modern H_2-dependent chemolithoautotrophs such as acetogenic bacteria and methanogenic archaea readily reduce CO_2 with electrons from H_2. This is fully in line with theories for autotrophic origins,[34,39,43] but H_2-dependent chemoautotrophs have to go to great lengths using enzymes that perform flavin-based electron bifurcation in order to generate reduced ferredoxin for CO_2 reduction.[48]

Table 1. Some redox potentials for $H_2 \rightarrow 2H^+ + 2e^-$ (values from Suppl. Table 6 in [59]). Note the large effect of pH on E. This is because at alkaline pH, the reaction of H^+ with OH^- serves as a pulling reaction, influencing the reaction quotient, and consequently the redox potential.

H_2 [atm]	pH	Temperature [°C]	E [mV]
10	10	100/200	−778/−986
1	10	100/200	−741/−939
0.1	10	100/200	−703/−892
10	9	100/200	−703/−892
1	9	100/200	−666/−845
0.1	9	100/200	−629/−798
10	8	100/200	−629/−798
1	8	100/200	−592/−751
0.1	8	100/200	−555/−704
10	7	100/200	−555/−704
1	7	100/200	−518/−657
0.1	7	100/200	−481/−610
10	6	100/200	−481/−610
1	6	100/200	−444/−563
0.1	6	100/200	−407/−516
0.01	6	100/200	−370/−469
0.001	6	100/200	−333/−423
0.0001	6	100/200	−296/−376

2.2. The importance of flavin-based electron bifurcation for early metabolism

Flavin-based electron bifurcation is a mechanism that enables cells to reduce low potential ferredoxin with electrons from H_2 at pH 7 by a flavin that splits (bifurcates) the electron pair from hydrogen across two acceptors: one with higher potential and another one with lower potential. The reduction of the higher potential acceptor is energetically favorable and is coupled to the endergonic reduction of a low potential acceptor which, in the case of CO_2 fixation, is ferredoxin. Though only discovered in 2008,[60] flavin-based electron bifurcation is essential and apparently universal in the physiology of strictly anaerobic prokaryotes.[61–62] The mechanisms of flavin-based electron bifurcation have been studied in some detail.[63–64]

Although not required for nonenzymatic CO_2 reduction by H_2 in alkaline conditions, electron bifurcation allows cells, both modern and ancient ones, to exploit the reductive potential of environmental H_2 at pH 6~7 even at low H_2 partial pressures near 10^{-5} atm[65] (compare Table 1), forging a link between metabolism and environment.[48] The issue of ferredoxin reduction with electrons from H_2 intuitively leads to thoughts about early evolution. Lipmann (1965) wrote *"I find it possibly of relevance that hydrogen activation […] is mediated by one of the more primitive catalysts, the recently discovered ferredoxin."*[41] In a similar vein, Eck and Dayhoff (1966) wrote of ferredoxin (which we today know to be an electron carrier, not a catalyst) "*It catalyzes the synthesis of pyruvate from carbon dioxide and acetylcoenzyme-A. This indicates its involvement with one of the simplest, most primitive synthetic processes in intermediary metabolism, the fixation of CO_2. It participates in nitrogen fixation and hydrogenase-linked reactions.*"[66] The findings that ferredoxin and other proteins with FeS clusters were, in essence, using rocks as their prosthetic group[67] clearly suggested their antiquity.

2.3. Origins in serpentinizing hydrothermal systems

The chemistry of submarine hydrothermal fields[68] aligned well with ideas about early physiological evolution and quickly led to explicit proposals for an origin of life at deep sea hydrothermal vents.[69–70] Theories of H_2-dependent, chemolithoautotrophic origins converge effortlessly with the chemistry of serpentinizing deep sea hydrothermal systems,[71–72] where geochemical reactions driven by and catalyzed by transition metals produce large amounts of H_2 and abiotic formate, the first intermediate of CO_2 fixation via the acetyl-CoA pathway,[52] as well as methane, the end product of methanogenesis via the acetyl-CoA pathway.[73] In serpentinization-dependent autotrophic theories, the main carbon converting geochemical reactions are homologous – similar by virtue of common ancestry – to biochemical reactions in the acetyl-CoA pathway.[74] The underlying premise is that the environment of the early Earth can give rise to biochemicals via geochemical reactions with the help of transition metal catalysts that resemble those in metabolism. This physiological constraint naturally generates a chemically continuous transition from non-life to life.

That brings us back to the Moon-forming impact and the source of carbon for the origin of metabolism, CO_2, and autotrophic theories. The Moon-forming impact gave rise to a pure and indefinitely stable, inert form of carbon in the gas phase, CO_2. In order to give rise to metabolism, CO_2 required activation on the surface of transition metal catalysts and reduction by H_2. In the laboratory, this generates formate, acetate and pyruvate, the backbone of microbial carbon and energy metabolism overnight.[49] Extension of the C3 carbon backbone by further ferredoxin-dependent CO_2 incorporations, in addition to the metal-catalyzed steps in the reverse TCA cycle,[75–77] or via an aldol condensation akin to the one in the gluconeogenic pathway,[78–80] generates the carbon backbones for amino acid biosynthesis. In metabolism, nitrogen is incorporated as NH_3 through reductive amination or transamination of 2-oxoacids at the final steps of amino acid biosynthesis, early metabolic evolution likely followed a similar path.[43,74,80–81]

In many enzymatic biosynthetic reactions, C–N bond formation involves nucleophilic attack of a carbonyl carbon by an amino group nitrogen, and often requires activation through phosphorylation by ATP. Phosphorylation aids the reaction in several ways, inter alia by increasing the electrophilicity of the carbonyl carbon and by generating a good leaving group that acts as a dehydrating agent.[82] However, recent studies show that C–N bond formation can take place in the absence of a phosphoryl donor under hydrothermal conditions using transition metal catalysts.[83] In that study, Ni–Fe nitrides were synthesized under ammonia flow at deep crust temperatures (300 to 400 °C). The inorganic catalysts contained N activated as nitrides, and generated formamide (and acetamide in some cases) over a range of temperatures and pH, starting from CO_2 and either water or H_2 as the electron source. The point here is that under geochemical conditions Fe–Ni catalysts can forge C–N bonds without the participation of phosphate. In addition, recent experiments have shown that serpentinization can be a stable source of ammonia,[84] which is generated during the process from N_2 (present in the primordial atmosphere)[17] and H_2 (produced through serpentinization). The presence of CO_2 accelerates ammonia formation.[84]

2.4. Energetics of prebiotic reactions in serpentinizing hydrothermal systems

The central pillar of autotrophic theories is that the reactions of primitive non-enzymatic microbial metabolism, starting from CO_2, had enough specificity and sufficient flux rates to support the origin of non-enzymatic (metal-catalyzed) protometabolic networks leading to amino acids, nucleobases and cofactors as building blocks and catalysts for further chemical evolution.[85–86] Most of the biosynthetic reactions of core metabolism are exergonic under the conditions of serpentinizing hydrothermal vents,[59] although some remain endergonic and involve the participation of a phosphoryl donor. Potential prebiotic energy currencies have been widely discussed in the literature.[74,87–91] Acyl phosphates stand out as obvious candidates because of their ability to phosphorylate ADP due to their higher phosphorylating potential.[92–93] In addition, acetyl phosphate is a much simpler molecule than ATP, and is synthesized during acetogenesis via the acetyl-CoA pathway.[74,88] Non-enzymatic acetyl phosphate formation from thioacetate has been reported.[94] Thioesters have also been considered as prebiotic energy currencies, not least because they are found as intermediates in reactions leading to substrate-level phosphorylation.[74,95–96] Recent studies suggest the possibility of abiotic thioester synthesis in Hadean deep-sea vent environments.[97] Another form of phosphorus, phosphite, was recently reported in serpentinite rocks, suggesting it can form during serpentinization.[98] It has been known for a while that some bacteria are capable of oxidizing phosphite to phosphate, which has been suggested to be an ancient trait.[99] Buckel proposed an ancient mechanism of substrate-level phosphorylation of ADP by phosphite via an acylphosphite and an acylphosphate intermediate.[100] In addition, phosphite is more soluble than phosphate, presenting a possible solution to the widely discussed 'phosphate problem' at the origin of life.[101] Recent metagenomic studies point to an enrichment in phosphonate and phosphite metabolizing and transporting proteins in microbial communities of reducing hydrothermal systems,[102] suggesting a potential role for phosphite in modern serpentinizing systems and possibly, by inference, at the origin of metabolism.

Some authors argue in favor of wet-dry cycles to circumvent the need for phosphoryl donors in order to make the first dehydration/condensation reactions energetically downhill.[103–105] Wet and dry polymerization is often thought to involve surfaces onto which molecules adsorb[106] increasing local concentrations or enhancing catalytic properties. For example, biological molecules can bind and chelate surface transition metal minerals or silica via carboxylate groups,[107–108] with multiple effects on the reaction parameters, such as making carbonyls more electrophilic, a function typically carried out by phosphorylation.[108]

There is a common misconception that deep-sea vent environments are necessarily high in water activity. Water is actually consumed by very dry rock during the serpentinization process, which can also lead to a local increase in salinity (ionic strength) and a decrease in water activity.[109] Serpentinization slows down and eventually comes to a halt at very low water activities, such that seawater has to diffuse from the outside into the rock pores through newly formed cracks in the crust in order for the process to continue – a scenario reminiscent of wet-dry cycles.[109–110]

Autotrophic theories currently posit that the central reactants of core microbial metabolism tend to unfold from CO_2, H_2 and NH_3 as a set of thermodynamically metastable intermediates in the presence of suitable inorganic catalysts. Given recent advances in laboratory CO_2 fixation with H_2,[49–51,111] in amino acid synthesis with transition metals,[75–76,112–113] in synthesis of nucleotide constituents[114–115] and in metal-dependent redox reactions involving cofactors,[116] such a proposition seems less radical now than it did 20 years ago. Abiogenic synthesis of amino acids has been reported from hydrothermal systems,[53,117] but abiogenic nucleic acid components have not. Nonetheless, congruence and overlap between reactions of serpentinizing systems connect the metabolism of primitive microbes to the chemistry of the early Earth.

3. Heterotrophic origins, starting from more reduced carbon sources

Theories for heterotrophic origins generally take root in the concept of organic soup presented by Oparin and Haldane in the 1920s.[118–119] The experiment by Miller and Urey that generated amino acids and other organic compounds from methane, ammonia and water under electric discharge provided a means of synthesizing organic soup.[120–121] Oró's synthesis of adenine from ammonium cyanide[122] provided a simple chemistry to nucleobases. Generations of chemists used similar conditions in laboratory experiments to synthesize the building blocks of life. In many cases these experiments require reduced starting compounds that could only be provided under a reducing early atmosphere.[21] Sometimes the reaction steps require *uv* radiation, which then ties the entire chemistry to a terrestrial surface,[123] in other cases the location for prebiotic chemistry is not strictly defined.[124] The synthesis of nucleobases from cyanide condensations[122] and nitriles[123,125] naturally joined with the concept of an RNA world, which had emerged with the discovery of catalytic RNAs in the early 1980s[126–127] and aligned well with Spiegelman's and Eigen's experimental and theoretical work from the late 1960s to the 1980s on *in vitro* selection among replicating RNA molecules.[128–132] With RNA demonstrably able to fulfill the informational function of DNA and some catalytic tasks usually attributed to proteins, the ability to synthesize nucleotides and RNA was regarded by many as key to solving the origin of life problem. In an RNA world, the circumstances surrounding the origin of enzymatic metabolism are of secondary importance. Recent studies highlight the potential for very interesting and informationally relevant chemistry at the interface of the RNA and the peptide world,[133] calling strict dichotomies between RNA and peptide evolution into question.

4. Autotrophic, heterotrophic, pros and cons

Divisions in schools of thought about origins are still evident.[134] In the same way that metabolism-first (autotrophic origin) theories fall short (so far) on the laboratory synthesis of nucleobases from CO_2 and NH_3 and therefore lack a mechanistic connection to replication, a shortcoming of the genetics-first view is that crucial ingredients used for the synthesis of nucleobases – nitrile moieties – do not occur in reactants or products of core microbial metabolism. Cyanide and nitriles are very efficient in the laboratory synthesis of bases, as is formamide,[135] but neither formamide nor nitriles occur in the biosynthetic routes used by cells, leaving no options to directly connect modern core biochemistry in an evolutionary inference to an origins scenario that starts from cyanide, nitriles or formamide.

4.1. The origin of reduced carbon compounds in a post-impact atmosphere

In favor of autotrophic origins, CO_2 meshes well with modern life and with primordial atmosphere. CO_2 directly interfaces with metabolism at over 400 reactions.[136] Life on Earth ultimately synthesizes all of its components from CO_2 and the most ancient pathway of CO_2 fixation entails a chemistry that merges seamlessly with that of serpentinizing hydrothermal systems. The requirement for CO_2 is in full agreement with current views regarding the composition of the atmosphere after the Moon-forming impact.[13–14,17] Organic syntheses from CO_2 necessarily require a reductant – H_2, and a nitrogen source – NH_3, for the generation of nitrogenous compounds. Both are continuously synthesized within the crust by serpentinization.[137–138] H_2 is the same reductant that the acetyl-CoA pathway uses and it was the source of electrons for primary production prior to the origin of photosynthesis. Conveniently, serpentinization provides H_2 exactly where it is needed for origins, at hydrothermal vents[74] such that H_2 and

CO_2 interface in an environment where the same minerals that catalyze CO_2 reduction are formed.[138]

In favor of cyanide, nitriles and formamide, they reliably and reproducibly enable the synthesis of nucleobases in the laboratory. If base synthesis is the main criterion for a prebiotic chemical scenario, cyanide and nitriles (and formamide) are the starting materials of choice. But cyanide and nitriles are not among the atmospheric constituents following the Moon-forming impact in current models.[13–15,27] In addition, volcanic plumes do not appear to be significant cyanide sources.[139] The RNA world in its current formulation struggles somewhat with the availability of the necessary precursors for its proposed organic syntheses on the early Earth. Because the early atmosphere was not a reducing atmosphere as Miller and Urey believed,[121] and because the necessary nitrile precursors could not form in an oxidizing atmosphere from CO_2, proponents of heterotrophic origins have suggested that additional impactors following the Moon-forming impact could have transiently transformed the atmosphere from an oxidizing state (H_2O, CO_2, N_2) to a short-lived reducing state (NH_3, CH_4, H_2). This would create an environment that could support origin of life scenarios that require reducing atmospheric conditions[21–22] and reduced precursors such as hydrogen cyanide and other nitriles in contact with sunlight.[21] In extreme formulations, this gives rise to immense and concentrated, but inferred, ’stockpiles’ of cyanide on the early Earth.[140]

4.2. What does LUCA say?

Life is a set of (bio)chemical reactions, the oldest of which date at least to the last universal common ancestor (LUCA). In hydrothermal versions of autotrophic origins, no major shifts in the basic chemical reactions of life are required in the transition from origins to LUCA and later to the first free-living cells: The first CO_2-reducing reactions set the pattern of products in the acetyl-CoA pathway, LUCA lived from gasses in a hydrothermal environment and made extensive use of both transition metals and cofactors, and the first free-living cells were acetogens and methanogens, which use the acetyl-CoA pathway and obtain both their carbon and energy from the reduction of CO_2 with H_2.[74,96,141–142] In this very explicit metabolism-first model, the exergonic reactions fueling the first organic syntheses and the first free-living cells remained constant while the nature of the catalysts changed as evolution progressed.[142] In genetics-first models, the connections between the first organic-synthetic reactions leading to RNA and the energetics of LUCA and the first cells are not readily specified, in part because genetics-first models account in great detail for replication and selection,[143–145] but not for carbon and energy metabolism that underpin the genetic process. The origin of nucleoside phosphates are one thing, the origin of cells, and of life are another.[134] Just as metabolism-first theories still fall short on the origin of genetic coding, genetics-first approaches have yet to naturally dock into the reactions of microbial metabolism.

4.3. Molecular fossils

The antiquity of RNA catalysis is indisputable – the ribosome itself is a ribozyme[146] and the evolution of all modern proteins postdates the origin of the ribosome and the genetic code.[147] However, at present only 21 catalytic RNA molecules are known across all life, according to the Ribocentre database.[148] Even though the catalytic efficiency of ribozymes can be shown to be comparable to protein enzymes for some reactions,[149–151] the types of reactions catalyzed by ribozymes in nature are limited: peptidyl-transfer in the ribosome, transesterifications or phosphate hydrolysis reactions.[152–153] Part of the popular appeal of genetics-first models resides in the application of Darwinian evolution to RNA, invoking natural variation and natural selection among molecules before the origin of cells. In that sense, genetics-first presents a universally tangible evolutionary mechanism – Darwinian evolution – that remains constant across the divide that connects the first organic synthesis to the first replicating cells. Many catalytic RNAs with demonstrable RNA polymerizing activities have been developed in the laboratory,[143,145,154] although the corresponding activities have not been identified in natural cells. If ribosomal RNA arose from replicating RNA, one might ask: where is its complementary strand? Curiously, the first hints for strand complementarity in molecular evolution trace to a protein-coding gene, in which the two complementary strands of the same DNA encode the ancestral forms – called “urzymes” by Carter, Wolfenden and colleagues – of the two classes of aminoacyl-tRNA synthetases.[155–157] In a world where RNA mainly synthesized protein, DNA might be more ancient than most of us currently think.[158]

While in the heterotrophic origins scenario modern ribozymes are seen as molecular fossils of a time when RNA catalyzed a broad set of reactions before the emergence of protein enzymes, autotrophic origins scenarios imply that the role of RNA, albeit important, was always limited to informational processing. In modern autotrophic theories, early metabolic reactions were catalyzed by transition metals, cofactors, and ultimately proteins, not RNA. Most redox reactions in life require redox-active transition metals (Fe, Ni, Co, Mo, Mn) and/or redox active organic coenzymes and prosthetic groups such as NAD^+, FAD, FMN and F_{420}, cofactors which are bound by enzymes today. These coenzymes and prosthetic groups likely represent molecular fossils of ancient chemistry catalyzed by transition metals[49,80,108,113,159–160] and by cofactors[116,161–163] in the time before the existence of genetically encoded peptides.

5. CO_2 and primary production underpin all ecosystems

5.1. CO_2 fixation and early metabolism

All carbon in today’s life ultimately stems from CO_2. In modern ecosystems, primary producers fix CO_2 by any of the seven carbon fixation pathways known.[39,164] The resulting reduced organic compounds comprise cell mass and metabolic end-products that serve as food for heterotrophs. Life forms exist today in environments with supercritical CO_2 concentrations, and such high environmental CO_2 can push metabolism in the direction of CO_2 fixation rather than respiration, if the cell has the proper enzymatic machinery.[165]

In modern ecosystems, the global rate of photosynthetic primary production (CO_2 fixation) is estimated to be roughly 10^5 times greater than that of H_2-dependent chemolithotrophs that populated the Earth in Hadean-Archaean times.[166] The Hadean-Archean rate of roughly 7×10^{11} g of assimilated inorganic C per year, averaged across the surface of the Earth (5×10^{14} m^2) corresponds to about 0.0014 g of C or 0.003 g of cells (dry weight) per m^2 per year or, very roughly, as cell size can vary by more than 2 orders of magnitude depending on growth rate, to about 10^{10} cells per m^2 per year. That is a substantial amount of autotrophic growth in Hadean times, sufficient to support the origin of heterotrophic lifestyles[167] and later photosynthesis.[168]

Primordial primary production had to have been H_2-dependent because there are no other environmentally available electron donors with sufficiently negative redox potentials to reduce CO_2 for growth. New forms of H_2-dependent metabolism are still being discovered in serpentinizing hydrothermal systems.[53] Though native metals such as Fe^0 can serve as the electron source to support growth of acetogens[169] and methanogens,[170] Fe^0 reacts with water to generate Fe^{2+} and H_2 such that both in modern environments and in an origins context, native metals generate H_2 as the reductant. Thus, from a modern perspective, the first autotrophs were probably chemolithoautotrophs, just as Mereschkowsky suspected,[34] but today we can be more specific with regard to their likely metabolism. They were hydrogenotrophic, most probably employing hydrogenotrophic methanogenesis[171–172] and hydrogenotrophic acetogenesis.[54,141]

In methanogens and acetogens growing on H_2, the acetyl-CoA pathway (Wood-Ljungdahl pathway) converts H_2 and CO_2 to formate, which is further reduced through a series of C1 compounds bound to pterin cofactors (tetrahydrofolate in acetogens and tetrahydromethanopterin in methanogens) to a methyl group that is transferred to a corrinoid iron-sulfur protein. This is the methyl branch, catalyzed by six enzymes. The carbonyl branch is catalyzed by only one enzyme, albeit an important one – bifunctional carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS).[39,173] CODH catalyzes the two-electron reduction of CO_2 to CO on its C-cluster (Ni–Fe–S), with ferredoxin as the electron donor.[174–175] The CO molecule diffuses through a gas channel to the ACS subunit. In the ACS active site, a condensation reaction of CO with the methyl group from the corrinoid iron-sulfur protein occurs on a Ni atom in the ACS A-cluster (Figure 2).[173,176] This generates a Ni-bound acetyl group that is removed from the enzyme by coenzyme A via thiolysis. The resulting acetyl-CoA can be converted to pyruvate as the central compound of metabolism by incorporation of one more CO_2 via the ferredoxin-dependent enzyme pyruvate:ferredoxin oxidoreductase (PFOR).[177] About

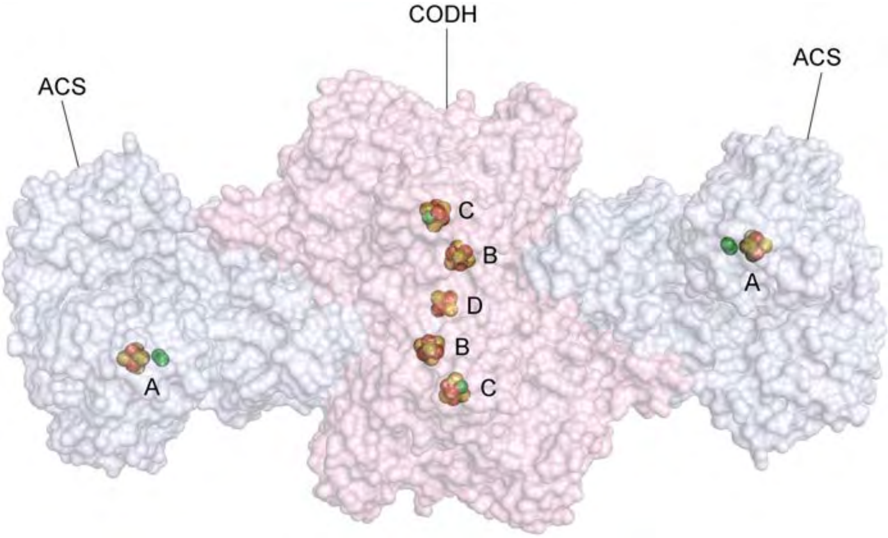


Figure 2. Crystal structure of the conserved bifunctional enzyme carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS) from the acetyl-CoA pathway (PDB ID: 7ZKJ).[181] The CODH homodimer is surrounded on both sides by an ACS subunit. The metal clusters are labeled, namely the D and B cubane Fe_4S_4 clusters of CODH that serve an electron transfer function, the C ($NiFe_4S_4$) cluster of CODH where the reaction occurs, and the active site A cluster of ACS, which is a Fe_4S_4-type cluster bridged to a binuclear Ni–Ni site.[176] The figure was prepared with PyMol (The PyMOL Molecular Graphics System, Version 2.5.4, Schrödinger, LLC).

50 % of the metabolic flux channeled into biosyntheses stems from acetyl-CoA and pyruvate.[39] Pyruvate synthesis allows for the carbon flux to be channeled into gluconeogenesis for sugars and into the incomplete (linear) reverse TCA, generating oxaloacetate and 2-oxoglutarate as central metabolic intermediates (Figure 3).[178–179] The acetyl-CoA pathway is a versatile metabolic route that can be employed in the assimilation of several simple organic compounds, such as formate, methanol, and formaldehyde.[39,180] In addition, it can release enough energy to allow cells to generate ion gradients without involving high-energy phosphorylated compounds. It is the only energy-releasing CO_2-fixation pathway, integrating ATP synthesis with CO_2 fixation, which makes it a likely candidate for the first metabolic pathway on Earth.[40]

5.2. A geochemical analogue of the acetyl-CoA pathway corroborates its antiquity

The acetyl-CoA pathway from H_2 and CO_2 to pyruvate requires 20 enzymes and 14 organic cofactors, coenzymes and C1 carriers (Figure 4). The synthesis of each of these depends on several enzymes (Supplementary Table 1). In total, the synthesis of formate, acetate, pyruvate and methane from H_2 and CO_2 in acetogens and methanogens requires 127 proteins at the bare

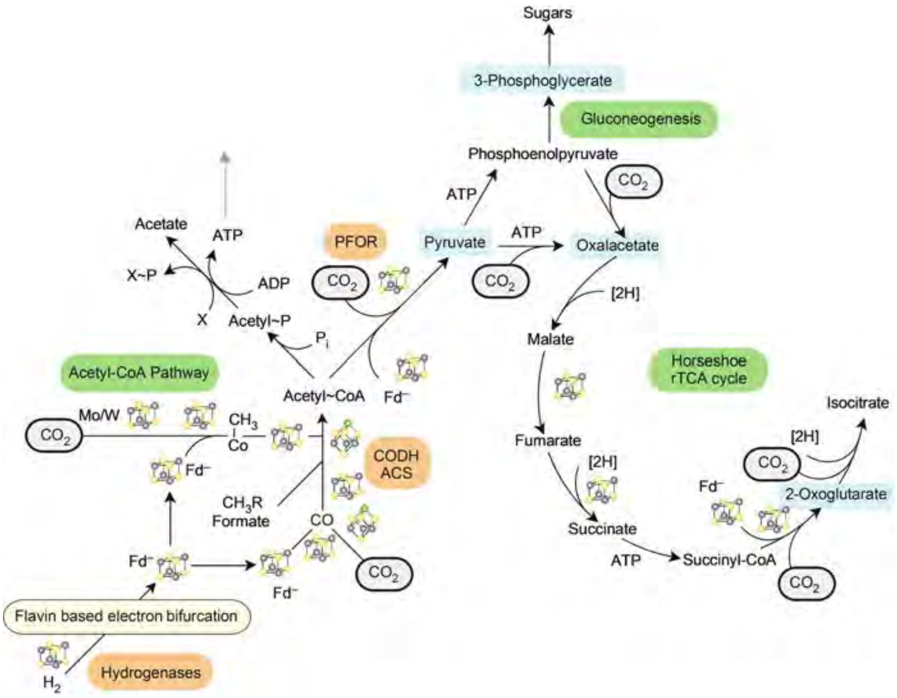
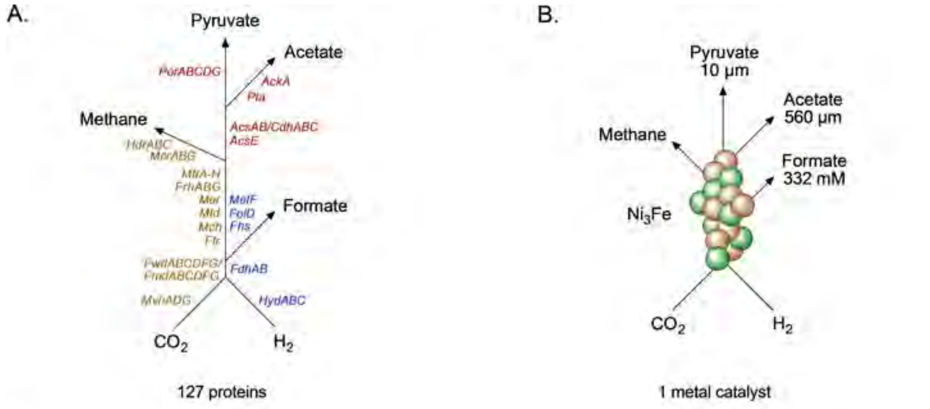
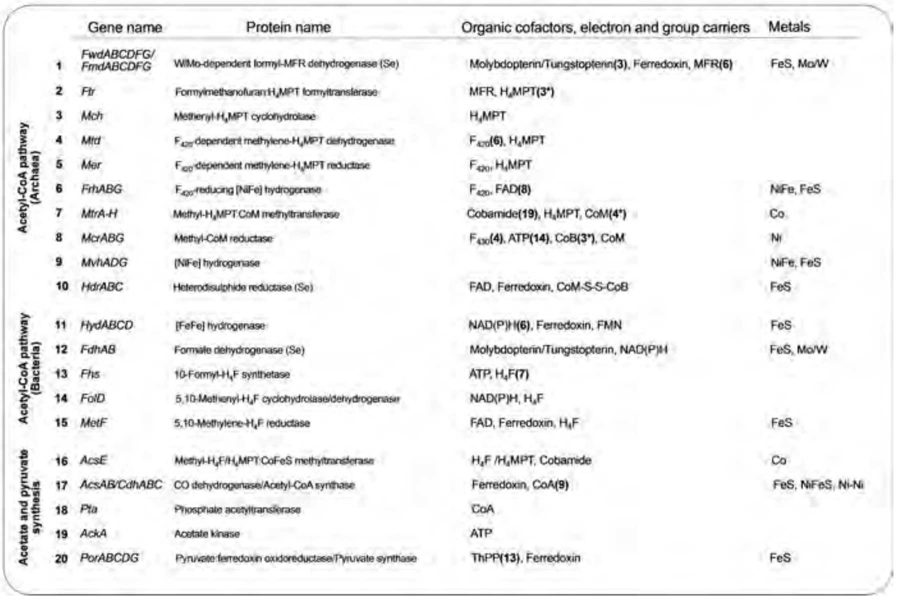


Figure 3. The enzymatic core metabolism of early life forms, modified from[74,179,182]. Environmentally produced H_2 from serpentinization could directly reduce CO_2 made accessible by the Moon-forming impact in metal-catalyzed geochemical reactions under hydrothermal conditions. The transition to an enzymatically catalyzed protometabolism, shown here, involved a gradual decrease in dependency on vent conditions, whereby H_2 became the electron donor for ferredoxin reduction catalyzed by hydrogenases, which employ flavin-based electron bifurcation in order to couple the endergonic reduction of ferredoxin with the exergonic reduction of a higher potential acceptor.[61,63–64] CO_2 is fixed in the acetyl-CoA pathway, with ferredoxin as the electron donor in reactions of both the methyl and the carbonyl branch.[39] While the methyl branch is catalyzed by a series of enzymes that are non-homologous in bacteria and archaea, the carbonyl branch is catalyzed by the conserved bifunctional carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS).[136,174,176,183] The product of the pathway, acetyl-CoA, is a high-energy thioester that can generate phosphoryl donors such as acetyl-phosphate, which has been proposed to be a primordial energy currency,[74,94] and ATP, via substrate-level phosphorylation. These reactions are catalyzed by phosphotransacetylase and acetate kinase, respectively.[184–185] In acetogens (bacteria) and methanogens (archaea) the reduction of CO_2 via the acetyl-CoA pathway is furthermore coupled to the generation of an ion gradient and the harnessing of its electrochemical potential for ATP synthesis by an ATP synthase.[65,186–187] The acetyl-CoA produced in the acetyl-CoA pathway is partly used to generate building blocks. Pyruvate:ferredoxin oxidoreductase (PFOR) catalyzes the reductive carboxylation of acetyl-CoA to pyruvate,[165,177] which is then directed into gluconeogenesis[78,188–189] and the incomplete (linear) rTCA pathway,[178] generating central metabolic intermediates for further synthesis of amino acids, cofactors and nucleobases. Six reactions of the rTCA cycle have been shown to proceed non-enzymatically, catalyzed by transition metals, and three of them (the sequence from oxaloacetate to succinate in the linear rTCA) have been obtained with hydrogen as the reductant.[75,77] Other reports of non-enzymatic variants of the reactions in the figure are mentioned in the text and summarized in[182]. Biological reductants other than Fd⁻ (reduced ferredoxin) are indicated with [2H]. Participation of FeS or FeNiS clusters in the enzymatic reactions is indicated.



C.



		Gene name	Protein name	Organic cofactors, electron and group carriers	Metals
Acetyl-CoA pathway (Archaea)	1	*FwdABCDFG/ FmdABCDFG*	W/Mo-dependent formyl-MFR dehydrogenase (Se)	Molybdopterin/Tungstopterin**(3)**, Ferredoxin, MFR**(6)**	FeS, Mo/W
	2	*Ftr*	Formylmethanofuran:H_4MPT formyltransferase	MFR, H_4MPT**(3*)**	
	3	*Mch*	Methenyl-H_4MPT cyclohydrolase	H_4MPT	
	4	*Mtd*	F_{420}-dependent methylene-H_4MPT dehydrogenase	F_{420}**(6)**, H_4MPT	
	5	*Mer*	F_{420}-dependent methylene-H_4MPT reductase	F_{420}, H_4MPT	
	6	*FrhABG*	F_{420}-reducing [NiFe] hydrogenase	F_{420}, FAD**(8)**	NiFe, FeS
	7	*MtrA-H*	Methyl-H_4MPT:CoM methyltransferase	Cobamide**(19)**, H_4MPT, CoM**(4*)**	Co
	8	*McrABG*	Methyl-CoM reductase	F_{430}**(4)**, ATP**(14)**, CoB**(3*)**, CoM	Ni
	9	*MvhADG*	[NiFe] hydrogenase		NiFe, FeS
	10	*HdrABC*	Heterodisulphide reductase (Se)	FAD, Ferredoxin, CoM-S-S-CoB	FeS
Acetyl-CoA pathway (Bacteria)	11	*HydABCD*	[FeFe] hydrogenase	NAD(P)H**(6)**, Ferredoxin, FMN	FeS
	12	*FdhAB*	Formate dehydrogenase (Se)	Molybdopterin/Tungstopterin, NAD(P)H	FeS, Mo/W
	13	*Fhs*	10-Formyl-H_4F synthetase	ATP, H_4F**(7)**	
	14	*FolD*	5,10-Methenyl-H_4F cyclohydrolase/dehydrogenase	NAD(P)H, H_4F	
	15	*MetF*	5,10-Methylene-H_4F reductase	FAD, Ferredoxin, H_4F	FeS
Acetate and pyruvate synthesis	16	*AcsE*	Methyl-H_4F/H_4MPT:CoFeS methyltransferase	H_4F /H_4MPT, Cobamide	Co
	17	*AcsAB/CdhABC*	CO dehydrogenase/Acetyl-CoA synthase	Ferredoxin, CoA**(9)**	FeS, NiFeS, Ni-Ni
	18	*Pta*	Phosphate acetyltransferase	CoA	
	19	*AckA*	Acetate kinase	ATP	
	20	*PorABCDG*	Pyruvate:ferredoxin oxidoreductase/Pyruvate synthase	ThPP**(13)**, Ferredoxin	FeS

Figure 4. A. The genes coding for the enzymes of the acetyl-CoA pathway are shown in order. Archaeal methanogenic enzymes are in brown, bacterial acetogenic enzymes are in blue, while enzymes present in both domains are shown in red. The latter include the carbonyl branch and the synthesis of acetate and pyruvate. In addition to the 20 enzymes of the pathway itself, 2 carrier proteins are required (ferredoxin and the corrinoid iron-sulfur protein) and at least 105 enzymes to synthesize the cofactors essential to the pathway (several cofactor biosynthesis pathways are not fully characterized). B. Analogues of the acetyl-CoA pathway have been obtained in the laboratory under alkaline hydrothermal vent conditions. Awaruite (Ni_3Fe) formed in serpentinizing systems catalyzes the formation of methane, formate, acetate and pyruvate, which are intermediates and products of the acetyl-CoA pathway, from CO_2 and H_2.[49,111] Reaction yields are shown for a 16 h reaction at 100 °C and pH 8 with 0.625 mmol Ni_3Fe.[49] Pyruvate has been found to react further to form citramalate,[50] a metabolic intermediate in some organisms (not shown).[198–199] Only one catalyst is required. C. The 20 genes and enzymes of the reductive acetyl-CoA pathway to pyruvate in bacteria and archaea are shown.[65,176,181,192,200–207] Selenoproteins are marked (Se). For each enzyme the cofactors and transition metals that participate in the reaction are listed. The number in brackets next to the first appearance of each cofactor shows the number of enzymes required for its biosynthesis. When the enzyme number is uncertain (likely an underestimate) because the biosynthetic pathway has not been fully characterized, the enzyme number is followed by an asterisk (*). Abbreviations: MFR: methanofuran; H_4MPT: tetrahydromethanopterin; CoM: coenzyme M; CoB: coenzyme B; H_4F: tetrahydrofolate; CoFeS: corrinoid iron-sulfur protein; CoA: coenzyme A; ThPP: thiamine pyrophosphate.

minimum. Kaster et al. have surmised that 200 genes are required for methanogenesis.[190] The enzymes of the acetyl-CoA pathway abound in transition metal clusters,[39,173–174] which need to be assembled and incorporated (Figure 4C). In addition, two protein carriers are indispensable for the pathway – ferredoxin and the corrinoid iron-sulfur protein CoFeS. CoFeS uses cobamide as its cofactor.

The synthesis of cobamide alone requires 19 enzymes (Supplementary Table 1). It is essential to the acetyl-CoA pathway in acetogens and methanogens. The tetrapyrrole-coordinated Co atom of cobamide in CoFeS accepts a methyl group from an N atom in a pterin cofactor (tetrahydrofolate or tetrahydromethanopterin), and then donates it to a Ni atom in the active site of CODH/ACS.[191] This cobamide-mediated methyl transfer reaction has a $\Delta G^{o\prime}$ of -4 kJ·mol-1.[39] Cobamide is also essential for the energy-conserving step of methanogenesis, the Na^+-pumping membrane complex MtrA-H, which catalyzes the transfer of a methyl group to coenzyme M.[153] In the MtrA-H reaction, the nitrogen-bound methyl group is transferred to CoM in a two-step process involving the corrinoid cofactor. The first step is the transfer of the methyl group from methyl-H_4MPT to Co(I) ($\Delta G^{o\prime} = -15$ kJ/mol) and then from methyl-Co(III) to CoM-SH ($\Delta G^{o\prime} = -15$ kJ/mol) to yield CoM–S–CH_3 (methyl-CoM). The transfer of the methyl group from methyl-Co(III) is Na^+-dependent, and is thus implicated in the Na^+ pumping process.[192] In acetogens[193] that lack cytochromes, net energy conservation from H_2-dependent CO_2 reduction is provided by pumping at the reaction catalyzed by Rnf. In methanogens that lack cytochromes, energy conservation occurs at MtrA-H, as previously described.[190,192,194] Figure 4 shows the synthesis of formate, acetate, pyruvate, and methane as products of primordial H_2-dependent CO_2 reduction reactions, not the coupled pumping reactions and chemiosmotic energy conservation via the rotor-stator ATP synthase, which are considered later evolutionary inventions. It is likely that primordial energy conservation via the acetyl-CoA pathway entailed acetyl phosphate synthesis and substrate level phosphorylation before the origin of chemiosmotic coupling.[49,74,195]

In hydrogenotrophic methanogens there are two routes for the reduction of methenyl-H_4MPT to methylene-H_4MPT.[192] The standard route, also used by other methanogens, involves Mtd which uses $F_{420}H_2$ provided by the activity of Frh, an F_{420}-reducing [FeNi] hydrogenase. However, under Ni-limiting conditions, hydrogenotrophic methanogens express an alternative enzyme for methenyl-H_4MPT reduction: H_2-forming methylene-H_4MPT dehydrogenase (Hmd). Hmd reduces methenyl-H_4MPT with H_2. Hmd is unique so far in that it is the only H_2-oxidizing enzyme known that does not reduce FeS clusters or ferredoxin. Instead, it catalyzes the direct transfer of a hydride from H_2 to methenyl-H_4MPT with the help of a unique cofactor, the FeGP (iron guanylylpyridinol) cofactor. The biosynthesis of the FeGP cofactor requires an additional seven enzymes,[196–197] which are not included in Figure 4 because it is an alternative pathway. Is the Hmd route ancient, or is it derived? From the standpoint of its constituents, the FeGP cofactor appears to be extremely ancient. The carbon atoms in the FeGP cofactor (excluding the GMP moiety) stem from CO_2 (including two CO ligands), a methyl group (from S-adenosyl methionine), acetate, and pyruvate. These are all direct products of the acetyl-CoA pathway, including its inorganic precursor[49] as shown in Figure 4, suggesting that the H_2-oxidizing FeGP cofactor traces to an early stage in (bio)chemical evolution.

The reactions of the acetyl-CoA pathway are replete with carbon metal bonds.[208] The pathway turns up at the center of genomic reconstructions of the last universal common ancestor LUCA.[141] How could such a complex biochemical pathway be genuinely primitive? In 2020 Preiner et al. reported an abiotic analogue of the acetyl-CoA pathway that synthesizes methane, formate, acetate and even pyruvate from H_2 and CO_2 without enzymes or organic cofactors. The catalysts were minerals that naturally form in serpentinizing hydrothermal vents,[209] such as awaruite (Ni_3Fe), magnetite (Fe_3O_4), and greigite (Fe_3S_4). Ni_3Fe (and Fe_3O_4) generated pyruvate from H_2 and CO_2 overnight at 100 °C (Figure 4B).[49] Wächtershäuser and others have proposed that single inorganic catalysts might be able to catalyze entire biochemical pathways, for example the reverse TCA cycle or purine synthesis on mineral surfaces.[43,108] In the case of the acetyl-CoA pathway from H_2 and CO_2 to pyruvate,[39,40,74,210] we finally have a concrete and reproducible example in hand where it actually happens, the function of 127 proteins (Figure 4) being replaced by a metal that, in nature, is synthesized in serpentinizing hydrothermal vents.[58]

The findings of Preiner et al.[49] are not an isolated report. Recent findings by the team of Harun Tüysüz at the Max-Planck Institute for Coal Research in Mülheim have characterized various parameters affecting transition metal-catalyzed H_2-dependent CO_2 reduction, as summarized in Table 2. By varying the catalysts, adding silica supports, or altering time and temperature of the reaction, they have been able to obtain high yields of formate and acetate and up to 200 μM pyruvate (Table 2).[51,111]

Why is 200 μM pyruvate so important? It is the physiological concentration of pyruvate [180 ± 40 μM] that accumulates in the cytosol of an acetogen, *Clostridium thermoaceticum*, growing on H_2 and CO_2.[204] In other words, starting from H_2 and CO_2, a solid state transition metal catalyst (Ni) not only produces the most central compound in carbon and energy metabolism, it produces pyruvate at exactly the same concentration as growing cells do. The metal just needs a week[111] (Table 2) instead of 24 hours,[205] the doubling time of *C. thermoaceticum*, to do the job. Do such findings, in sum, identify the origin of autotrophic metabolism from H_2 and CO_2 via reactions of the acetyl-CoA pathway?[40] The most direct answer is 'yes'.

These H_2-dependent CO_2 reductions under simulated hydrothermal conditions work without the help of flavin-based electron bifurcation because the redox potential of H_2 under conditions of serpentinizing vents becomes sufficiently negative (Table 1) to reduce CO_2 without the need for an additional, supporting oxidant. Although the reactants involved (H_2 and CO_2) as well as the catalysts (minerals) are inorganic (Table 2), the organic products are more or less exactly the backbone of carbon and energy metabolism in anaerobic autotrophs (Figure 4). The finding that a geological analogue of the acetyl-CoA pathway, which is the backbone of carbon and energy

Table 2. Products of aqueous CO_2 reduction with H_2 obtained using mineral catalysts.

H_2:CO_2 ratio[a]	Temp. [°C]	Time [h]	Catalyst[b]	Product yields[c]			Ref.
				Formate [mM]	Acetate [mM]	Pyruvate [μM]	
4:1[a]	100	24	Fe_3S_4	2.98	0.43	n.d.[d]	[49]
2:3	100	16	Ni_3Fe	332	0.56	10	[49]
2:3	100	16	Fe_3O_4	0.05	0.18	10	[49]
2:3	100	16	Fe_3O_4/Fe^0	1.37	0.27	10	[49]
2:1[a]	180	72	Co[e]	3.6	1.2	n.d.[d]	[51]
2:3	25	24	Ni_3Fe	26.7	0.04	20	[50]
2:3	100	8	Ni^0	5.8	0.1	20	[111]
2:3	100	24	Ni_3Fe	55.5	0.2	40	[111]
2:3	100	24	Ni^0	36.5	0.7	110	[111]
2:3	100	168	Ni^0	36.1	0.8	200	[111]

[a] 25 bar in all cases except: In ref. [49] the experiments with greigite were performed at 2 bar; In ref. [51] 2 MPa (20 bar) were used; [b] In most cases nanoparticular catalysts were used, see references for specifics of catalyst synthesis; [c] Mean of values reported for the conditions specified; [d] Not detected; [e] Cobalt on Ti-modified silica.

metabolism of methanogens and acetogens, unfolds spontaneously from H_2 and CO_2 activated on transition metal surfaces in hydrothermal conditions, suggests that this pathway is both ancient and the starting point of metabolism. This also indicates that the salient chemical reactions of the acetyl-CoA pathway are older than the enzymes that catalyze them.[40,210]

The idea that core metabolic reactions preceded genes in evolution[33] is at the heart of metabolism-first theories for origins and goes back in evidence at least to Degani and Halmann's 1967 report of non-enzymatic glycolytic reactions,[211] notwithstanding early work on cofactor-catalyzed nonenzymatic reactions from the 1950s.[74] This principle helps to explain the otherwise puzzling observation that most of the enzymes in the archaeal and bacterial versions of methyl synthesis in the acetyl-CoA pathway are not evolutionarily related, even though the chemical reactions and cofactors involved are very similar.[212] The reactions leading to products of the acetyl-CoA pathway (Table 2; Figure 4) start from CO_2, the product of the Moon-forming impact, and present a strong case for a natural hydrothermal chemistry underlying autotrophic origins, without the need for a late veneer or impactors that generate transiently reducing conditions, because serpentinization, which can also reduce N_2,[84] has been taking place since there was water on Earth.[213]

5.3. Rocks and water and CO_2

Throughout Earth history, H_2 has always been continuously produced in hydrothermal vents through serpentinization,[213–214] a process in which Fe^{2+}-bearing minerals in ultramafic rocks in the Earth's crust reduce circulating water to molecular hydrogen, generating minerals such as Fe_3O_4 and Ni_3Fe in the process. This process of H_2 synthesis has been ongoing since the first oceans condensed because the Earth's ancient crust was largely composed of minerals that can undergo serpentinization[213] and because the process of serpentinization is itself exergonic.[215–216]

Starting from pure CO_2, present in the atmosphere and the oceans as a consequence of the Moon-forming impact, and pure H_2 from serpentinization, Ni_3Fe and Fe_3O_4 can replace the function of more than 127 enzymes in the synthesis of pyruvate from H_2 and CO_2 (Supplementary Table 1). The concept that non-enzymatic versions of chemical reactions at the origin of metabolism were originally catalyzed by minerals,[33] metals[66,67] or alkaline conditions[211] and that they later came to be catalyzed by cofactors and enzymes has been around for over six decades, but the extent to which this very robust principle is realized in core carbon metabolism of microbes continues to be surprising.

Kitadai et al. showed in the laboratory that the production of native metals such as Fe^0 from the corresponding sulfides can take place under hydrothermal conditions.[76] This is relevant because Varma et al. showed CO_2 fixation with native metals, such as Fe^0, Ni^0 and Co^0 as catalyst and reductant.[217] A promising prospect involves exploring the chemical capacity of different metal catalysts.[51,111] Another approach relevant to CO_2 fixation in hydrothermal systems focuses on the electrical currents that are generated between the hydrothermal fluid and seawater, across the mineral deposits of the vents, which act as conductors.[218–219] The discovery of multiple microbial species capable of extracellular electron transfer directly from a solid electron source such as an electrode, rather than a soluble electron-donating molecule,[220–222] opens up questions concerning the potential role for electrotrophic microbial growth on the early Earth. The potentials generated by H_2 under serpentinizing conditions (Table 1) are themselves a source of electric current, provided that acceptors are available.

6. Conclusions

The Moon-forming impact was a key event in Earth's planetary history. Without it, life on Earth might never have come into existence. Carbon brought to Earth by carbonaceous chondrites was initially present in the form of inert polyaromatic hydrocarbons (PAH).[223] That inert carbon was converted to pure, clean, and reactive atmospheric CO_2 by the Moon-forming impact. CO_2 kept the Earth warm enough to maintain liquid surface water, mitigating the faint young Sun, but its main impact was to provide a CO_2 reservoir that was an accessible carbon source available to kick-start life. When the oceans rained out from the H_2O-rich atmosphere, CO_2 started dissolving in the oceans and was destined to subduction as carbonates. The CO_2 that remained in seawater and that was bound in the crust was available for the first organic syntheses. These required reductant, supplied as H_2 by serpentinization initiated by water's first encounters with primordial crust. According to autotrophic theories, synthesis did not occur in a primordial soup. Specific environments and solid state catalysts were required. Today's submarine hydrothermal vents generate highly localized reducing conditions and allow for the concentration of reactants in far-from-equilibrium environments where H_2 is continuously formed. Hydrogen gas serves as the reductant for CO_2, made available by the Moon-forming impact. Transition metal catalysts deposited *in situ* can convert H_2 and CO_2 to intermediates and end-products of the acetyl-CoA pathway, such as pyruvate. The acetyl-CoA pathway is assumed to be ancient, likely the first C fixation pathway, and is employed by the oldest microbial lineages – acetogens and methanogens. Theories of autotrophic origins generally aim for congruence between Earth's early history and geochemistry, on one hand, and microbial physiology, on the other, in order to generate chemical continuity (Morowitz called it 'historical continuity'[224]) in the transition from the first organic chemical reactions to life. Serpentinizing hydrothermal vents link geochemistry with biochemistry in that they combine CO_2 from the early oxidized atmosphere with a stable source of reductant (H_2) for organic syntheses in reactions that unfold spontaneously on transition metal surfaces to yield organics that constitute the backbone of carbon and energy metabolism in ancient microbes. They also provide the redox potentials required for CO_2 reduction without flavin-based electron bifurcation[58] and – were that not enough – they also deposit native metals and native metal alloys *in situ* as inorganic catalysts that specifically accelerate those reactions.[138,225] Life need not have started that way, but had it done so we would be able to recognize the imprint of its origin in reactions of H_2-dependent chemolithoautotrophic metabolism.

Supporting Information

The authors have cited additional references within the Supporting Information.[226–229]

Author Contributions

Conceptualization: W. F. M., N. M.; Writing – Original Draft: W. F. M, N. M.; Writing – Review & Editing: W. F. M., N. M., J. L. E. W., L. S., M. B.; Visualization: W. F. M., N. M.; Funding acquisition: W. F. M.; Supervision: W. F. M.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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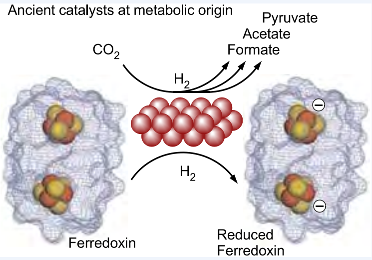

CONSPECTUS: Life is an exergonic chemical reaction. The same was true when the very first cells emerged at life's origin. In order to live, all cells need a source of carbon, energy, and electrons to drive their overall reaction network (metabolism). In most cells, these are separate pathways. There is only one biochemical pathway that serves all three needs simultaneously: the acetyl-CoA pathway of CO_2 fixation. In the acetyl-CoA pathway, electrons from H_2 reduce CO_2 to pyruvate for carbon supply, while methane or acetate synthesis are coupled to energy conservation as ATP. This simplicity and thermodynamic favorability prompted Georg Fuchs and Erhard Stupperich to propose in 1985 that the acetyl-CoA pathway might mark the origin of metabolism, at the same time that Steve Ragsdale and Harland Wood were uncovering catalytic roles for Fe, Co, and Ni in the enzymes of the pathway. Subsequent work has provided strong support for those proposals.

In the presence of Fe, Co, and Ni in their native metallic state as catalysts, aqueous H_2 and CO_2 react specifically to formate, acetate, methane, and pyruvate overnight at 100 °C. These metals (and their alloys) thus replace the function of over 120 enzymes required for the conversion of H_2 and CO_2 to pyruvate via the pathway and its cofactors, an unprecedented set of findings in the study of biochemical evolution. The reactions require alkaline conditions, which promote hydrogen oxidation by proton removal and are naturally generated in serpentinizing (H_2-producing) hydrothermal vents. Serpentinizing hydrothermal vents furthermore produce natural deposits of native Fe, Co, Ni, and their alloys. These are precisely the metals that reduce CO_2 with H_2 in the laboratory; they are also the metals found at the active sites of enzymes in the acetyl-CoA pathway. Iron, cobalt and nickel are relicts of the environments in which metabolism arose, environments that still harbor ancient methane- and acetate-producing autotrophs today. This convergence indicates bedrock-level antiquity for the acetyl-CoA pathway. In acetogens and methanogens growing on H_2 as reductant, the acetyl-CoA pathway requires flavin-based electron bifurcation as a source of reduced ferredoxin (a 4Fe4S cluster-containing protein) in order to function. Recent findings show that H_2 can reduce the 4Fe4S clusters of ferredoxin in the presence of native iron, uncovering an evolutionary precursor of flavin-based electron bifurcation and suggesting an origin of FeS-dependent electron transfer in proteins. Traditionally discussed as catalysts in early evolution, the most common function of FeS clusters in metabolism is one-electron transfer, also in radical SAM enzymes, a large and ancient enzyme family. The cofactors and active sites in enzymes of the acetyl-CoA pathway uncover chemical antiquity in metabolism involving metals, methyl groups, methyl transfer reactions, cobamides, pterins, GTP, *S*-adenosylmethionine, radical SAM enzymes, and carbon−metal bonds. The reaction sequence from H_2 and CO_2 to pyruvate on naturally deposited native metals is maximally simple. It requires neither nitrogen, sulfur, phosphorus, RNA, ion gradients, nor light. Solid-state metal catalysts tether the origin of metabolism to a H_2-producing, serpentinizing hydrothermal vent.

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INTRODUCTION

Life is an exergonic chemical reaction that branches out into roughly 1000 catalyzed and interconnected partial reactions (metabolism) and that, given sufficient reactants, produces a functional copy of its catalyst set (a daughter cell).[4] Understanding how life could have arisen involves forging links between the chemical reactions of living cells and chemical environments on the early Earth. The number of chemical environments on the early Earth where the origin of life was theoretically possible is vast. By contrast, the number of chemical environments on the early Earth that directly interface with the chemical reactions of living cells is limited. The physiology of modern cells can provide constraints that help to discriminate between the kinds of environmental catalysts that could have accelerated the primordial chemical reactions that gave rise to metabolism. Physiology can also help to identify ancient lifestyles, ancient reactions and ancient catalysts in metabolism.[5−7] Because no one was around to observe the origin of life 4 billion years ago, the physicochemical environment of origins is debated. But physiology adds needed constraints. For example, all cells, without exception, have to satisfy their basic requirements for carbon (CO_2 vs organics; autotrophy vs heterotrophy), electron donors (inorganic vs organic; lithotrophy vs organotrophy) and energy (photons vs chemical reactions; phototrophy vs chemotrophy) in order to grow. Given what we know about early Earth environments, which of these combinations is ancient?

Carbon

All ecosystems today start from CO_2 with autotrophs providing the reduced carbon compounds required by heterotrophs. Carbon from space is too reduced and structurally too heterogeneous to support fermentations,[8] and the Moon-forming impact transformed all accreted carbon on the early Earth into CO_2.[9,10] That means that CO_2 was the starting material for the first organic syntheses and the source of carbon for primary producers (autotrophs) that fueled the first ecosystems, the foundation of autotrophic theories for origins.[11−14] The Moon-forming impact generated a primordial atmosphere rich in CO_2 that dissolved in the ocean, generating a pH of roughly 6.5 from carbonic acid.[15] That localizes primordial CO_2 to the Earth's entire surface and oceans, but provides no direct clues about the specific environment where the CO_2 reduction process might have gotten started. The source of reductants narrows down the possibilities.

Electrons

The list of possible reductants for the first organic synthesis from CO_2 and the source of electrons for autotrophic pathways is short. Among the environmentally available reductants on the early Earth, only H_2 has a sufficiently negative midpoint potential to reduce CO_2 directly, but it requires alkaline pH and catalysts in order to do so. High pH (≥8) pulls the equilibrium in the reaction $H_2 \rightarrow 2H^+ + 2e^-$ to the right by removing protons, which shifts the midpoint potential of the H_2 oxidation reaction from −414 mV under standard physiological conditions (25 °C, 1 atm H_2, pH 7) to −533 mV (25 °C, 1 atm H_2) at pH 9, for example, using the Nernst equation. The midpoint potential of CO_2 reduction to formate is on the order of −430 mV.[16] In the absence of catalysts, H_2 in alkaline water will not react with dissolved CO_2 at significant rates. But if the solid-state transition metals Fe^0, Co^0 or Ni^0 or their alloys are included as catalysts for the reaction, H_2 readily chemisorbs onto the metal surface and quickly reduces CO_2 to a modest spectrum of specific organic compounds.[2,17−20] The reducing power of H_2 links the site of organic synthesis to the proximity of serpentinizing hydrothermal systems,[21,22] which naturally generate alkaline effluent (pH 9−11) with concentrations of H_2 reaching 10 mM or more.[23] The requirement for a solid-state catalyst (and high pH) furthermore ties organic synthesis rather specifically to the physical site of a serpentinizing vent, because the reducing conditions of serpentinizing systems actively deposit the native metals Fe^0, Co^0, Ni^0 and their alloys,[24] thereby providing the diffusible reductant (H_2, an inorganic electron donor), the alkaline pH needed to favor H_2 oxidation, and the native metal catalysts that allow chemisorbed H_2 and CO_2 to react.

Energy

All cells have a main exergonic chemical reaction that allows the cell to conserve energy. At origins, the first reactions to synthesize organics also had to be exergonic, otherwise the reactions would not have gone forward. Most autotrophs have separate and independent pathways of carbon and energy metabolism, with ATP from the latter energetically financing the former.[25] The search for ancient forms of energy metabolism is relatively simple because there are only two lineages of microbes that combine carbon and energy metabolism, obtaining their ATP from the reduction of CO_2 with H_2. They are strict anaerobes and they both employ the most ancient among CO_2 fixing pathways, the acetyl-CoA pathway:[12,14,26] acetogens and methanogens. In acetogen and methanogen metabolism, the main intermediates and products of the acetyl-CoA pathway are formate (an intermediate), pyruvate (the main source of carbon for biosynthesis), acetate and methane (the end products of acetogens and methanogens, respectively).[27,28] The acetyl-CoA pathway is linear, exergonic, the only CO_2-fixing pathway that occurs in bacteria and archaea[12,14] and can simultaneously support CO_2 fixation and ATP synthesis, and it is the only multistep biochemical pathway that can be replaced entirely by single metals as catalysts. The acetyl-CoA pathway requires a total of 127 enzymes—about 20 enzymes of the pathway itself (in acetogens and methanogens) plus >100 enzymes for the synthesis of required cofactors.[10] That massive enzymatic demand might not seem ancient at first sight. But solid-state

Fe^0, Co^0 or Ni^0 or their alloys—as sole catalysts—convert H_2 and CO_2 to formate, acetate, pyruvate and methane overnight in alkaline water at 25−100 °C[2,17−20,29] (Figure 1). The

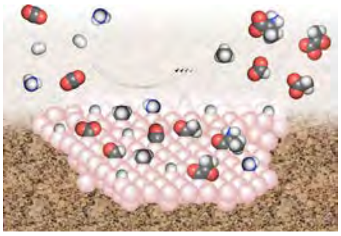

Figure 1. Schematic showing synthesis of methane, formate, acetate, pyruvate, and alanine on native metal catalysts deposited on mineral surfaces in hydrothermal pores at the onset of metabolism. The corresponding reactions (synthesis of formate, acetate and pyruvate from H_2 and CO_2, see text) including amino acid synthesis from 2-oxo acids, NH_3 and H_2,[30] symbolized here with alanine, take place in laboratory experiments, whereby the exact nature of the metal-bound intermediates is not known, although chemisorption of H_2 onto transition metal surfaces and the subsequent diffusion of hydrogen atoms on metal surfaces are well studied.[31] In the laboratory, Fe, Co, Ni and their alloys are catalytically active, the metal surface sketched represents generic transition metals. The scheme is based on the mechanisms proposed by Varma et al.[29] and Preiner et al.[2] and the findings of Kaur et al.[30]

reactions go forward because they are exergonic.[2,27,28] As a source of energy and physiologically central carbon compounds, the acetyl-CoA pathway is ancient. Its requirement for enzymes and cofactors in cells does not preclude its antiquity, because enzymes, once they arose in chemical evolution, merely accelerate reactions that tend to occur anyway: The exergonic chemical reactions catalyzed by ancient enzymes are older than the enzymes themselves.

Catalysis

In order to harness carbon, energy and electrons for synthesis, microbial metabolism requires enzymes as catalysts. The scheme in Figure 1 depicts recently observed[2,17−20,29,30] product formation in reactions of H_2 and CO_2 catalyzed by native metal surfaces on an inorganic support, which could represent a silicate support for a heterogeneous catalyst in the laboratory[17,20,30] or an environmental (host) rock in a serpentinizing environment[21−24] with the inorganic catalysts serving as precursors for similar reactions that later came to be catalyzed by enzymes.[26] In practice, solid-state catalysis is not as simple as sketched in Figure 1, as recent work by the team of Harun Tüysüz underscores. They have performed many such reactions, obtaining various amounts of formate, acetate, pyruvate under a variety of conditions with characterization of the surface of Fe, Co, Ni, and alloy catalysts post-reaction.[2,17−20] Across several independent studies, they found no significant surface alteration in most cases in particular when H_2 was used as reductant, but in the absence of H_2 evidence of oxide or oxyhydroxide formation in some cases, metal dissolution in some cases, and metal carbonate formation in others could be observed.[2,17−20] The degree to which the catalysts are altered can depend upon pH, temperature, time, pressure, the presence of H_2, the metals themselves, and whether different metals are alloyed or simply mixed.

In some combinations of catalysts and conditions, the CO_2-reducing reactions go forward without the addition of exogenous H_2 whereby the metal serves as reductant. For example, using Ni_3Fe as catalyst, CO_2 reduction to organic acids was observed[18] without exogenous H_2, though with yields that were lower by an order of magnitude or more compared to those obtained with the addition of H_2. In the absence of exogenous H_2, the reactions can be accompanied by dissolution of Fe as Fe^{2+} from the Ni_3Fe alloy, which is not observed when H_2 is supplied as reductant.[2,18] Varma et al.[29] reported efficient CO_2 reduction in the absence of exogenous H_2, where Fe^0 was serving as reductant, probably generating H_2 from water on the catalyst surface especially at high pH.[1,2] Reaction mechanisms on the surface of heterogeneous catalysts are notoriously difficult to study, but Henriques Pereira et al.[32] were able to show using ^2H_2O (D_2O) and $^1H_2$ gas with NAD^+ as an electron acceptor that Ni^0 acts as a true catalyst, generating NAD^1H, while Fe^0 can generate NAD^2H from D_2O in addition to NAD^1H from $^1H_2$. A complicating aspect is that the standard midpoint potentials at pH 7 for H_2 formation from water (−414 mV),[16] CO_2 reduction to formate (−430 mV),[16] and Fe^{2+} reduction to Fe^0 (−440 mV)[18] are not only very close to one another, but also vary with temperature, pressure and in particular pH so as to overlap in some conditions but not in others. A recent report[33] stated that several studies surveyed here provided no evidence for catalysis, because the turnover numbers they calculated were <1; however, they counted each atom of solid-state catalysts as "catalytically" active,[33] regardless of particle size. For H_2 activation, about 1% of the atoms on the surface of a typical heterogeneous metal catalyst such as iron are active,[34] and for 25 nm nanoparticles, only about 4% of the atoms are on the surface (H. Tüysüz, Personal communication); accordingly, the turnover numbers calculated[33] were roughly 1000-fold too low for the solid-state catalysts considered here when H_2 was used as the reductant. The implementation of heterogeneous catalysis for organic synthesis is still new in the origins field. It opens up many new avenues of pursuit, and it can be implemented using substrates, catalysts and products that align well with chemistry observed at H_2-producing hydrothermal vents.[23]

METALS INSTEAD OF FeS MINERALS

Conventional wisdom has it that early in biochemical evolution, inorganic surfaces served as catalysts that preceded cofactors and enzymes.[35] Much modern thinking on early biochemical evolution is centered around the idea that such inorganic surfaces were environmentally formed FeS minerals which served as ancient catalysts that preceded FeS clusters in evolution. This idea stems from the discovery of ferredoxin,[36] the first protein found to contain covalent Fe−S bonds[37] and FeS clusters. Ferredoxin is the strongest long-lived reductant in the cytosol and the main soluble one-electron carrier in anaerobes.[16] For decades, ferredoxin was implicated in the origin of metabolism as a link between the organic and inorganic worlds.[5,6,38] However, the surfaces that functionally predate the acetyl-CoA pathway (Figure 1) do not consist of iron sulfides, they consist of pure metals and the reactions do not require the presence of sulfur in any form.

The native transition metals that catalytically substitute for the entire acetyl-CoA pathway are exactly the same ones that are coordinated in the active sites of the modern enzymes in this ancient pathway: Fe, Co and Ni (Figure 2). Nickel is catalytically active in carbon monoxide dehydrogenase,[39] acetyl-CoA synthase,[40−43] [FeNi] hydrogenase,[44] and the Ni-containing tetrapyrrole F_{430} used in the final methane-synthesizing step of methanogenesis.[45] Cobalt is used in the methyl-transferring cobamide cofactor of the corrinoid iron−sulfur protein CoFeS[46] that donates methyl groups to acetyl-CoA synthase. Iron is used in [FeFe] hydrogenase[47] and [Fe] hydrogenase.[48] These ancient enzymes generate covalent bonds between carbon and the active site transition metal during the reaction mechanism, or harbor carbon−metal bonds in active site ligands (Figure 2). While iron sulfur clusters are common in proteins,[49] carbon−metal bonds are generally rare in biological reactions. Those involved in core carbon metabolism in acetogens and methanogens are ancient[50] (Figure 2), relicts from a time when the onset of metabolism (Figure 1) involved native metals as catalysts.

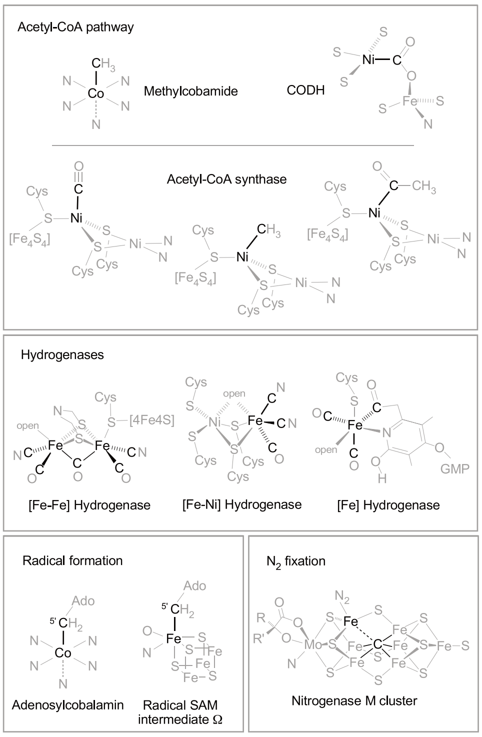

Figure 2. Carbon−metal bonds in active sites of ancient enzymes. The structures are from refs 39, 43, 44, 46−48, 51, and 52. The three intermediates in the acetyl-CoA synthase reaction that have been spectroscopically captured are from refs 41−43.

Of the structures shown in Figure 2, in addition to the metal cluster prosthetic groups shown, corrins and S-adenosylmethionine (SAM), acting through the radical SAM intermediate Ω,[52] are cofactors required by acetogens and methanogens. Several of the structures have an additional ancient property in common in that radical SAM enzymes are involved in their synthesis: the corrin backbone of cobalamin, the iron-guanylyl pyridinol cofactor of [Fe] hydrogenase, the active site of nitrogenase and of [Fe−Fe] hydrogenase all require radical SAM enzymes for the synthesis of the active cofactor.[53−56] Radical SAM enzymes harbor a 4Fe4S cluster that coordinates S-adenosylmethionine (SAM) via its amino and carboxyl groups. The 4Fe4S cluster in the enzyme initiates radical formation via the covalent intermediate Ω,[57] followed by homolytic cleavage of the carbon−iron bond to yield a 5′-deoxyadenosyl radical (5′dAdo●) that typically abstracts H● from the substrate to initiate the reaction mechanism.

All radical SAM enzymes contain a 4Fe4S cluster. They are reported as the largest enzyme family known[57] and in all cases, the function of the 4Fe4S cluster is one-electron transfer that forms the 5′dAdo● radical that initiates the radical reaction mechanism with the substrate. This kind of radical-initiating, one-electron transfer is a fundamentally different function from the myriad catalytic and electrostatic surface-binding functions initially envisioned for FeS minerals in the FeS world proposed by Wächtershäuser.[58] The metal-catalyzed acetyl-CoA pathway from H_2 and CO_2 to pyruvate (Figure 1) also departs sharply from Wächtershäuser's iron−sulfur world proposal in that (i) the reactions lack sulfur altogether, (ii) the primordial CO_2 fixation pathway is the acetyl-CoA pathway, not a reverse TCA cycle consisting of thioacids,[58] and (iii) the reductant is H_2, which was categorically excluded as the initial electron source "*since its reducing potential is not sufficient for reducing* CO_2".[59]

The midpoint potential of H_2 is a crucial parameter in biological H_2 utilization. How cells use H_2 as a reductant in the acetyl-CoA pathway was a puzzle for several decades because under standard conditions the midpoint potential of H_2 (−414 mV)[16] does indeed require electrons to flow energetically uphill to CO_2 (−430 mV),[16] even more so to reduce ferredoxin (−450 mV),[16] which is the physiological donor for CO_2 reduction in cells that use the acetyl-CoA pathway.[60] Yet modern cells readily use H_2 as a reductant for CO_2, whereby acetogens and methanogens even obtain energy from CO_2 reduction with H_2. Physiology always obeys the laws of thermodynamics. To reduce CO_2 with electrons from H_2, cells employ an elegant thermodynamic mechanism called flavin-based electron bifurcation, which splits the electron pair in H_2, sending one electron uphill to ferredoxin and the other downhill to a high potential acceptor (like NAD^+ or CoM−S−S−CoB) so that the overall reaction of H_2-dependent CO_2 reduction is exergonic.[61] Before the existence of enzymes, electron bifurcation was not required, because H_2 readily reduces CO_2 to formate, acetate and pyruvate in the presence of native Fe, Co and Ni catalysts at alkaline pH (Figure 1). But the acetyl-CoA pathway employs ferredoxin-dependent enzymes.[62] The electron donor for those enzymes is always reduced ferredoxin with a midpoint potential of near −500 mV.[16,61,63,64] How did metabolism generate reduced ferredoxin before the origin of the complex protein machinery involved in electron bifurcation? Again, native metals, which are naturally deposited in hydrothermal vents,[23] can replace enzymes for this ancient reaction. Brabender et al.[1] recently showed that H_2 can reduce *Clostridium pasteurianum* ferredoxin in the presence of Fe^0 as catalyst.

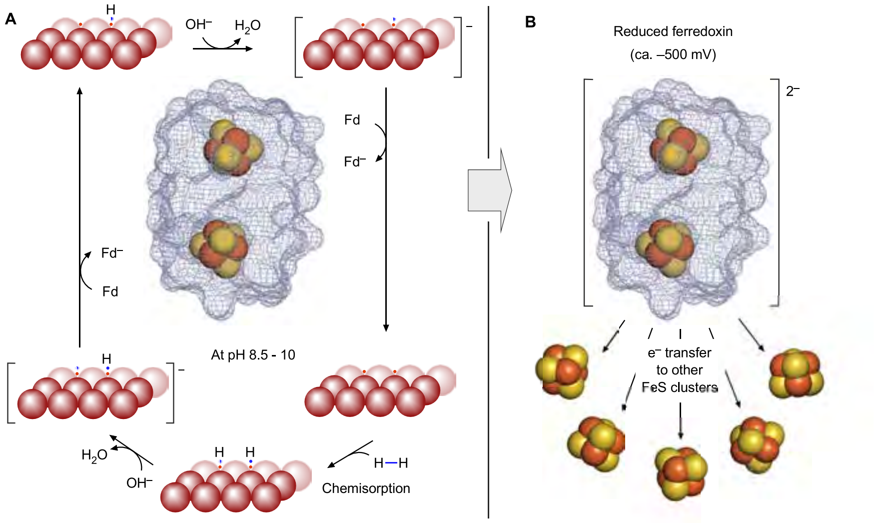

Figure 3. Proposal for the role of ferredoxin in early evolution. **A.** Proposed schematic mechanism for ferredoxin reduction by H_2 over Fe^0 in alkaline (pH 8.5 to 10) conditions.[1] Electrons indicated as small dots are solely for illustration purposes. Because iron conducts, the site of H_2 oxidation and ferredoxin (Fd) reduction need not be identical. The figure makes no statement about physical position of H atoms on the metal surface, distance between metal surface and Fd, or rates of electron transfer. **B.** A primordial role of ferredoxin could have been to transfer electrons from hydride-bearing metal surfaces (solid-state hydrogenases) to the aqueous phase as a soluble electron carrier for enzymes requiring a strong electron donor (see text). Though sometimes referred to as a catalyst in older literature,[6] ferredoxin is not a catalyst, it is an electron donor and acceptor for enzymatic reactions involving FeS clusters or cofactors[1] (in particular flavins, which transduce one-electron and two-electron transfers in metabolism).[16]

■ ELECTRON TRANSFER: THE PRIMORDIAL FUNCTION OF FeS CLUSTERS

Although cells can encounter extremely strong reductants in the environment, for example phosphite,[65] ferredoxin is the strongest reductant that is generated during metabolism of anaerobic chemotrophic cells and it requires electron bifurcation for synthesis.[16,64,66] From the 4Fe4S clusters of reduced low potential ferredoxin, electron transfer to all other FeS clusters in metabolism is energetically downhill. As with CO_2 reduction (Figure 1), the mechanism of ferredoxin reduction by H_2 and Fe^0 is not known, but one simple possibility is outlined in Figure 3A, involving chemisorption of H_2 onto the iron surface, H^+ removal by the alkaline aqueous phase, and electron transfer to ferredoxin.

The transfer of electrons from the solid-state metal surface to the 4Fe4S clusters of ferredoxin in solution[1] probably involves similar mechanisms as occur between FeS clusters in proteins: tunneling.[67] This is schematically drawn in Figure 3A. Though FeS clusters are traditionally discussed in the context of catalysis in early evolution, in modern metabolism their main function is electron transfer: in radical SAM enzymes and in redox enzymes generally. A striking example is the enzyme formylmethanofuran dehydrogenase, which contains 46 4Fe4S clusters in the structure of the active enzyme[68] that serve as electron conduits from reduced ferredoxin to the active site.

The ability of Fe^0 to catalyze the transfer of electrons from H_2 to ferredoxin suggests the existence of an intermediate state in early biochemical evolution in which ferredoxin became a soluble and diffusible single electron donor for early redox-dependent processes (Figure 3B), including CO_2 reduction via the acetyl-CoA pathway and N_2 reduction via nitrogenase, such that reactions that were once physically tied to the Earth's crust by virtue of a solid-state catalyst[1] became soluble, hence exportable from the site of life's origin to life as a free-living cell. Before the origin of free-living cells, other ancient proteins with FeS clusters in addition to ferredoxin, such as enzymes of the acetyl-CoA pathway,[1] could have interacted directly with hydride-laden metal surfaces, in a similar manner as sketched for ferredoxin in Figure 3A. If metabolism arose via redox reactions catalyzed on surfaces of Fe^0, Co^0 and Ni^0 (Figure 1), the first main function of FeS proteins would not have been catalysis, but the same as it is today: single electron transfer (Figure 3A and B).

■ THE BIOSYNTHETIC CORE OF 400 REACTIONS

In order for the first free-living cells to emerge, they had to be able to synthesize all of the essential building blocks of life in stoichiometrically useful amounts and more or less specific form, meaning that the rates of the reactions that generate the building blocks of life had to become similar by virtue of catalysts.[69] How many organic catalysts did that involve? Starting from H_2, CO_2, NH_3, H_2S, P_i, and water and salts, the synthesis of the 20 canonical amino acids, the eight nucleobases of DNA and RNA (excluding modifications), and the 18 main cofactors used by modern cells involves about 400 reactions that are thermodynamically favorable under the reducing conditions of serpentinizing hydrothermal vents.[3] One can collectively designate those reactions as the autotrophic biosynthetic core.[70] The biosynthetic core does not include synthesis of the ribosome,[71] tRNA modifications[72] or nucleic acid handling, it just comprises synthesis of the monomeric components.

The reverse citric acid cycle (rTCA) is a central hub of primordial metabolism as it provides the carbon backbones for amino acid synthesis, which in turn are the starting point for cofactor and nucleobase biosynthesis. Almost all of the reactions of the reverse citric acid cycle have been shown in recent work by Joseph Moran's group to operate in water using only metals and metal ions as catalysts.[30,73−75] Notably, the amino group of amino acids is almost always added as the last step in amino acid synthesis via reductive amination of the 2-oxo moiety of the corresponding 2-oxo acid. That can occur without enzymes using pyridoxal phosphate, by using metal catalysts via transamination reactions[76,77] or by using NH_3 and H_2 in the presence of Ni^0 as a solid-state catalyst in water at 25 °C, generating high yields and specific synthesis of 10 different amino acids from their 2-oxo precursors.[30]

We looked among the 400 reactions of the autotrophic core[3] for ancient traits in the form of FeS clusters and radical reactions. We identified 44 reactions catalyzed by FeS proteins (Supplementary Table 1), which is a conservative number due to database incompleteness and lack of characterization for all proteins underlying the reactions. Notwithstanding, FeS proteins were found to be involved in a wide range of core metabolic pathways, spanning from H_2 oxidation to central carbon metabolism including the acetyl-CoA pathway and the reverse tricarboxylic acid cycle, to cofactor biosynthesis (in the pathways for biotin, coenzyme B, cobalamin, coenzymes F_{420} and F_{430}, MoCo, NAD, thiamine diphosphate and tetrahydromethanopterin), amino acid and nucleobase biosynthesis. In *E. coli* about 2−3% of all proteins have been described as FeS proteins, the vast majority adopting a [4Fe4S] geometry,[49,78] the estimate increasing to roughly 5% when including candidate cases.[49] About 11% of reactions in the autotrophic core involve enzymes with FeS clusters. Nonetheless, the overrepresentation is substantial and not unexpected, as redox reactions are also overrepresented in the autotrophic core,[70] the reason being that carbon in CO_2 has to be reduced to the state of alcohols, carbonyls, methenyl, methylene, and methyl groups to make amino acids, cofactors and bases, while peripheral metabolism predominantly involves polymerization reactions of monomers, conjugations, acetal and hemiacetal formations, water eliminations and the like, which are typically redox neutral. Phosphorylations are also almost always redox neutral in metabolism, with one recently characterized and possibly very ancient exception involving ADP synthesis from AMP and phosphite (HPO_3^{2-}) using NAD^+ as the electron acceptor.[65]

Enzymes containing FeS clusters typically require FeS cluster assembly proteins—the Nif, Suf or Isc systems[79]—which incorporate iron as Fe^{2+} from the cytosol and S from cysteine into enzymes typically via cysteine sulfhydryl ligands. Enzymes of the Nif, Suf and Isc systems[79] typically contain FeS clusters, which are not counted here. For the synthesis of some FeS clusters, such as those in the active site of [FeFe] hydrogenase, additional FeS cluster-containing maturases are involved,[54] they are also not counted here. Among the 44 reactions involving FeS proteins, 10 of them were identified as SAM-dependent (~23%), with the FeS cluster initiating a radical reaction in almost all cases (Supplementary Table 1 and Supplementary Table 2).

■ RADICAL REACTIONS

A closer look into the autotrophic core reaction network reveals 14 reactions employing a radical mechanism (Supplementary Table 2), a conservative estimate, as databases are not complete and the properties of the enzymes are not known in all cases. It is generally held that radical enzymes are employed to catalyze demanding reactions to which there is no mechanistic alternative.[53,80] Radicals are highly reactive due to their unpaired electron, and can wreak havoc among molecules of the cell if not properly sequestered and controlled. In enzymes, radicals are generated by homolytic bond cleavage or one-electron transfer from metals and metal clusters or other one-electron carriers.

Radical SAM (rSAM) enzymes are the most common radical enzymes in the autotrophic core (Supplementary Table 2). The generation of the 5′−deoxyadenosyl radical (5′dAdo●) is initiated by one-electron transfer from a [4Fe4S] cluster to SAM, with the cytosolic electron donor being ferredoxin or flavodoxin.[80] The radical can act directly on the substrate, or induce a glycyl radical on the protein. Radical SAM enzymes are ancient, dating back to LUCA,[81] and possibly belonging to the first enzyme families.[53] It is therefore not surprising they accept an electron from ferredoxin, which is also ancient[5,6,38] and can acquire electrons directly from metal surfaces under H_2[1] (Figure 3). In modern *E. coli* cells, rSAM enzymes comprise over 10% of all iron−sulfur proteins,[49] and new ones are constantly being discovered. They are versatile and fulfill diverse cellular functions; they are known to take part in central pathways such as the biosynthesis of the [FeFe] hydrogenase H-cluster, the nitrogenase active site, the molybdopterin cofactor, thiamine diphosphate, biotin, modified tetrapyrroles, but also in RNA modification, DNA repair and others.[53] These pathways are essential to and had to exist before the first free living cells emerged.[70,81] They are ancient.

The energetic barrier for one-electron transfer to SAM is high. In enzymes this is compensated by coordination to the FeS cluster, active site polarity and other solutions.[53] In a prebiotic world it is possible that one-electron reductions of a small organic molecule (SAM) were easier to achieve, with iron-dependent mechanisms perhaps similar to that sketched in Figure 3. Although there are striking similarities between the B_{12} radical reactions and those catalyzed by radical SAM enzymes,[53,82] B_{12} is involved in only about 12 reaction classes in all of known metabolism,[53] while the rSAM family catalyzes over 100 different documented chemical reactions.[57] In adenosylcobalamin-dependent enzymes, the same 5′−deoxyadenosyl radical as the one derived from SAM is induced by homolytic bond cleavage of a cobalt−carbon bond.[80] Radical SAM reactions proceed through an organometallic intermediate called Ω, with a covalent bond being formed between iron of the [4Fe4S] cluster and SAM.[83] Homolytic cleavage of the Fe−C bond liberates the radical, in a similar fashion as for B_{12}, narrowing the gap between the mechanism employed by rSAM and B_{12} enzymes.[57]

One radical reaction that is not formally part of the biosynthetic core,[3] because it is not part of an amino acid, base or cofactor biosynthetic pathway, is included in this analysis nonetheless: methyl-CoM reductase (MCR). MCR catalyzes the terminal step in the methanogenic pathway[28] via a methyl radical intermediate[45] formed at the active site by a Ni-containing tetrapyrrole, F_{430}. By generating methane, MCR catalyzes a radical pulling reaction ($\Delta G^{\circ\prime} = -30$ kJ·mol^{-1})[28]

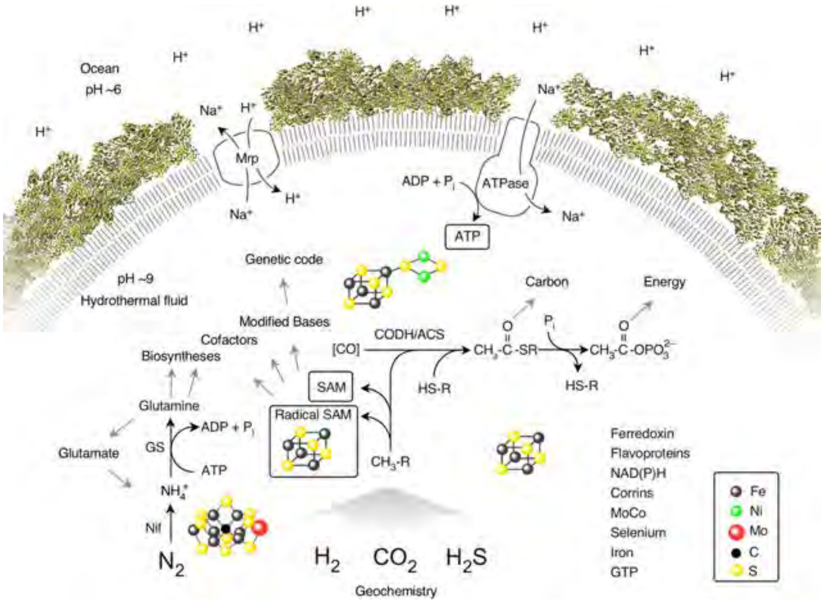

Figure 4. A schematic representation of a noncellular LUCA,[81] reproduced from ref 90, available under a Creative Commons CC BY 4.0 license (http://creativecommons.org/licenses/by/4.0). Copyright 2018 Weiss et al.

for cobamide (a Co-tetrapyrrole)-dependent ion pumping at the MtrA-H methyltransferase reaction (also $\Delta G^{\circ\prime} = -30$ kJ·mol^{-1})[28] that synthesizes methyl-CoM and conserves energy as an ion gradient for ATP synthesis.[28] From that perspective, and looking at Figure 1, the involvement of metal-dependent reactions involving methyl groups at the core of methanogen energy metabolism makes sense: metabolic evolution has not found catalytic alternatives to these metal-catalyzed reactions, it has simply solubilized the metal-dependent methyl reactions with the help of tetrapyrroles and enzymes.

■ ANCIENT METHYL GROUPS PULL COFACTORS DEEP

The corrins required by acetogens and methanogens in the acetyl-CoA pathway are a set of diverse cobamides which differ in lower ligand[84,85] structure, whereby the corrin in the CoFeS protein initially isolated by Ragsdale and team[86] from *Clostridium thermoaceticum* had no lower ligand in the methyl-Co(III) or in the Co(II) or Co(I) forms, identifying 'base-off' corrins in proteins. Cobamides are required for methyl transfer reactions in which the methyl groups are bound by the free (upper) coordination site of the CoFeS Co atom[84,85] and transferred to a Ni atom in the active site of acetyl-CoA synthase, a rare metal-to-metal methyl transfer reaction.[46] The acetyl-CoA pathway, both in nonenzymatic (Figure 1) and enzymatic form,[14,62] is a pathway of methyl synthesis and methyl transfer: to a hydrogen atom in methanogenesis[45] and to CO in the acetyl-CoA pathway.[43] Methyl groups are ancient. There are eight SAM-dependent methylation steps involved in the synthesis of the corrin ring, suggesting that SAM is older than corrins. Methyl groups are also essential for the genetic code to work, with RNA methylations being essential for translation.[71,72,81]

GTP is ancient.[87] The genetic code requires the operation of the ribosome, whereby the ribosome is ancient, more ancient than any protein coding gene, as all protein coding genes are translated on ribosomes. GTP was the energy currency at the origin of translation[87] and it is the aromatic substrate for pterin synthesis (the GTP cyclohydrolase reaction). Pterins are ancient as they are the methyl carriers in the acetyl-CoA pathway of acetogens and methanogens, respectively, tetrahydromethanopterin differing from tetrahydrofolate by the C6 substituent and by additional methyl groups on the pterin ring.[88]

If we accept the robust inference that the acetyl-CoA pathway to pyruvate[14] of acetogens and methanogens is ancient, given its complete replacement by native forms of the metals (Figure 1) that serve in its enzymes and cofactors,[88] it follows that the following cofactors are also ancient: iron, nickel, cobalt, cobamides, SAM, MoCo, NAD(P)+ and flavins, coenzymes A, B and M, ATP and GTP, TPP, ferredoxin, methanofuran, tetrahydrofolate and tetrahydromethanopterin, F_{420} and F_{430}. They are required for the pathway to operate using proteins instead of solid-state metals as catalysts.

Otherwise extremely rare in biology, nickel is very common in ancient metabolism, not only in the acetyl-CoA pathway (Figure 2). The last step of methanogenesis entails the Ni-containing tetrapyrrole F_{430} in the MCR radical mechanism.[45] Yet Ni^0 alone catalyzes methane formation from H_2 and CO_2 in water[18] ($4H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$, $\Delta G^{\circ\prime} = -131$ kJ·mol^{-1}), condensing the entire methanogenic pathway, which methanogens use in a stepwise manner to generate carbon backbones for biosynthesis and ion pumping for ATP synthesis,[28] into a single reaction. Why use a multistep pathway when a single catalyst can perform the reaction? As metabolism evolved from solid−state catalysts to proteins with complex cofactors, highly exergonic single step reactions were split into series of linked reactions, each requiring more sophisticated catalysts that could introduce specificity and rate control.[89]

Perhaps unsurprisingly, the acetyl-CoA pathway pulls almost all universal cofactors into LUCA (Figure 4) except pyridoxal phosphate (transaminations) and biotin (carboxylations). The latter two are however essential for biosynthesis of amino acids[77] and bases. Heme and siroheme, which are essential for cytochrome-dependent electron transfers, are not present in the biosynthetic core and are not universal cofactors as they are not required either by hydrogenotrophic acetogens[27] or by hydrogenotrophic methanogens.[28]

■ CONCLUSION

The list of cofactors and catalysts that the acetyl-CoA pathway draws down into ancient biochemical evolution is almost identical to the list of cofactors identified in an earlier, phylogeny-based approach to the physiology of the last universal common ancestor LUCA.[81] A difference is that we now know that the overall reaction sequence of the acetyl-CoA pathway unfolds from H_2 and CO_2 overnight in the laboratory over transition metals in water under the alkaline conditions of serpentinizing hydrothermal vents,[2,17−20,29] and that several examples of acetogens and methanogens that grow in serpentinizing hydrothermal systems have been discovered.[91−94] These developments converge on one pathway, the acetyl-CoA pathway, and one kind of H_2-producing environment with native metal catalysts[23] at the origin of metabolism, helping to close the gaps between early Earth and early life.[89]

What do we not see in ancient metabolism? We do not see cyanide or nitrile-dependent[95] reactions in primary metabolism, ancient or otherwise. The CN and CO ligands of [FeFe] hydrogenase (Figure 2) are derived from the aliphatic moiety of tyrosine in a specific hydrogenase maturation process[54] although they can also be derived from glycine.[96] Cyanide is never observed in natural environments, not even in volcanic gases,[97] but abiotic glycine is produced by serpentinizing hydrothermal vents in amounts sufficient to support microbial growth.[94] Oró reported cyanide-dependent purine synthesis in 1960.[98] In the 60 years since, the gap between cyanide chemistry and life chemistry has widened, while the gap between the chemistry of hydrothermal vents and metabolism has narrowed. We also do not see RNA bases performing catalytic functions in core metabolism. The bases of RNA do not themselves catalyze any reactions in the acetyl-CoA pathway or in the biosynthetic core, although they do play an essential role in the peptidyl transferase reaction at the ribosome[99] and newer findings mechanistically implicate the modifications of tRNA in primordial peptide synthesis reactions.[100] GTP serves as a precursor for pterin synthesis (cyclohydrolase) and for DNA synthesis.[101] RNA bases are often attached to cofactors, and even though every radical SAM reaction requires bound adenosyl, the base plays no role, the active moiety of the reaction is the $5' - CH_2\bullet$ of ribose.[57] FeS clusters are mainly cofactors of electron transfer (Figure 3), but also serve to coordinate Ni, which exerts catalytic function in reactions central to the acetyl-CoA pathway.[43]

Native Fe, Co, or Ni can replace the entire acetyl-CoA pathway without the help of nitrogen, sulfur or phosphorus. For the bedrock-level origin of metabolism, reactions of H_2 and CO_2 in water on metal surfaces at alkaline pH (conditions of serpentinizing systems) are sufficient.[2,17−20,29] While meteorites can also deliver native metals as catalysts,[102] serpentinizing hydrothermal vents also provide a continuous stream of H_2 as reductant[23] in addition to microcompartments[25] and temperature gradients that can concentrate products of synthesis for further reaction.[103] Though the ion gradients of serpentinizing systems could have powered primitive ATP synthases prior to the origin of biological ion pumping,[23,25,81,89] we see no requirement for ion gradients[104] at the origin of the biosynthetic core, with metabolism emerging from reactions of aqueous H_2 and CO_2 interfacing with solid-state catalysts (Figure 1). The first organic compounds in this view were organic acids, including 2-oxoacids,[74] with the incorporation of N taking place exactly as in metabolism, but with H_2 as reductant and Ni as catalyst.[30] The incorporation of S is facile.[79] The incorporation of phosphate, however, is still not resolved. Using metabolism as a guide, it might have entailed reactions of phosphate with reactive carbonyl moieties[26] or it might have involved reactions of phosphite, as suggested by phosphite-dependent substrate phosphorylations recently discovered in organisms that use the acetyl-CoA pathway.[65] That Ni, Co, and Fe served as catalysts in the enzymatic reactions of the acetyl-CoA pathway,[40] and that these reactions are ancient,[12] was evident 40 years ago. Since that time, serpentinizing hydrothermal vents[105] have conjoined the metals in their native catalytic form (i) with the source of reductant (H_2) that acetogens and methanogens still use today and (ii) with specific products of the acetyl-CoA pathway, marking chemical antiquity in metabolism that connects life to Earth.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.accounts.4c00226.

> Supplementary Table 1 with a list of reactions of the autotrophic core[3] catalyzed by FeS proteins, Supplementary Table 2 with a list of reactions of the autotrophic core[3] catalyzed by radical enzymes, and supplementary references (PDF)

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Notes

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Supporting Information for

Chemical antiquity in metabolism

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Supplementary tables 1 and 2
Supplementary references

KEGG	**E.C.**	**Reaction**	**Pathway**
R00019	1.12.7.2, 1.12.99.- [106,107]	Hydrogen + 2 Oxidized ferredoxin <=> 2 Reduced ferredoxin + 2 H+	Ferredoxin
R01195	1.18.1.2 [106,107]	2 Reduced ferredoxin + NADP+ + H+ <=> 2 Oxidized ferredoxin + NADPH	Ferredoxin
R07157	1.2.7.4 [106,107]	CO2 + 2 Reduced ferredoxin + 2 H+ <=> CO + H2O + 2 Oxidized ferredoxin	Ferredoxin
R00134	1.17.1.10 [108]	Formate + NADP+ <=> CO2 + NADPH + H+	WL
R07168	1.5.1.20 [109]	5-Methyltetrahydrofolate + NAD+ <=> 5,10-Methylenetetrahydrofolate + NADH + H^+	WL
None *	1.5.7.- [110]	5-Methyltetrahydrofolate + Oxidized ferredoxin <=> 5,10-Methylenetetrahydrofolate + Reduced ferredoxin	WL
R10243	2.1.1.258, 2.3.1.169 [106]	5-Methyltetrahydrofolate + CoA + CO <=> Tetrahydrofolate + Acetyl-CoA	WL
R10866	1.2.7.- [107]	Acetyl-CoA + CO2 + Reduced flavodoxin <=> Pyruvate + CoA + Oxidized flavodoxin	rTCA
R01196	1.2.7.1, 1.2.7.11 [106,107]	2 Reduced ferredoxin + Acetyl-CoA + CO2 + 2 H+ <=> 2 Oxidized ferredoxin + Pyruvate + CoA	rTCA, WL
R01197	1.2.7.11, 1.2.7.3 [106,107]	2 Reduced ferredoxin + Succinyl-CoA + CO2 + 2 H+ <=> 2 Oxidized ferredoxin + 2-Oxoglutarate + CoA	rTCA
R01082	4.2.1.2 [111]	(S)-Malate <=> Fumarate + H2O	rTCA
R01325	4.2.1.3 [106,107]	cis-Aconitate + H2O <=> Citrate	rTCA
R01900	4.2.1.3 [106,107]	Isocitrate <=> cis-Aconitate + H2O	rTCA
R11634	1.1.98.6 [106,107,†]	ATP + Formate <=> dATP + CO2 + H2O	Purine
R11633	1.1.98.6 [106,107,†]	GTP + Formate <=> dGTP + CO2 + H2O	Purine
R01072	2.4.2.14 [112]	L-Glutamine + 5-Phospho-alpha-D-ribose 1-diphosphate + H2O <=> 5-Phosphoribosylamine + Diphosphate + L-Glutamate	Purine
R11636	1.1.98.6 [106,107,†]	CTP + Formate <=> dCTP + CO2 + H2O	Pyrimidine
R01869	1.3.1.14 [106]	(S)-Dihydroorotate + NAD+ <=> Orotate + H+ + NADH	Pyrimidine
R01867	1.3.98.1 [113]	(S)-Dihydroorotate + Fumarate <=> Orotate + Succinate	Pyrimidine
R07460	2.8.1.7 [106]	[Enzyme]-cysteine + L-Cysteine <=> [Enzyme]-S-sulfanylcysteine + L-Alanine	TPP
R03472	4.1.99.17 [106,†]	Aminoimidazole ribotide + S-Adenosyl-L-methionine <=> 4-Amino-2-methyl-5-(phosphooxymethyl)pyrimidine + 5'-Deoxyadenosine + L-Methionine + Formate + CO	TPP
R10246	4.1.99.19 [106,†]	L-Tyrosine + S-Adenosyl-L-methionine + Reduced acceptor <=> Iminoglycine + 4-Cresol + 5'-Deoxyadenosine + L-Methionine + Acceptor	TPP
R10392	4.2.1.114 [106,107]	(R)-(Homo)2-citrate <=> cis-(Homo)2-aconitate + H2O	CoB
R10395	4.2.1.114 [106,107]	(R)-(Homo)3-citrate <=> cis-(Homo)3-aconitate + H2O	CoB
R10393	4.2.1.114 [106,107]	cis-(Homo)2-aconitate + H2O <=> (-)-threo-Iso(homo)2-citrate	CoB
R10396	4.2.1.114 [106,107]	cis-(Homo)3-aconitate + H2O <=> (-)-threo-Iso(homo)3-citrate	CoB
R03444	4.2.1.114, 4.2.1.- [106,107]	(R)-2-Hydroxybutane-1,2,4-tricarboxylate <=> (Z)-But-1-ene-1,2,4-tricarboxylate + H2O	Lys
R04371	4.2.1.114, 4.2.1.36 [106,107]	(Z)-But-1-ene-1,2,4-tricarboxylate + H2O <=> Homoisocitrate	Lys
R03968	4.2.1.33 [106,107]	alpha-Isopropylmalate <=> 2-Isopropylmaleate + H2O	Leu

R04001	4.2.1.33 [106,107]	2-Isopropylmaleate + H2O <=> (2R,3S)-3-Isopropylmalate	Leu
R03898	4.2.1.35 [107]	2-Methylmaleate + H2O <=> D-erythro-3-Methylmalate	Ile
R03896	4.2.1.35 [107]	(R)-2-Methylmalate <=> 2-Methylmaleate + H2O	Ile
R05070	4.2.1.9 [114]	(R)-2,3-Dihydroxy-3-methylpentanoate <=> (S)-3-Methyl-2-oxopentanoic acid + H2O	Ile
R04441	4.2.1.9 [114]	(R)-2,3-Dihydroxy-3-methylbutanoate <=> 3-Methyl-2-oxobutanoic acid + H2O	Val, Ile
R00996	4.3.1.19 [106]	L-Threonine <=> 2-Oxobutanoate + Ammonia	Ile
R00248	1.4.1.3, 1.4.1.4, 1.4.1.13 [115]	2-Oxoglutarate + Ammonia + NADPH + H+ <=> L-Glutamate + NADP+ + H2O	Glu
R09394	4.1.99.22 [106,107, †]	GTP + S-Adenosyl-L-methionine + Reduced acceptor <=> (8S)-3',8-Cyclo-7,8-dihydroguanosine 5'-triphosphate + 5'-Deoxyadenosine + L-Methionine + Acceptor	MoCo
R11372	4.6.1.17 [106,107, †]	(8S)-3',8-Cyclo-7,8-dihydroguanosine 5'-triphosphate + H2O <=> Precursor Z + Diphosphate	MoCo
R12161	2.5.1.147 [116, †]	5-Amino-6-(1-D-ribitylamino)uracil + L-Tyrosine + S-Adenosyl-L-methionine <=> 5-Amino-5-(4-hydroxybenzyl)-6-(D-ribitylimino)-5,6-dihydrouracil + Iminoglycine + L-Methionine + 5'-Deoxyadenosine	F420
R12162	4.3.1.32 [116, †]	5-Amino-5-(4-hydroxybenzyl)-6-(D-ribitylimino)-5,6-dihydrouracil + S-Adenosyl-L-methionine <=> 7,8-Didemethyl-8-hydroxy-5-deazariboflavin + Ammonia + L-Methionine + 5'-Deoxyadenosine	F420
R04292	2.5.1.72 [106]	Iminoaspartate + Glycerone phosphate <=> Quinolinate + 2 H2O + Orthophosphate	NAD
R10802	1.5.99.15 [106]	7,8-Dihydromethanopterin + Reduced acceptor <=> 5,6,7,8-Tetrahydromethanopterin + Acceptor	H_4MPT
R11580	2.1.1.272 [106]	S-Adenosyl-L-methionine + Cobalt-factor III + Reduced acceptor <=> S-Adenosyl-L-homocysteine + Cobalt-precorrin 4 + Acceptor	Cobalamin
R01078	2.8.1.6 [106, †]	Dethiobiotin + Sulfur donor + 2 S-Adenosyl-L-methionine + 2 e- + 2 H+ <=> Biotin + 2 L-Methionine + 2 5'-Deoxyadenosine	Biotin

Supplementary Table 1. Reactions of the autotrophic core[3] catalyzed by enzymes that contain iron-sulfur clusters. Iron-sulfur proteins were identified by searching the BRENDA[106] and InterPro[107] databases with the E.C. numbers from the autotrophic core reaction list[3], and parsing the BRENDA comments section and the InterPro domain and family entries for keywords (“Fe4S4”, “Fe2S2”, “Fe3S4” and other notation variants). Several reactions known to be catalyzed by FeS proteins but not identified in the database search were added manually (references are provided in the E.C. column). SAM-dependent reactions were identified by looking for SAM as a reactant/product, and by searching the BRENDA comments section and the InterPro domain and family entries for keywords (SAM, S-adenosylmethionine, AdoMet and other notation variants). The “Pathway” column specifies the metabolic pathway the reaction belongs to, and includes the reverse TCA cycle (rTCA), the acetyl-CoA pathway (WL), and biosyntheses of cofactors, amino acids, nucleobases. * Not present in the analysis in [3]; [†] SAM-dependent reaction.

KEGG	E.C.	Reaction	Pathway
R01196	1.2.7.1, 1.2.7.11 [63]	2 Reduced ferredoxin + Acetyl-CoA + CO2 + 2 H+ <=> 2 Oxidized ferredoxin + Pyruvate + CoA	rTCA, WL
R04541 *	2.8.4.1 [45]	Coenzyme B + 2-(Methylthio)ethanesulfonate <=> Coenzyme M 7-mercaptoheptanoylthreonine-phosphate heterodisulfide + Methane	WL
R11634	1.1.98.6 [106,107,†]	ATP + Formate <=> dATP + CO2 + H2O	Purine
R11633	1.1.98.6 [106,107,†]	GTP + Formate <=> dGTP + CO2 + H2O	Purine
R11636	1.1.98.6 [106,107,†]	CTP + Formate <=> dCTP + CO2 + H2O	Pyrimidine
R03444	4.2.1.114, 4.2.1.- [107]	(R)-2-Hydroxybutane-1,2,4-tricarboxylate <=> (Z)-But-1-ene-1,2,4-tricarboxylate + H2O	Lys
R01714	4.2.3.5 [106]	5-O-(1-Carboxyvinyl)-3-phosphoshikimate <=> Chorismate + Orthophosphate	Trp, Phe, Tyr
R00946	2.1.1.13 [117]	5-methyltetrahydrofolate + L-homocysteine <=> tetrahydrofolate + L-methionine	Met
R10246	4.1.99.19 [106,107,†]	L-Tyrosine + S-Adenosyl-L-methionine + Reduced acceptor <=> Iminoglycine + 4-Cresol + 5'-Deoxyadenosine + L-Methionine + Acceptor	TPP
R01078	2.8.1.6 [106,107,†]	Dethiobiotin + Sulfur donor + 2 S-Adenosyl-L-methionine + 2 e- + 2 H+ <=> Biotin + 2 L-Methionine + 2 5'-Deoxyadenosine	Biotin
R12161	2.5.1.147 [106,107,†]	5-Amino-6-(1-D-ribitylamino)uracil + L-Tyrosine + S-Adenosyl-L-methionine <=> 5-Amino-5-(4-hydroxybenzyl)-6-(D-ribitylimino)-5,6-dihydrouracil + Iminoglycine + L-Methionine + 5'-Deoxyadenosine	F420
R12162	4.3.1.32 [106,107,†]	5-Amino-5-(4-hydroxybenzyl)-6-(D-ribitylimino)-5,6-dihydrouracil + S-Adenosyl-L-methionine <=> 7,8-Didemethyl-8-hydroxy-5-deazariboflavin + Ammonia + L-Methionine + 5'-Deoxyadenosine	F420
R09394	4.1.99.22 [106,107,†]	GTP + S-Adenosyl-L-methionine + Reduced acceptor <=> (8S)-3',8-Cyclo-7,8-dihydroguanosine 5'-triphosphate + 5'-Deoxyadenosine + L-Methionine + Acceptor	MoCo
R11372	4.6.1.17 [107,†]	(8S)-3',8-Cyclo-7,8-dihydroguanosine 5'-triphosphate + H2O <=> Precursor Z + Diphosphate	MoCo

Supplementary Table 2. List of reactions in the autotrophic core[3] catalyzed by radical enzymes. Radical enzymes were identified by searching the BRENDA[106] and InterPro[107] databases with the E.C. numbers from the autotrophic core reaction list[3], and parsing the BRENDA comments section and the InterPro domain and family entries for keywords ("radical"). Several reactions known to be catalyzed by radical enzymes but not identified in the database search were added manually (references are provided in the E.C. column). SAM-dependent reactions were identified by looking for SAM as a reactant/product, and by searching the BRENDA comments section and the InterPro domain and family entries for keywords (SAM, S-adenosylmethionine, AdoMet and other notation variants). The "Pathway" column specifies the metabolic pathway the reaction belongs to, and includes the reverse TCA cycle (rTCA), the acetyl-CoA pathway (WL), and biosyntheses of cofactors, amino acids, nucleobases. * Not present in the analysis in [3]; [†] SAM-dependent reaction.

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10.4 Publication 4

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LUCA and the Origins of Cellular Life

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Key Points

- LUCA (the Last Universal Common Ancestor) is an inferred transitional state between the origin of life and the first microbial fossils.
- LUCA is the common ancestor of bacteria and archaea, which means it is the common ancestor of all extant life.
- Universal traits are widespread and conserved across life, but they cannot provide information about some aspects of LUCA's physiology.
- Lateral gene transfer between archaea and bacteria makes tracing specific genes to LUCA challenging.
- According to recent reconstructions, LUCA had a genetic code and was a chemolithoautotroph, but was half-alive, dependent on geochemistry.
- Present data suggest that LUCA existed in serpentinizing hydrothermal vents that provided H_2 as reductant, CO_2 as carbon source for the acetyl-CoA pathway, transition metals as catalysts, methyl groups and gradients.

Glossary

(Anti)Codon A Codon is a sequence of three nucleotides in mRNA that encodes a specific amino acid. An anti-codon is a sequence of three nucleotides in an amino acid-carrying tRNA that pairs with a codon during protein synthesis, translating the genetic code into amino acid sequence.

(Poly)Peptide A chain of 2–50 amino acids. A polypeptide has at least 51 amino acids.

Acetogens Strictly anaerobic bacteria that obtain their carbon and energy from the reaction of H_2 and CO_2 to form acetate using the acetyl CoA pathway.

Acetyl-CoA pathway The most ancient pathway of CO_2 fixation, present in bacteria and archaea. When it functions autotrophically, it generates acetyl-CoA or pyruvate from CO_2 and H_2. Widespread in many obligate anaerobes such as methanogens, acetogens and others.

Adenosine triphosphate (ATP) The universal energy currency. It can be employed to promote metabolic reactions that require energy input. ATP is a nucleotide with two high-energy phosphoanhydride bonds.

Aminoacylation The attachment of an amino acid to a tRNA.

Archaea One of the two domains of prokaryotes. They differ from bacteria in many ways, most notably in the composition of their lipid membranes, which contain isoprene ethers of glycerol-1-phosphate.

Autotrophy Autotrophic organisms have the ability to produce organic compounds using CO_2 as the source of carbon. The energy can come from light or chemical reactions.

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Bacteria One of the two domains of prokaryotes. They differ from archaea in many ways, most notably in the composition of their lipid membranes, which contain fatty acid esters of glycerol-3-phosphate.

Carbon fixation The process by which some organisms transform CO_2 to organic compounds.

Catalyst A substance that increases the rate of a chemical reaction without being changed in the process.

Cell wall A structural layer present outside the cytoplasmic membrane. It gives structural strength and protection from osmotic lysis to the cell. The composition of cell walls is significantly different between bacteria, archaea and eukaryotes.

Chemolithoautotroph An organism that is at the same time a chemotroph (obtains energy from chemical reactions, not light), a lithotroph (a chemotroph that gets energy from inorganic compounds, not organics) and an autotroph (uses CO_2 as the source of carbon, not organics).

Chemotroph An organism that gets its energy from chemical compounds, not light.

Class One of the classifications of taxonomy. The highest taxon is a domain. Then in descending order there are: kingdom, phylum, class, order, family, genus and species.

Cofactor An additional chemical component bound by an enzyme and required for its activity. Cofactors can be metal ions or more complex organic molecules (coenzymes).

Differential gene loss An evolutionary process which results in a certain gene being lost in some lineages, but retained in others. This means that a gene originating in an ancestral lineage is not present in all of its descendents.

DNA Deoxyribonucleic acid, the molecule that contains the genetic material (genes) of all cells and some viruses. The universal code of life.

Domain Highest taxonomic classification in biology.

Electron transfer Electrons are atomic particles that orbit around the atomic nucleus, and that can get transferred during redox reactions from atoms of an electron donor to atoms of an electron acceptor.

Enzyme A biological catalyst made up of proteins. Each enzyme increases the rate of a specific reaction or group of reactions.

Eukaryote A cell or organism whose cells have a nucleus and organelles. The nucleus and organelles have their own membranes. All true multicellular organisms are eukaryotes.

Family One of the classifications of taxonomy. See "Class".

Gene family A group of genes that evolved by duplication from one original gene. All genes in a gene family are therefore evolutionarily related, and so are the proteins in the corresponding protein family.

Gene A segment of DNA (or RNA in some viruses) that encodes a specific protein.

Genome The total of all genetic information of a cell or virus.

Geochemistry The study of the chemistry of Earth.

GTP Guanosine triphosphate, an energy currency used in translation and ribosome biogenesis.

Habitat An environment within a larger ecosystem in which an organism lives.

Heterotrophy Heterotrophic organisms need an organic substance as their carbon source.

Homologous Similar by common ancestry. Proteins in different species that are similar in sequence and/or structure because they have a common ancestor are homologous proteins.

Hydrogenase An enzyme capable of using H_2 as the electron donor for another compound, almost always the small protein ferredoxin. Found in anaerobic organisms.

Hydrothermal vents Springs at the bottom of the ocean that emit warm or hot water and usually other compounds that have seeped out of the rocks because of the hot water venting through them. They are usually close to crustal spreading zones on the sea floor.

Intra- and inter-domain Intra = within/inside, inter = between/among. Intra-domain LGT denotes transfers of genes between different bacteria, or between different archaea. Inter-domain LGT denotes gene transfers between bacterial and archaeal cells.

Ion A positively or negatively charged molecule or atom.

Lateral gene transfer (LGT) A process by which prokaryotes transfer genes from one cell (organism) to the other independently of vertical parent-to-offspring inheritance. LGT can occur by conjugation (the transfer of regions of DNA from one organism to the other via specialized plasmids), transformation (the direct uptake of environmental DNA) or transduction (the transfer of DNA segments via viral vectors).

Lineage A temporal sequence of individuals of given taxonomic rank that have a continuous line of common descent. Examples are the primate lineage, the insect lineage, the bacterial lineage, etc.

Membrane In generic terms, a thin sheet or layer. In biological or biochemical terms, a membrane refers specifically to a lipid bilayer. Membranes separate the contents of a cell from the environment. They sustain ion gradients and harbor transmembrane proteins.

Metabolism The sum of biochemical reactions in a cell. These reactions can be involved in breaking down compounds and harnessing energy (catabolism) or in the synthesis of cell parts (anabolism). Acetogens and methanogens have the unusual property of harnessing energy from the synthesis of organic compounds from H_2 and CO_2.

Methanogens Strictly anaerobic archaea that obtain their carbon and energy from the reaction of H_2 and CO_2 to form methane using the acetyl-CoA pathway.

Monophyly Descent from a single common ancestor.

MtrA-H A membrane-integral enzyme (a methyltransferase) in methanogens. It transfers a nitrogen bound methyl group to CoM (coenzyme-M) and simultaneously pumps Na^+ out of the cell. It generates the ion gradient that methanogens use to drive their ATP synthase.

Nucleobase A chemical compound made up of one or two rings containing C, O, H and N. There are 5 nucleobases in the genetic code (including DNA and RNA): Adenosine (A), Guanine (G), Cytosine (C), Thymine (T) and Uracil (U). A forms a basepair with T/U and C forms a basepair with G.

Nucleotide A monomeric unit of a nucleic acid made up of a sugar (ribose in RNA, deoxyribose in DNA), a phosphate group and a nucleobase.

Organelle A membrane-enclosed structure within a eukaryotic cell. Examples are the mitochondrion and the chloroplast.

Organic compound A chemical molecule that contains at least one carbon-hydrogen or carbon-carbon bond.

Phototroph An organism that gets energy from light.

Phylogenetic tree Phylogeny is the history of evolution of all life on Earth. A phylogenetic tree is the most common way in which phylogeny can be portrayed. Because it consists of nodes and branches, it is called a tree.

Phylum One of the classifications of taxonomy. See "Class".

Prokaryote A single-cell organism that lacks organelles and usually has its genome in a single circular molecule instead of multiple nucleus-enclosed chromosomes like eukaryotes. Bacteria and Archaea are prokaryotes.

Proton gradient A spacial difference between the concentration of protons (a positively charged hydrogen atom), often generated by a combination of proton pumps and membrane separation. The protons can spontaneously (without energy input) move only in one direction, from higher to lower concentration.

Radicals Highly reactive chemical compounds with unpaired electrons. They are formed in some chemical reactions, including some enzymatic reactions.

Redox reactions Reactions where the number of electrons in one or more atoms effectively changes. An electron transfer between an electron donor and an electron acceptor is a redox reaction.

Replication DNA synthesis from an existing DNA strand.

Reverse Krebs cycle A cyclic metabolic pathway of CO_2 fixation that produces organic compounds from carbon dioxide by using electron donors such as ferredoxin or NADPH. It requires energy input (ATP hydrolysis). It is used by some bacteria. Also known as the reverse tricarboxylic acid cycle (rTCA).

Ribosome Component of the cell that is made up of proteins and ribosomal RNA (rRNA). It produces proteins by linking amino acids together by reading the codons provided by the mRNA with the aid of tRNAs. They are responsible for the translation process in cells.

Ribozyme Short for ribonucleic acid enzyme. An RNA molecule capable of catalysis.

RNA Ribonucleic acid. There are multiple forms of RNA, the most well known ones are messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA). It is essential for protein synthesis.

Rnf Short for *Rhodobacter* nitrogen fixation. A membrane protein (oxidoreductase) in acetogens that transfers electrons from reduced ferredoxin to NAD^+ and pumps Na^+ out of the cell. It generates the ion gradient that acetogens use to drive their ATP synthase.

Serpentinization A reaction of metal minerals in the Earth's crust with ocean water that produces H_2 and alters the composition of the minerals.

Species In eukaryotes that reproduce sexually a species is roughly defined as all organisms that can reproduce with one another and produce viable and fertile offspring. In prokaryotes the definition is more challenging. It is usually a group of strains that share major traits and differ from other groups of strains in one or more major traits. All members of a species have a relatively recent common ancestor.

Substrate-level phosphorylation A mechanism that involves the direct synthesis of ATP (adenosine triphosphate) from ADP (adenosine diphosphate) and phosphate, with the energy to carry out this reaction coming from the hydrolysis of a high-energy bond in a high-energy compound such as 1,3-bisphosphoglycerate or acetyl-phosphate, not from a chemiosmotic transmembrane gradient.

Trait A very broad term to define the characteristics of an individual. This can refer to anything, from morphological characteristics such as shape, to metabolic adaptations. Most traits are determined by genes and/or the habitat the organism lives in.

Transcription mRNA synthesis from a complementary DNA strand.

Transition metals Metals from groups 3−12 of the periodic table, such as copper, iron, nickel and cobalt. They have unpaired electrons in their d-orbitals, which determines their reactivity.

Translation Protein synthesis on the ribosome using mRNA as a template.

Tree of Life The phylogenetic tree of all life on Earth.

Abstract

The last universal common ancestor (LUCA) is the ancestor of all life on Earth. It is an inferred intermediate state between the origin of life and the first free living cells. Lateral gene transfer being common among prokaryotes makes it difficult to determine what LUCA looked like and because of these challenges, many hypotheses exist about the nature of LUCA and the universal tree of life. Recent studies suggest that LUCA most likely lived from chemical reactions (not sunlight), obtained its carbon from CO_2 and used H_2 as a source of energy and electrons along a reaction sequence that strongly resembles the modern acetyl-CoA pathway. It probably arose and lived in a serpentinizing (H_2-producing) hydrothermal vent which provided CO_2 as its carbon source and H_2 as its reductant, transition metals as catalysts and cofactors as well as ion- and temperature-gradients.

Introduction: What is LUCA?

The Earth is 4.55 billion years old, liquid water existed on Earth roughly 4.2 billion years ago and the first traces of life appear in rocks that are 3.9—3.5 billion years of age (Arndt and Nisbet, 2012). The last universal common ancestor, LUCA, is the inferred link between geochemical reactions on an uninhabited planet and a young Earth teeming with microbial life. All chemical reactions of life take place in water, or in the water-free active site of enzymes that are dissolved in water. Considering that LUCA is a water-based organism, it must have existed sometime between the first appearance of water and the origin of the two prokaryotic domains: Bacteria and Archaea. There is no direct evidence in fossils or rocks that would reveal what LUCA looked like or where it lived. But we can explore the attributes, the biology and the lifestyle of LUCA using evolutionary inference and phylogenetic reconstructions. Based on what we know about modern and ancient lineages, we can also explore the kind of habitats in which LUCA might have existed.

An early concept of LUCA was called the progenote (Woese and Fox, 1977). The progenote (from Greek: "*pro*", before, and "*genēs*", of specified kind) is a hypothetical primitive entity that had not yet evolved a link between genotype and phenotype. Modern prokaryotes are complex free-living cells and are assigned either to Bacteria or Archaea in classification systems. In primordial evolution, there had to be intermediate states en route to cellular organization that were not yet free-living, hence not assignable either to Bacteria or Archaea. The progenote had smaller and less complex proteins and genomes and was just in general far less complex than the cells we know today. Prokaryotes then evolved from the progenote. Today, the term progenote has largely been replaced by the term LUCA.

There are many different theories for the origin of life and how LUCA ultimately came to be. There are those focused on the genetic material and the concept of self-replicating RNA molecules (Joyce, 2009; Cech, 2012). These give rise to a concept of LUCA that existed more or less as a kind of "living" nucleic acid. RNA can be synthesized in laboratory experiments but under conditions that most geochemists would deem unlikely to have ever existed on the primitive Earth (Pahlevan et al., 2019). Furthermore, RNA-centric views of LUCA focus on the origin of genetic material rather than metabolism and therefore do not readily connect to metabolism or the environment. There is also a biological approach to LUCA from a comparative viewpoint. Traits that are common to all cells should be present in LUCA, and this is where newer findings have led to major changes in how LUCA is reconstructed (Weiss et al., 2016, 2018).

Main Sets of Ideas on the Nature of LUCA

The idea that all life arose from a common ancestry can be traced back to Darwin who first proposed the Tree of Life (TOL). Different kinds of trees that try to relate the different species to each other have since been proposed. Until recently the three-domain system suggested by Woese and colleagues (Woese and Fox, 1977; Woese et al., 1990) has been the most widely accepted tree of life. They suggest that LUCA is the common ancestor of Bacteria, Archaea and Eukaryotes with Archaea and Eukaryotes branching off later and having a common ancestor. However, in recent years more and more research suggests that we are actually dealing with a two domain system, with LUCA being the common ancestor of Bacteria and Archaea and Eukaryotes then either branching off from a phylum of Archaea (Williams et al., 2013) or coming from endosymbiotic events of Archaea and Bacteria (Weiss et al., 2018).

The question of what LUCA actually looked like, or to which modern cells it was most similar in terms of physiology, is a strongly debated topic. There is the bacterial root case, with Archaea branching off at a later point (Cavalier-Smith, 2006) (**Fig. 1**i). The reverse case was also suggested (Caetano-Anollés et al., 2014) (**Fig. 1**ii). There was also the suggestion that LUCA was very complex, having for example different kinds of lipids in the cell membrane, and Bacteria and Archaea eventually branched off from this complex LUCA (Wächtershäuser, 2003) (**Fig. 1**iii). Newer studies have pointed to a geochemical, half-alive LUCA, which was more primitive than either Bacteria or Archaea and was not yet capable of being a free-living cell, requiring chemical input from its geochemical environment (**Fig. 1**iv), but already had many functions inherent to life (Weiss et al., 2016, 2018).

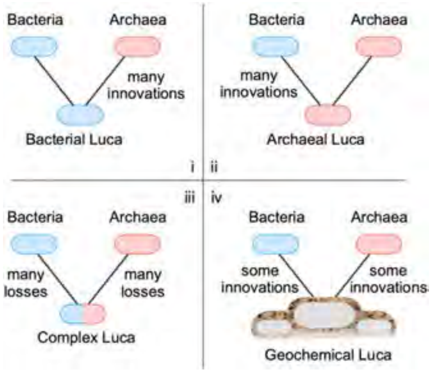

Fig. 1 Different views of the relationship of the last universal common ancestor LUCA to modern cells. See text for details.

LUCA's Universal and Non-universal Traits

Universal traits are widespread across all organisms of the tree of life, which makes them naturally traceable to LUCA. In other words, if all living organisms, from bacteria to human, have genes stored in their DNA, it is clear-cut to surmise that their common ancestor had genes stored in its DNA. This trait was then simply inherited from one generation to the next, over hundreds of millions of years and even billions of years. New species, families, classes, phyla of organisms emerged, always inheriting the trait. However, an inference of the characteristics of LUCA based on universal traits paints too narrow a picture, because many biological properties cannot be inferred by looking at what is universal across the tree of life. Even though all prokaryotes are water-based unicellular organisms with no organelles, they are very diverse, in that they exhibit a wide range of ecological, physiological and metabolic adaptations (Madigan et al., 2018), the driving forces behind their evolutionary success.

DNA, RNA and Metabolism

All life on Earth has DNA and a transcription, translation and replication apparatus, and while the transcription and translation machinery has a highly conserved, homologous core in all domains of life, the DNA replication enzymes are not homologous. This seems to imply LUCA could not replicate its DNA in the same way modern cells do.

LUCA also had a core metabolism for the synthesis of amino acids, cofactors and nucleotide bases, with 402 core reactions required to synthesize those compounds from H_2, CO_2, NH_3, H_2S, phosphate and mineral salts (Wimmer et al., 2021). But not every prokaryote on Earth actually uses these 402 core reactions, because many organisms obtain important nutrients from their environment. For example, humans (and all animals) obtain their B vitamins and half of their amino acids from their diet. In early stages of evolution, before LUCA had a fully developed metabolism, LUCA also must have acquired many essential building blocks of life from the environment.

All cells are surrounded by lipid membranes, but the chemical constituents of cell membranes in bacteria and archaea are different: bacteria synthesize fatty acid esters via enzymatic pathways, archaea synthesize isoprene ethers via enzymatic pathways (Koga et al., 1998). As lipids, LUCA probably used hydrophobic compounds provided by the environment before the enzymatic pathways of bacteria and archaea evolved (Martin and Russell, 2003).

The components that are responsible for performing cellular functions sometimes differ across bacteria and archaea but the underlying function they perform is for the same purpose. To account for these differences, which impact the ribosome (the central protein-synthesizing machine of the cell) (Fox et al., 1980), the lipid membrane, the cell wall (Albers and Meyer, 2011) and more, biologists typically resort to one of the four basic schemes outlined in **Fig. 1**. Either (i) archaea underwent many transitions and evolved from a bacterial ancestor, or (ii) bacteria underwent many transitions and evolved from an archaeal ancestor, or (iii) LUCA had all such traits and bacteria and archaea evolved via loss rather than invention, or (iv) LUCA was simple, not free living, chemically supported by the environment and the ancestors of both lineages evolved the differences that distinguish bacteria from archaea today. Although there is no fossil LUCA in existence to provide final answers, the simplest explanation is that LUCA was a primitive organism that had primordial or geochemically supplied versions of many of these functions. All theories for the origin of life require that chemical components had to be provided by the environment before cells could synthesize all of their constituents. In that sense, LUCA was likely a progenote, a not-yet free-living cell, because of the significant differences between Bacteria and Archaea, even though many of the functions and (proto-) proteins common among cells were already present in LUCA (Di Giulio, 2023).

Because all cells use the same genetic code, we can infer that LUCA had a functional (possible primitive) ribosome that could form peptide bonds and synthesize proteins. Despite there being differences between ribosomes of bacteria and archaea, parts of the ribosome are remarkably conserved, including the overall 3D structure and several ribosomal proteins and ribosomal RNAs. In terms of structure and function, the most strongly conserved portion of the ribosome is a 70 nucleotide stretch containing the peptidyl-transferase site (the site that makes peptide bonds during translation) called the protoribosome (Yonath, 2009). Although the protoribosome corresponds to only about 1% of a modern ribosome's total mass, it can bind aminoacyl-tRNAs and form peptide bonds from them (Bose et al., 2022). But the protoribosome can also bind aminoacyl minihelices, 7−11 nucleotide long RNA molecules that correspond to the stem of tRNA where amino acids are bound. Minihelices are possibly the simpler evolutionary precursors of tRNAs, they can also be aminoacylated by aminoacyl-tRNA synthetases (Tamura, 2015; Schimmel, 2018).

Lateral Gene Transfer

Inheritance in prokaryotes is not always vertical. That is, prokaryotes can, and do, readily transfer genes across lineages without regard for lineage affiliation. Lateral gene transfer was discovered in the 1950s and 1960s in hospitals, as antibiotic resistance spread rapidly from one pathogen to the next, making antibiotic treatments increasingly difficult. The reason turned out to be lateral gene transfer: the antibiotic resistance genes were encoded on small extrachromosomal DNA circles (plasmids) that were passed from donor to recipient regardless of species boundaries (Freeman, 1951; Akiba et al., 1960). Prokaryotes are capable of harnessing chemical energy or light energy and evolved the ability to use a wide variety of chemical compounds as electron donors and acceptors. Considering the diversity of prokaryotic metabolism, universality is clearly not a good indicator of the metabolic traits of the last universal common ancestor. Most of the metabolic adaptations in prokaryotes have originated long after LUCA and reflect the flexibility of prokaryotes to adapt to different habitats and lifestyles. Their evolution cannot be easily traced on a phylogenetic tree because of the prevalence of intra− and interdomain LGT (lateral gene transfer) in prokaryotes (Nelson-Sathi et al., 2015). LGT makes it extremely challenging to distinguish whether the presence of a trait in two evolutionarily distant lineages is the result of lateral gene transfer between members of these lineages, or of the presence of the trait in a common ancestor, with differential loss of the trait in the lineages that do not exhibit it. Coupled with gene loss, these are main reasons why determining which traits were present in LUCA and in what form, and which were not, remains difficult to this day. Note that LGT can only explain the phylogenetic distribution of shared traits, it does not explain the origin of novel traits.

LUCA's Carbon Source

Cells are made up of roughly 50% carbon by dry weight. In order to obtain the carbon to build up their cells, prokaryotes can metabolize preexisting organic compounds (heterotrophs) or reduce environmental CO_2 to organic carbon (autotrophs). The only carbon compound that was continuously bioavailable on the early Earth was CO_2 (Sossi et al., 2020; Mrnjavac et al., 2023), the carbon source of all modern ecosystems. What about organisms that cannot fix CO_2 (heterotrophs)? Even today, all cells acquire carbon either from CO_2 or from organic compounds that were at some point produced by autotrophs from CO_2. This all points to heterotrophs appearing late in evolution by inventing mechanisms to tap the organic compounds that autotrophs were producing and releasing into the environment. Under this premise, LUCA was an autotroph capable of carbon fixation. It was once thought that glycolysis, the breakdown of glucose (C6) to pyruvate (C3) in heterotrophs, was the first pathway of carbon metabolism (Degani and Halmann, 1967). However, the early Earth lacked substrates from which cells could extract energy for heterotrophic growth (Schönheit et al., 2016) while pathways that supply energy from reactions of H_2 and CO_2 were abundant on the primordial Earth and probably provided the energy for the origin of LUCA (Weiss et al., 2016).

LUCA's Energy Source and Energy currencies

To obtain the energy necessary to fuel the reactions that sustain life, modern prokaryotes conserve energy from energy-releasing (exergonic) reactions in chemical compounds (chemotrophs) or from photons emitted either by the sun or by thermal light emitted from hydrothermal vents (phototrophs) (Martin et al., 2018). In most cases energy is released in a series of electron transfer reactions (redox reactions) in which electrons move from the initial electron donor (reductant) to an electron acceptor (oxidant), which can either be an environmental compound such as SO_2 (respiration) or produced during metabolism such as pyruvate (fermentation). Phototrophy originated post-LUCA in the domain bacteria, while chemotrophy is considered to be the ancestral mechanism of energy conservation. Few of these compounds were available on the early Earth, but CO_2 and H_2 were both abundant in serpentinizing hydrothermal vents (Schwander et al., 2023).

Modern theories for the origin of life assume the existence of a LUCA that was a chemolithoautotroph, meaning it used CO_2 as a carbon source and relied on environmental inorganic electron donors for biosynthesis and chemical reactions to satisfy its energy needs. In all modern cells (hence in LUCA), energy is conserved in the form of adenosine triphosphate (ATP). ATP is the most well-known energy currency, but it is not the only one. In all modern cells (hence in LUCA) GTP is employed by enzymes that are involved in the assembly and maturation of the ribosome, and by proteins that move the ribosome along the mRNA during translation. GTP at the ribosome is equally universal as ATP in biochemical pathways. Because all proteins are synthesized by the ribosome, including the main enzyme that catalyzes ATP synthesis—the ATP synthase—and because translation is GTP dependent, it would appear that GTP (which powers ribosome biogenesis and function) is the more ancient energy currency, with ATP appearing after the origin of the ATP synthase. During the early evolution of life before LUCA, GTP became established as the universal energy currency of translation, while ATP became the main energy currency of metabolism (Mrnjavac and Martin, 2024).

Extrapolating From Genomes: A Geochemical LUCA

As outlines above, LGT can hinder the inference of which proteins were present in LUCA because genes present in bacteria and archaea can either be the result of vertical inheritance from LUCA or the result of LGT. How to distinguish between the two? One approach is to identify and remove cases of lateral gene transfer between bacteria and archaea. One such study identified from over 250,000 gene families present in bacteria and archaea 355 gene families that, in their respective phylogenetic tree, showed a clear separation of bacteria and archaea (the domains are monophyletic), and that were present in at least two distinct lineages of each domain (Weiss et al., 2016). These 355 genes were probably not the result of LGT and hence were likely present in the last universal common ancestor. The LUCA reconstructed from those genes was not a free-living cell, which require about 1000 genes to survive. Rather, it was half-alive and was heavily dependent upon geochemistry (**Fig. 2**). It had a DNA-based genetic code and ribosomal translation. In order to satisfy its energetic requirements, it harnessed geochemically generated ion gradients via an ATP synthase, but also used substrate-level phosphorylation (SLP).

Out of the 7 known CO_2 fixation pathways (Sánchez-Andrea et al., 2020), only genes for the acetyl-CoA pathway turned up in the LUCA reconstruction. The acetyl-CoA pathway was previously proposed to be the most ancient metabolic pathway of CO_2 fixation (Fuchs and Stupperich, 1985), it is the only one present in both archaea and bacteria, and it can release energy instead of consuming it. The acetyl-CoA pathway is at the core of modern chemolithoautotrophic theories for the origin of life (Martin and Russell, 2007). The electron donor for the pathway is H_2, and hydrogenases required to harness H_2 gas by prokaryotes were also present in the LUCA gene set. The pathway is highly dependent on transition metals, in that they make up the metal clusters in the active sites of several enzymes of the pathway and are bound by several cofactors. It also relies on organic cofactors such as flavins, pterins, corrins (which also require cobalt), and others. Genes for the synthesis of several cofactors were found in the LUCA set.

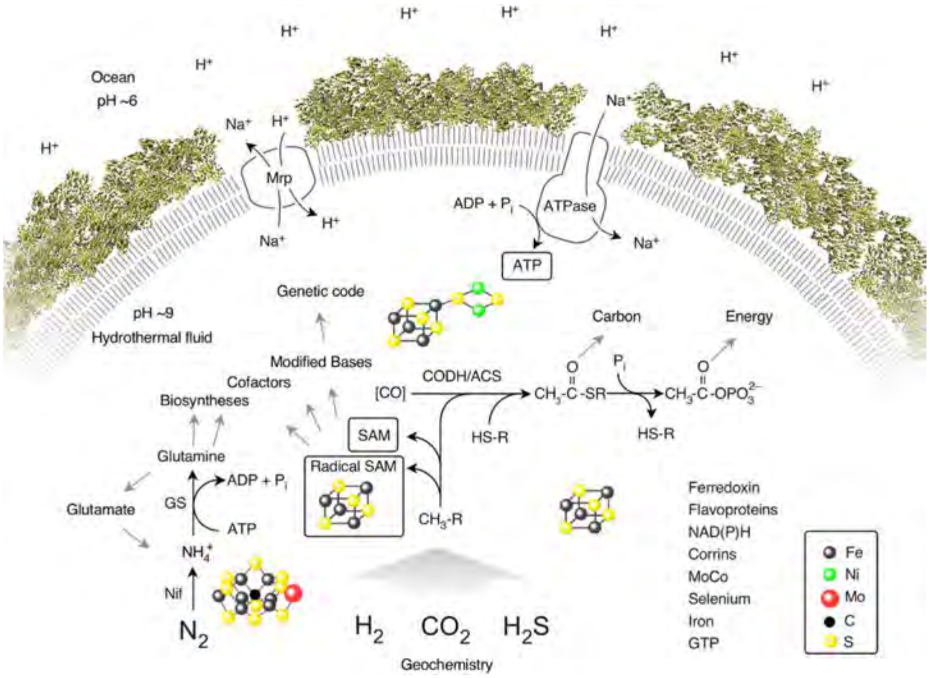

Fig. 2 A geochemical LUCA, as reconstructed in Weiss et al. (2016). LUCA was not a free-living cell; it existed enclosed in pores in hydrothermal vent chimneys. According to this reconstruction, it was a half-alive transition state between the onset of metabolism and free-living cells (Bacteria and Archaea). Reproduced from Weiss et al. (2018) under a creative commons CC BY 4.0 license. © 2018 Weiss et al.

Some common cofactors bound by the proteins of LUCA include iron-sulfur clusters, which are known for their role in electron transfer in proteins, and the organic cofactor *S*-adenosyl methionine (SAM) which enables the transfer of methyl groups, $-CH_3$, between compounds, sometimes involving a radical mechanism. Several enzymes that catalyze the introduction of chemical modifications (with methyl group additions being the most common) on nucleobases that make up RNA nucleotides were also identified. This points to the antiquity of RNA base modifications, which is not surprising, as RNA modifications are required for codon-anticodon recognition, i.e. for the functioning of the genetic code. It is also possible that direct chemical interactions between the chemical ligands of modified bases preceded the genetic code in primitive translation processes (Müller et al., 2022).

Habitat and Physiology

Hydrothermal Vents and Ancient Microbes

At the bottom of the ocean there are hydrothermal vents that undergo a process called serpentinization. Serpentinization is a geochemical process in which water circulating through the Earth's crust reacts with metal-containing minerals and produces hydrogen (Schrenk et al., 2013). Serpentinizing hydrothermal vents exist today but also existed on the early Earth (Tamblyn and Hermann, 2023). This hydrogen can then be used as an electron donor for reactions that produce simple organic compounds that are identical to intermediates and end products of the acetyl-CoA pathway (Schwander et al., 2023). Serpentinizing systems in deep-sea hydrothermal vents are an environment that provides CO_2 and H_2 for the acetyl-CoA pathway and transition metals for the enzymes' metal centers and cofactors. At present, they are most likely the habitat in which LUCA could have arisen. The ubiquity of methylations in LUCA's RNA and metabolism suggests that the genetic code and protometabolism arose in an environment where reduced C1 intermediates (such as methyl groups) were readily available. RNA methylations in LUCA (and modern cells) reflect the chemical nature of the environment where the genetic code arose. This is in agreement with previous proposals of deep-sea vents as sites for life's origin and early evolution (Baross and Hoffman, 1985).

Serpentinizing systems provide all essential components for life: carbon, hydrogen, nitrogen, oxygen, phosphorus, sulfur, metals and minerals, as well as energy, temperature, ion and pH gradients (Schwander et al., 2023). Even compounds that were thought difficult to abiotically synthesize such as a nitrogen source for amino acid production and a phosphorus source have been synthesized in a simulated hydrothermal environment through serpentinization by Shang et al. (2023) (ammonia) and Pasek et al. (2013) (phosphite). Under the conditions of serpentinizing hydrothermal vents, the synthesis of amino acids takes place readily (Kaur et al., 2024).

Another strong indicator for serpentinizing hydrothermal vents being the habitat that hosted LUCA is the identification of bacterial acetogens (Clostridia) and archaeal methanogens as the bacterial and archaeal lineages that appeared to be the most ancient based on their basal branching in phylogenetic trees (Weiss et al., 2016), in line with previous proposals (Decker et al., 1970). They are part of microbial communities that inhabit hydrothermal vents today (Colman et al., 2022; Nobu et al., 2023) and are able to employ the acetyl-CoA pathway for both carbon fixation and energy conservation (Schöne et al., 2022). They live off CO_2 and H_2, like LUCA did, and under chemolithoautotrophic theories for the origin of life they represent living fossils, reflecting ancestral metabolic traits.

Geochemical Origins

One can occasionally read that all life depends on energy from the sun, but that is not true because life in serpentinizing hydrothermal vents thrives in total darkness, powered by pure chemical energy, the reaction of H_2 with CO_2. It is true, however, is that all life on Earth depends on organisms that fix CO_2 (also called primary producers). It is therefore likely that life has also started out that way.

CO_2 was made widely available on the early Earth through the Moon-forming impact (Mrnjavac et al., 2023). This planetary collision between the young Earth and a Mars-sized body (Theia) gave origin to the Moon, but also significantly modified the Earth. It melted all of the Earth's mantle, and vaporized ~20% of it, which resulted in a large amount of carbon dioxide degassing into the atmosphere. When the planet cooled down, the water vapor from the atmosphere condensed and rained down to form the oceans. CO_2 from the atmosphere dissolved in the oceans, the same way gasses from the modern atmosphere, such as N_2 and O_2, dissolve in the oceans today. The CO_2 dissolved in the early ocean was made available to react with H_2 on metal catalysts in the Earth's crust. Such reactions have been studied in laboratory experiments, using reactors to simulate temperature and pressure conditions at deep-sea hydrothermal vents. Results show that in the presence of H_2 and metal catalysts (native transition metals Ni^0, Fe^0 or Co^0 or their alloys) that naturally occur in serpentinizing systems, CO_2 is rapidly reduced to formate (an organic acid with one C atom, $HCOO^-$), acetate (an organic acid with 2 C atoms, CH_3COO^-) and pyruvate (an organic acid with 3 C atoms, CH_3COCOO^-) in aqueous solution by hydrogen-driven reactions (Varma et al., 2018; Preiner et al., 2020; Belthle et al., 2022; Beyazay et al., 2023a,b). The reactants (CO_2 and H_2) and organic products derived from CO_2 in the experiments coincide with the substrates, intermediates and products of the acetyl-CoA pathway employed by modern acetogens and methanogens. In addition, the enzymes and cofactors of the acetyl-CoA pathway harbor Fe, Co, and Ni in their active sites. This resemblance implies that the experimentally investigated CO_2 reduction with H_2 might be a true prebiotic analog of the acetyl-CoA pathway. Going one step further, recent work has shown that H_2 in the presence of nickel drives a series of reactions analogous to the microbial reverse Krebs cycle, the metabolic pathway that follows the acetyl-CoA pathway and is fed by its products or their derivatives. The reverse Krebs cycle precursor generates ketoacids, from which several amino acids are synthesized in the presence of ammonia (Kaur et al., 2024). These reactions occur in one pot under the same reaction conditions, which makes them a natural reaction sequence in a prebiotic setting, as they could have occurred in the same chimney pore of a hydrothermal vent.

Evolving Metabolic Complexity

The first step in the origin of life was generating the first organics from the available inorganic material. As the diversity of organic molecules increased, more chemical transformations became possible, and some small organic molecules started catalyzing reactions, in concert with the metal surfaces, or independently from them. The proposal that surfaces and cofactors predate enzymes is not new (Eakin, 1963). In some cases, metal ions and cofactors act in concert to accelerate chemical reactions, as in the case of non-enzymatic transaminations catalyzed by pyridoxal phosphate, a cofactor that catalyzes the transfer of amino groups, $-NH_2$, to generate amino acids (Dherbassy et al., 2023).

In addition to pyridoxal phosphate, several other biological cofactors have been studied in a prebiotic setting. The redox cofactor NAD is a hydride (H^-) carrier in cells and can be reduced to NADH without enzymes, by hydrogen gas on a surface of solid-state iron, nickel or cobalt (Henriques Pereira et al., 2022). Metals in serpentinizing hydrothermal vents could have catalyzed NAD reduction by H_2 before the origin of enzymes that catalyze this reaction in modern cells.

Another ancient redox carrier in cells is a small protein named ferredoxin. It contains one or more iron-sulfur clusters which can be reduced or oxidized, and it acts as a soluble electron donor, transferring electrons to cellular proteins and their substrates. In acetogens and methanogens it is reduced enzymatically with electrons from H_2, but this reaction cannot proceed directly, because under physiological conditions H_2 is not a strong enough reductant. Cells are forced to employ an intricate mechanism that involves separating the electron pair from hydrogen, with one electron going to a better electron acceptor, which allows the other electron to reduce ferredoxin (Herrmann et al., 2008). It was found, however, that at conditions resembling alkaline hydrothermal vents, ferredoxin can be reduced without enzymes by adding metallic iron and H_2 to the reaction mixture (Brabender et al., 2024). Why is H_2 capable of reducing ferredoxin directly under these conditions, but not in cells? The ability of H_2 to donate electrons depends on temperature and pH. In alkaline hydrothermal vents, the ability of H_2 to donate electrons increases (in chemical terms: its midpoint potential becomes more negative), which makes ferredoxin reduction with H_2 possible (Boyd et al., 2020). This reduction of ferredoxin by H_2 is a (so far rare) example of interaction between a protein and a solid-state metal surface, pointing to a transition phase in early evolution when proteins were present, but were still in contact with metal surfaces, before LUCA's metabolism evolved to become fully independent of the solid state.

At the onset of metabolism, prebiotic chemical reactions were catalyzed by environmental metal surfaces. Such reactions likely brought forth LUCA to start, and as LUCA progressed in complexity, the system slowly transitioned to include organic catalysts generated by the reaction network (Sousa et al., 2015; Xavier et al., 2020), although organic cofactors could not always replace metal catalysts. Metal clusters in the active sites of modern enzymes of the acetyl CoA pathway are likely relics of a metal-catalyzed protometabolism. The increase in the number and complexity of reactions and their products was followed by a similar increase in the diversity and complexity of catalysts. Before LUCA had evolved a protoribosome, amino acids could still be polymerized into oligopeptides of reproducible sequence by non-ribosomal protein synthesis (Lipmann, 1973). Genetic information was probably first encoded in RNA. The origin of a replicating genetic code and protein synthesis on the ribosome allowed standard Darwinian evolution and selection to set in.

The core missing elements for cellularity are cellular membranes. There are indications that LUCA was able to harness ion gradients via the universally conserved rotor-stator ATP synthase (Lane et al., 2010), but no indications that LUCA was able to synthesize lipids (Weiss et al., 2016). As outlined in **Fig. 1**, membranes of archaea and bacteria are chemically different, and are synthesized by different, non-related enzymes. This means that modern membranes likely originated after the two domains separated, in other words, after LUCA. However, simpler membranes separating the system from the surrounding environment before domain separation could have been present. These membranes might have been made up of hydrocarbons or simpler lipids such as fatty acids, which have been shown to form abiotically under alkaline hydrothermal conditions (He et al., 2021; Purvis et al., 2024). These ancestral membranes could have served to compartmentalize reactants and products, maintain ion gradients and embed early membrane proteins. LUCA probably did not harbor fully functional membrane electron transfer chains made up of multiple intricate protein complexes, because these are not conserved across that bacterial-archaeal divide, and LUCA likely powered its ATP synthase with environmental ion gradients. The protein that H_2-dependent chemolithoautotrophic archaea (methanogens) use to generate ion gradients (a methyltransferase called MtrA-H) is not related to the protein that H_2-dependent chemolithoautotrophic bacteria—acetogens—use to generate ion gradients (a NADH:ferredoxin oxidoreductase called Rnf) (Martin and Kleinermanns, 2024). This circumstance, in addition to the independent origins of bacterial and archaeal membrane synthesis, indicates that the process of cellularization and escape occurred independently in the ancestors of bacteria and archaea, the free-living descendants of LUCA.

Conclusion

Defining LUCA and the origins of cells remains a complex but actively studied topic. Approaches to studying the nature of LUCA using gene phylogenies alone are impacted by lateral gene transfer and gene loss, which make it difficult to determine which genes really originated in LUCA and which were invented later. Part of these problems are rooted in the circumstance that protein sequences are not well conserved for many gene families. There is hope that the use of protein structural information will provide more insights. Genome-based studies point to LUCA's reliance on the acetyl-CoA pathway, a H_2-dependent, chemolithoautotrophic lifestyle and its use of a genetic code that included modified bases in tRNA. Approaches to LUCA that rely on strict conservation of traits in all prokaryotes indicate that LUCA had ribosomes that used GTP during translation, relied upon GTP for ribosome biogenesis and had a rotor-stator ATP synthase that operated in membranes of hydrophobic compounds provided by the environment. LUCA also had the 20 amino acids, the eight main nucleotides of DNA and RNA and the roughly 18 organic cofactors that are universal to modern metabolism. Approaches to studying the nature of LUCA using chemical reactions that simulate the conditions of H_2-producing hydrothermal vents converge on a H_2-dependent chemolithoautotrophic lifestyle and the acetyl-CoA pathway, as suggested by genomic inferences. These studies suggest that LUCA was not a free-living cell, but was half-alive instead in that it had protein synthesis and genes and hence could evolve, but was confined to inorganic compartments and was dependent upon nutrients and energy provided by a serpentinizing hydrothermal system, the catalysts and chemistry of which supported the origin and early evolution of LUCA's metabolism. Ion and pH gradients provided an energy source that early life learned to harness, while temperature gradients could locally increase the concentrations of reactants via a process called thermophoresis (Matreux et al., 2024). These conditions could make some reactions possible that would otherwise be unlikely to occur and could impose a certain degree of specificity and directionality to protometabolic reactions. Serpentinizing systems provide the core elements present in all life, with CO_2 as the main carbon source, as well as metal catalysts and H_2, a strong electron donor. The physiology of LUCA aligns well with laboratory simulations of prebiotic reactions and with the geochemistry of serpentinizing vents.

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10.5 Publication 5

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Conversion of pyridoxal to pyridoxamine with NH_3 and H_2 on nickel generates a protometabolic nitrogen shuttle under serpentinizing conditions

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Serpentinizing hydrothermal vents are likely sites for the origin of metabolism because they produce H_2 as a source of electrons for CO_2 reduction while depositing zero-valent iron, cobalt, and nickel as catalysts for organic reactions. Recent work has shown that solid-state nickel can catalyze the H_2-dependent reduction of CO_2 to various organic acids and their reductive amination with H_2 and NH_3 to biological amino acids under the conditions of H_2-producing hydrothermal vents and that amino acid synthesis from NH_3, H_2, and 2-oxoacids is facile in the presence of Ni^0. Such reactions suggest a metallic origin of metabolism during early biochemical evolution because single metals replace the function of over 130 enzymatic reactions at the core of metabolism in microbes that use the acetyl-CoA pathway of CO_2 fixation. Yet solid-state catalysts tether primordial amino synthesis to a mineral surface. Many studies have shown that pyridoxal catalyzes transamination reactions without enzymes. Here we show that pyridoxamine, the NH_2-transferring intermediate in pyridoxal-dependent transamination reactions, is generated from pyridoxal by reaction with NH_3 (as little as 5 mM) and H_2 (5 bar) on Ni^0 as catalyst at pH 11 and 80 °C within hours. These conditions correspond to those in hydrothermal vents undergoing active serpentinization. The results indicate that at the origin of metabolism, pyridoxamine provided a soluble, organic amino donor for aqueous amino acid synthesis, mediating an evolutionary transition from NH_3-dependent amino acid synthesis on inorganic surfaces to pyridoxamine-dependent organic reactions in the aqueous phase.

Introduction

Nitrogen is essential to life. By dry weight, modern cells consist of ~ 55% protein and ~ 20% nucleic acids at the level of polymers [1], with ~ 50% carbon and ~ 10% nitrogen by elements [2]. At life's origin, amino acids and bases had to be supplied by prebiotic reactions without the help of enzymes, but how? The two main competing theories for how C and N entered prebiotic chemistry are genetics first and metabolism first. Genetics-first theories posit that highly reactive cyanide or alkyl cyanides (nitriles) generated from meteorite impacts [3] were the source of nucleic acid bases [4,5], amino acids [6,7], and more recently some cofactors [8], with both C and N entering prebiotic chemistry via nitrile bonds. Nitrile moieties do not,

Abbreviations
PL, pyridoxal; PLP, pyridoxal 5′-phosphate; PM, pyridoxamine; PMP, pyridoxamine 5′-phosphate.

however, occur in any substrate or product among the 400 reactions that generate amino acids, bases, and cofactors in the biochemistry of cells [9], such that genetics-first theories do not speak to the origin of reactants, catalysts, or compounds in metabolism. Metabolism first theories hold that C and N entered prebiotic chemistry along reaction routes that strongly resemble some, but not all, modern enzymatic pathways, that inorganic surfaces and cofactors served as catalysts at origins [10], and that the first organisms were autotrophs that obtained their carbon from CO_2 [11–14].

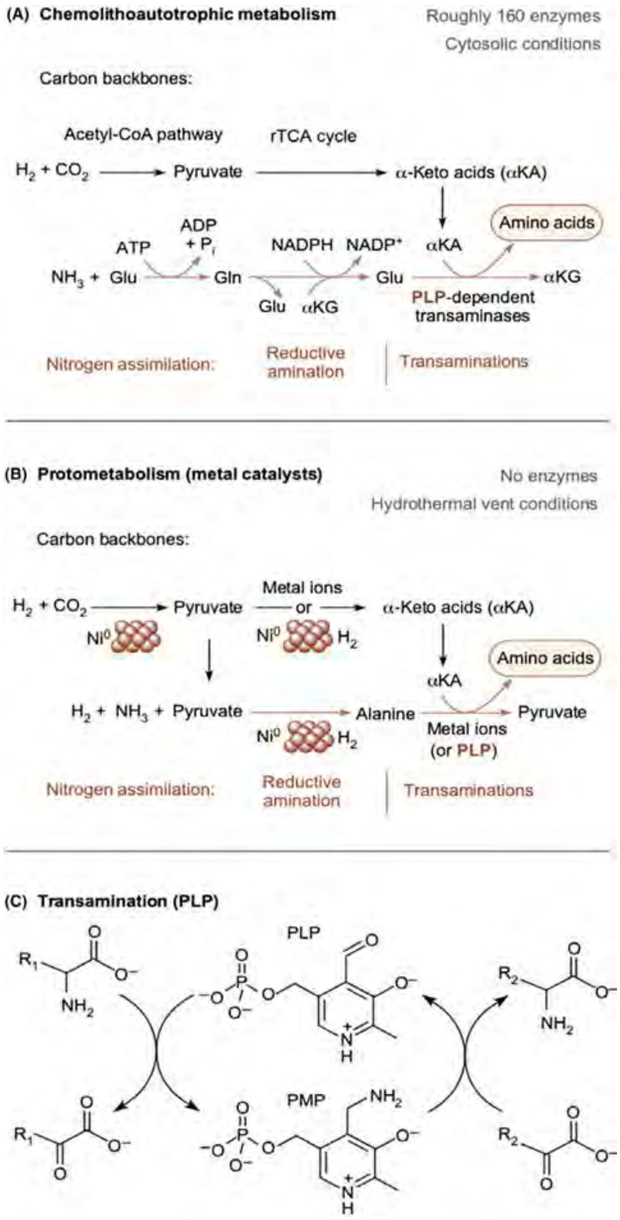
For carbon, a coherent picture of primordial CO_2 assimilation in hydrothermal vents is emerging in metabolism first theories. Among the known pathways of CO_2 fixation [15,16], the acetyl-CoA pathway is the oldest [12,15]. It is the only pathway of CO_2 fixation that occurs in bacteria and archaea [17], and the only route that simultaneously supports CO_2 fixation and ATP synthesis [18,19]. Its reactions from H_2 and CO_2 to pyruvate proceed without enzymes [20–24] under the conditions of serpentinizing hydrothermal vents [25] using only solid-state transition metals Fe^0, Co^0, Ni^0 [20], and their alloys as catalysts. These native metals are naturally deposited [26] by the highly reducing (H_2-rich) conditions of serpentinizing hydrothermal systems [27,28]. The same transition metals are coordinated in the active sites of the enzymes [29,30] and cofactors [31–33] of the acetyl-CoA pathway, providing continuity of substrates, catalysts, and products between its metal-catalyzed and enzymatic versions [34].

For nitrogen, a coherent picture of primordial N incorporation into protometabolism under hydrothermal conditions is also coming into focus. In laboratory serpentinization reactions, NH_4^+ is readily generated from N_2 and H_2 in water reactions with peridotite, the host rock of serpentinizing systems [35]. As a hydrothermal route of NH_3 incorporation into metabolism, Kaur *et al.* [36] recently showed that in the presence of H_2 and as little as 6 mM NH_4^+, Ni^0 will catalyze reductive aminations of 2-oxoacids to form amino acids. These reductive aminations produced glycine, alanine, aspartate, glutamate, valine, leucine, and isoleucine with yields of up to approximately 50% over 72 h at room temperature and a pH of 7–8. Both native Ni and the amount of H_2 that Kaur *et al.* [36] employed (5 bar) are observed in actively serpentinizing hydrothermal vents [25,27,28]. Abiotic glycine synthesis in a serpentinizing system has also been reported [37] (Fig. 1B). These reactions generate an ample supply of amino acids under primordial hydrothermal vent conditions. Yet they tether amino acid synthesis to solid-state metal (mineral) surfaces before the origin of enzymes.

In metabolism, nitrogen incorporated as amino acids is distributed into other amino acid biosynthesis via transaminases, enzymes that require the cofactor pyridoxal phosphate (PLP). PLP interconverts 2-oxoacids and amino acids (Fig. 1A). In a primordial protometabolism, transaminations can be catalyzed without enzymes by PLP alone [38] or even by metal ions in solution alone, without PLP, albeit at reduced rates [39,40]. Recent work on PLP-dependent transaminations in the context of early evolution has uncovered specific reaction mechanisms [39–42] and shown that soluble metal ions can significantly impact the PLP-dependent nonenzymatic reactions [39,40,43,44], as can the inclusion of small peptides instead of metal ions [45]. While pyridoxal-dependent transaminations efficiently interconvert 2-oxoacids and amino acids [39,40,43,44], they are dependent upon reductive aminations for net N incorporation. The mechanisms of PLP-dependent transaminations always generate pyridoxamine phosphate (PMP) as the intermediate N-donating species [41] (Fig. 1C).

Though Ni^0 and H_2 can incorporate NH_4^+ into protometabolism as a primordial source of organic nitrogen

Fig. 1. Biological and prebiotic N incorporation. (A) In organisms that live from the reduction of CO_2 with H_2, carbon backbones for amino acid synthesis are provided by the acetyl-CoA pathway and the incomplete reverse citric acid cycle [12,15,34]. Nitrogen is assimilated as ammonium via N activation by glutamine synthase (GS), and reductive amination by glutamate synthase (GluS). Glutamate donates the amino group to various a-keto acids via PLP-dependent transaminases. In some organisms, the aminotransferase activity of GS is circumvented by glutamate dehydrogenase (GDH), which generates Glu from α-ketoglutarate, ammonium, NAD^+, and NAD(P)H. Another route of N incorporation entails the synthesis of carbamoyl phosphate (required in *de novo* pyrimidine synthesis) from NH_3 and CO_2 [46]. (B) Pyruvate is synthesized from H_2 and CO_2 under hydrothermal vent conditions using various native metals including Ni (shown), Fe, Co, and their alloys as catalysts [20–24]. Native Ni catalyzes reductive amination of various a-keto acids with H_2 including pyruvate [36]. Metal ions (Fe^{2+}) will convert pyruvate and glyoxylate to citric cycle intermediates including α-ketoglutarate [47], but Ni–H_2 will catalyze the same sets of TCA cycle reactions [36]. Transamination reactions can be catalyzed by PLP or PL alone or by metal ions alone, (including Ni^{2+}), or by combining PL with metal ions [40]. PL, pyridoxal; PLP, pyridoxal phosphate. (C) General mechanism of a PLP-dependent transamination. In transaminases, PLP is enzyme-bound as an aldimine to a lysyl side chain amino group [39] but is shown here as the free aldehyde for simplicity.


(A) Chemolithoautotrophic metabolism
Roughly 160 enzymes
Cytosolic conditions
Carbon backbones:
Acetyl-CoA pathway
rTCA cycle
H2 + CO2
Pyruvate
α-Keto acids (αKA)
ATP
ADP + Pi
NH3 + Glu
Gln
NADPH
NADP+
Glu
αKG
Glu
αKA
Amino acids
αKG
PLP-dependent transaminases
Nitrogen assimilation:
Reductive amination
Transaminations
(B) Protometabolism (metal catalysts)
No enzymes
Hydrothermal vent conditions
Carbon backbones:
H2 + CO2
Ni0
Pyruvate
Metal ions or
Ni0
H2
α-Keto acids (αKA)
αKA
Amino acids
H2 + NH3 + Pyruvate
Alanine
Ni0
H2
Metal ions (or PLP)
Pyruvate
Nitrogen assimilation:
Reductive amination
Transaminations
(C) Transamination (PLP)
PLP
PMP
R1
R2
NH2
O
O−
P
N+
H

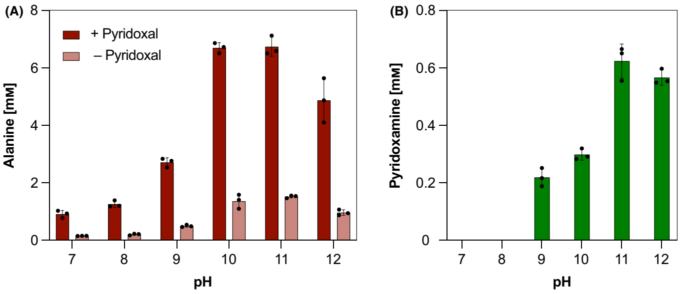


Fig. 2. Effect of pH and addition of pyridoxal on the nickel-catalyzed reductive amination of pyruvate at 5 bar H_2 and 80 °C over 18 h (Table S1). Pyruvate concentration was set to 20 mM and the ratio between pyruvate, ammonium chloride, and pyridoxal was 1 : 1 : 1. The amount of nickel nanopowder was 1.5 mmol in a total volume of 1.5 mL. The reaction was performed at 80 °C for 18 h under a 5 bar hydrogen atmosphere. Error bars in the figure represent the standard deviation (SD). Each reaction was performed in triplicate. (A) Concentration of alanine in presence and absence of pyridoxal. (B) Concentration of pyridoxamine correlating with the concentration of alanine (A) in presence of pyridoxal.

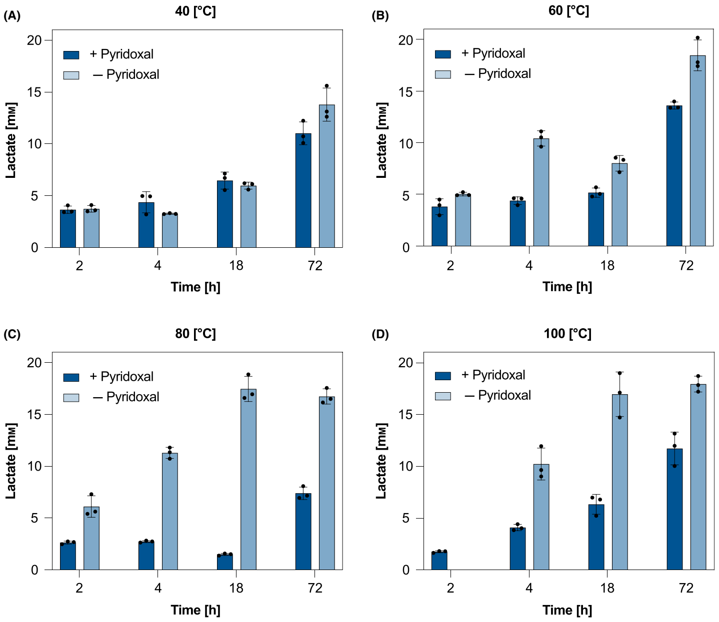


Fig. 3. Effect of temperature, time, and addition of pyridoxal on the nickel-catalyzed accumulation of lactate (Table S2). Pyruvate, ammonium, and pyridoxal concentrations were set to 20 mM. The catalyst (nickel nanopowder) was added as 1.5 mmol of undissolved solid phase powder in a total reaction volume of 1.5 mL. The reaction was performed under a 5 bar hydrogen atmosphere at (A) 40 °C, (B) 60 °C, (C) 80 °C, and (D) 100 °C. The pH was set to 11 with KOH. Error bars in the figure represent the standard deviation (SD). Each reaction was performed in triplicate. The corresponding alanine concentrations are given in Fig. 5.

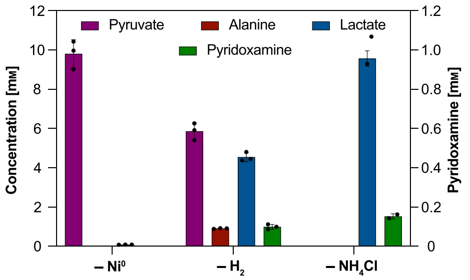


Fig. 4. Alanine synthesis requires the presence of nickel catalyst. Pyruvate, pyridoxal, and ammonium were reacted in the presence of nickel catalyst (Table S3). Product accumulation after the removal of nickel nanopowder, hydrogen, and ammonium chloride is shown. Pyruvate, ammonium, and pyridoxal concentrations were set to 20 mM (or 0 in the corresponding control). The catalyst (nickel nanopowder) was added as 1.5 mmol of undissolved solid phase powder in a total reaction volume of 1.5 mL. The reaction was performed under a 5 bar hydrogen atmosphere or 5 bar argon in the control, pH was set to 11 with KOH, the temperature was set to 100 °C, and the reaction time was 18 h. Error bars in the figure represent the standard deviation (SD). Each reaction was performed in triplicate. Accumulation of alanine and lactate in the absence of hydrogen stems from nickel-dependent reduction, the midpoint potential of the pyruvate to lactate reduction (E_0') is −190 mV, the midpoint potential (E_0') of Ni^0 to Ni^{2+} oxidation is −260 mV [67]. Pyridoxamine concentrations are shown on the right-hand *y*-axis.

[36], the catalyst physically ties both NH_4^+ incorporation and amino acid synthesis to solid-state metal surfaces in or on the Earth's crust. For microbial cells to emerge, the reactions of metabolism had to become soluble, and the catalysts (enzymes) had to become independent of solid-state catalysts. To explore possible intermediate steps in the transition from metal-catalyzed reductive amination to pyridoxal-catalyzed transamination, we investigated the ability of Ni^0 to catalyze H_2-dependent reductive amination of pyruvate to alanine in the presence or absence of pyridoxal to determine if pyridoxal impacts reductive amination under conditions of active serpentinization. We also tested whether H_2 and NH_4^+ over Ni^0 will reductively aminate pyridoxal to pyridoxamine as a freely diffusible, active amino donor for soluble transamination reactions.

Results and Discussion

Pyridoxal promotes reductive amination over nickel

Actively serpentinizing hydrothermal systems can harbor alkaline effluent with a pH of 8–10 or, in hyperalkaline systems, up to pH 12 or greater [25]. We first examined the effect of pH on reductive amination of pyruvate to alanine in the presence or absence of pyridoxal with all reactants at 20 mM and H_2 at 5 bar (or ~ 3.9 mM using Henry's law) in the presence of commercial nickel powder (Ni^0) as the catalyst. After 18 h at 80 °C, we observed a 7.6% conversion of pyruvate to alanine at pH 11 and a 34% conversion in the presence of pyridoxal (Fig. 2A) using ^1H NMR to assay products (see Materials and methods). This indicates that at pH 11, alkalinity observed in actively serpentinizing hydrothermal vents [25], pyridoxal is compatible with Ni^0, that is, it is not sequestered by the metal, it is stable during the reaction, and it does not inactivate the catalyst. Under these conditions, pyridoxal (PL) increases the alanine yield by a factor of 4.4 (Fig. 2A). This increase is due in part to the effect of pyridoxal on the competing lactate synthesis reaction. Without PL, 87% of pyruvate is converted to lactate, but only 7.5% conversion to lactate is observed in the presence of PL at 80 °C (Fig. 3). We also monitored pyridoxamine (PM) accumulation (Fig. 2B) via ^1H NMR. At pH 11, under the addition of PL, only 3.1% of PL accumulates as PM while 34% of pyruvate is converted to alanine. An increase in PL-dependent alanine accumulation at a low steady-state PM concentration suggests that PM is serving as a shuttle, undergoing cycles of reductive amination via the nickel catalyst, converting pyruvate to alanine, and regenerating PL in the process. In the absence of nickel catalyst, no alanine accumulates (Fig. 4).

Pyridoxal promotes amination at higher temperature

Serpentinizing hydrothermal vents exhibit temperature gradients as effluent interfaces with seawater [48–50]. In the range of 0–100 °C, most uncatalyzed biological reactions take place more rapidly with increasing temperature, such that catalysts can increase rate more effectively at lower temperatures than at higher temperatures, itself an argument in favor of an origin of metabolism at high temperature [51]. At 40 °C and pH 11, the addition of PL apparently inhibited alanine accumulation as we could only detect alanine accumulation in the absence of PL (Fig. 5). The enhancing effect of PL on alanine accumulation increased with temperature, at 80 °C and 100 °C up to 48% of pyruvate was converted to alanine, while temperature had little effect on alanine accumulation in the absence of PL at pH 11.

Pyridoxamine accumulation was also temperature dependent (Fig. 5), with alanine accumulating after

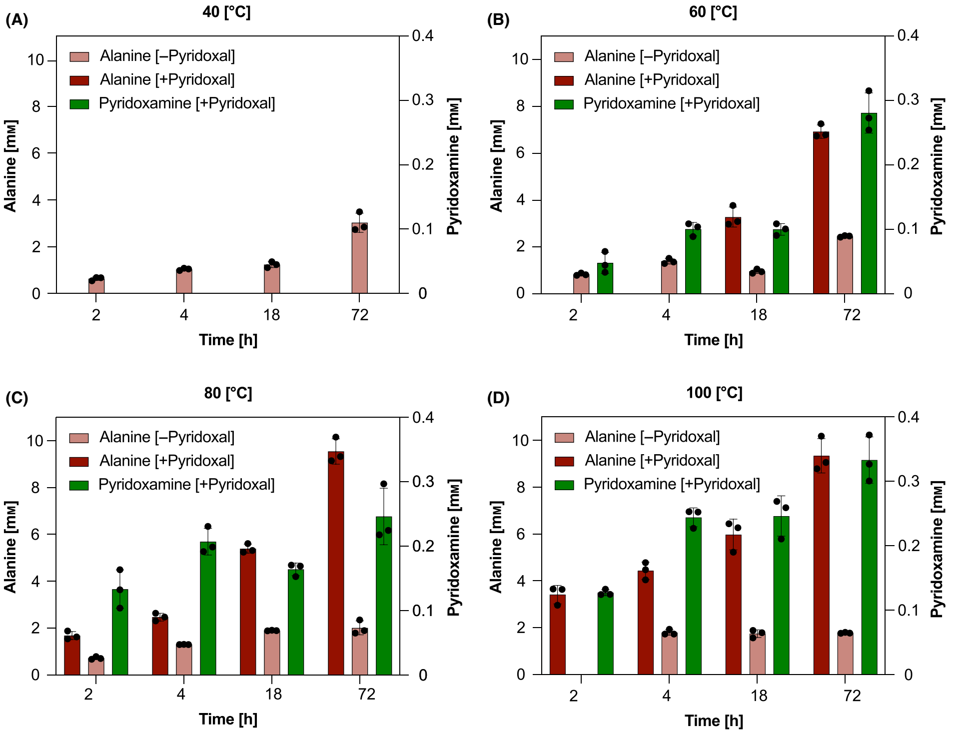


Fig. 5. Effect of temperature, time, and addition on the formation of pyridoxamine in nickel-catalyzed reductive amination of pyruvate (Table S4). Pyruvate concentration was set to 20 mM and the ratio between pyruvate, ammonium chloride, and pyridoxal was 1 : 1 : 1. Pyridoxamine concentrations are given on the right axis. The amount of nickel nanopowder was 1.5 mmol in a total volume of 1.5 mL. The reaction was performed under a 5 bar hydrogen atmosphere at (A) 40 °C, (B) 60 °C, (C) 80 °C and (D) 100 °C. The pH was set to 11 with KOH. Error bars in the figure represent the standard deviation (SD). Each reaction was performed in triplicate. We noticed that after 18 h at 80 °C, the PM concentration is three times lower than in Fig. 2, likely due to the use of different batches of nickel. No PM was detected in reactions performed at 40 °C.

18 h only in reactions that led to PM accumulation of 1.2%. This is consistent with the role of PM as the aminating agent in PL-dependent pyruvate conversion to alanine in the present samples. A main effect of PL was to favor reductive amination of pyruvate over pyruvate reduction to lactate. After 72 h, 9.4% pyruvate conversion to alanine by H_2, NH_3, and Ni^0 alone was observed without PL, while under the addition of PL alanine and lactate accumulated in equal proportions (Fig. 6).

The addition of PL had a pronounced effect at low ammonium concentrations. At 5 mM ammonium, we detected 11% pyruvate conversion after 18 h at 100 °C but only 1.9% of pyruvate converted to alanine without PL using commercial nano-nickel powder (Fig. 7A). For comparison, Kaur *et al.* [36] detected 16.7% pyruvate conversion to alanine at 6 mM NH_4^+ and 25 °C after 72 h using silicate-supported nano-nickel catalysts. Above 100 mM NH_4^+, alanine accumulation showed no significant difference with or without the addition of PL. However, below 100 mM NH_4^+, the addition of PL significantly enhanced alanine formation compared to the H_2 and NH_3-driven reaction, with the effect becoming more pronounced as NH_4^+ concentration decreased (Fig. 7A). This is also consistent with a role for PM in PL-enhanced reductive amination over nickel.

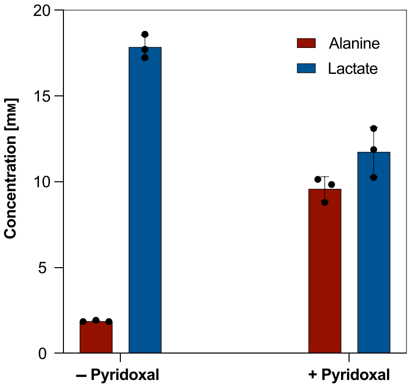


Fig. 6. Effect of addition of pyridoxal on the product ratio between alanine and lactate in nickel-catalyzed reductive amination of pyruvate (Table S5). Pyruvate concentration was set to 20 mM and the ratio between pyruvate, ammonium chloride, and pyridoxal was 1 : 1 : 1. The amount of nickel nanopowder was 1.5 mmol in a total volume of 1.5 mL. The reaction was performed at 100 °C for 72 h under a 5 bar hydrogen atmosphere, and pH was set to 11. Error bars in the figure represent the standard deviation (SD). Each reaction was performed in triplicate.

In cells, PL is highly reactive and typically bound to the ε-amino group of lysine in enzymes through a Schiff base linkage [52], such that the content of the free cofactor is very low. We tested pyridoxal concentrations ranging from 1 to 20 mM (Fig. 8). The yield of alanine generally decreased with decreasing PL concentrations. Up to 5 mM PL, there was an increased alanine yield compared to the control without PL. However, below 5 mM, no significant effect from PL addition could be detected.

Conclusion

Biochemical views on the origin of metabolism posit that inorganic surfaces and cofactors served as catalysts before the advent of enzymes [10]. Pyridoxal is a textbook case of cofactor-only catalysis in that a pyridoxal-containing transaminase will accelerate the rate of the uncatalyzed reaction by a factor of 10^{18}, with PLP alone contributing a factor of 10^{10} to rate enhancement and the enzyme contributing the remaining 10^{8}-fold increase [53]. Iron sulfides were long thought to be the inorganic forerunners of enzymes [13,54]. FeS minerals will catalyze some CO-dependent reactions [55] as well as some reductive aminations in the laboratory, but the latter only at NH_4^+ concentrations above 1 M [56]. Recent work indicates that for prebiotic CO_2 reduction and reductive amination, native Fe, Co, and Ni and their alloys are more effective and more versatile catalysts than FeS minerals and in many cases generate products that are identical to compounds of microbial metabolism [20–24].

Like Kaur *et al.* [36], we found that inorganic surfaces of Ni^0 can catalyze the reductive amination of pyruvate with H_2. The alanine yield increased under conditions that correspond closely to those of actively serpentinizing hydrothermal vents (pH 11, 80 °C) [28,57,58] (Fig. 2). Reductive amination of pyruvate with H_2 over Ni resulted in a 4.4-fold increase in alanine yield in the presence of PL (Fig. 5). We also found that in the presence of H_2 and 20 mM NH_4^+, Ni^0 will reductively aminate PL to PM (Fig. 5), the amino-donating intermediate of transamination reactions. The yield of the reductive amination of pyridoxal depends on the pH (Fig. 2), time, and temperature (Fig. 5). The variation in PM concentrations in both figures can be attributed to the impact of these factors on the rate of reductive amination [41]. The PM amounts at pH 11, 18 h, and 80 °C differ across different experiments (Figs 2 and 5), which are attributable to different nickel catalyst batches from the supplier (Sigma-Aldrich, St. Louis, Missouri, USA). Pyruvate, the substrate converted to alanine in our experiments, is readily produced from H_2 and CO_2 over nickel and nickel-iron alloys at 20–100 °C and pH 8–10 in laboratory simulations of serpentinizing conditions [20–24].

The addition of PL increased pyruvate conversion to alanine relative to the competing reaction to lactate at lower NH_4^+ concentrations down to 5 mM NH_4^+ over 18 h (Fig. 7A,B). Although abiogenic NH_4^+ synthesis has not been reported in modern serpentinizing systems, laboratory simulations of rock-water interactions during serpentinization show that peridotite (a rock substrate for serpentinization) will react with water, H_2, and N_2 (300 °C, 50 bar N_2) to generate NH_3 at amounts corresponding to 350 μM over 29 days [35]. An abiotic source of PL has not been reported, but compounds similar to PL are readily obtained from heating glycolaldehyde with ammonia, though other routes from sugars and ammonia are also possible [59]. The present results show that PL and PM are compatible with native Ni, as PL enhances the Ni-catalyzed reaction (Fig. 1), and PM-dependent pyruvate amination is not inhibited by nickel (Fig. 9). Cofactor compatibility with native Ni, Co, and Fe was also observed for NADH [60,61], and compatibility with Fe^0 was also observed for ferredoxin, an electron carrier protein with two redox-active 4Fe4S clusters [62].

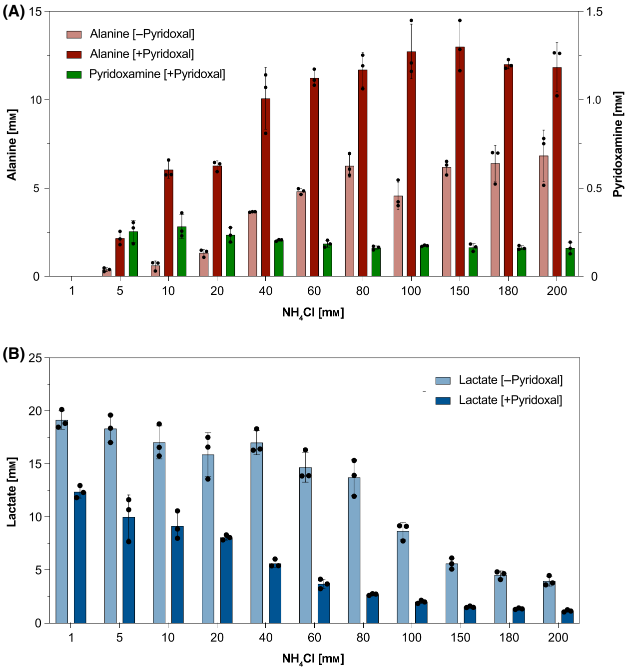


Fig. 7. Effect of ammonium chloride concentration and addition of pyridoxal on the nickel-catalyzed reductive amination of pyruvate (Tables S6 and S7). Pyruvate and pyridoxal concentrations were set to 20 mM. Pyridoxamine concentrations are given on the right axis. The amount of nickel nanopowder was 1.5 mmol in a total volume of 1.5 mL. The reaction was performed at 100 °C for 18 h under a 5 bar H_2 atmosphere, and pH was set to 11. Error bars in the figure represent the standard deviation (SD). Each reaction was performed in triplicate. (A) Concentration of alanine in presence and absence of pyridoxal; and concentration of pyridoxamine in presence of pyridoxal. (B) Concentration of lactate in presence and absence of pyridoxal.

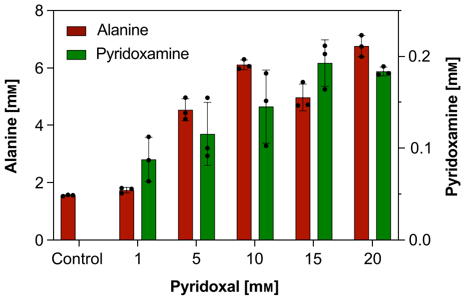


Fig. 8. Effect of the pyridoxal concentration on the Ni-catalyzed reductive amination of pyruvate (Table S8). Pyruvate and ammonium concentrations were set to 20 mM. Pyridoxamine concentrations are given on the right axis. The amount of nickel nanopowder was 1.5 mmol in a total volume of 1.5 mL. Error bars in the figure represent the standard deviation (SD). Reactions were performed in triplicate at 100 °C for 18 h under a 5 bar H_2 atmosphere, and pH was set to 11.

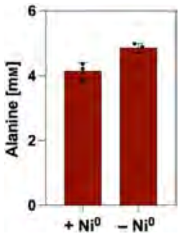


Fig. 9. PM-dependent pyruvate amination is not inhibited by nickel (Table S9). Pyruvate and PM concentrations were set to 20 mM. The reaction was performed under 5 bar argon atmosphere, pH was set to 11 with KOH, the temperature was set to 100 °C, and the reaction time was 18 h in the presence or absence of nickel catalyst (1.5 mmol of undissolved solid phase powder in a total reaction volume of 1.5 mL). Error bars in the figure represent the standard deviation (SD). Each reaction was performed in triplicate.

Pyridoxal is a widespread cofactor in metabolism. It is estimated that PLP is used by roughly 1% of all protein-coding genes in prokaryotes [63]. Pyridoxal is clearly the most versatile of all cofactors in terms of reaction types. In enzymatic reactions involving amino acid, oxoacid, and amine substrates, PLP catalyzes transaminations, Claisen condensations, β- and γ-eliminations, β- and γ-substitutions, epimerizations,

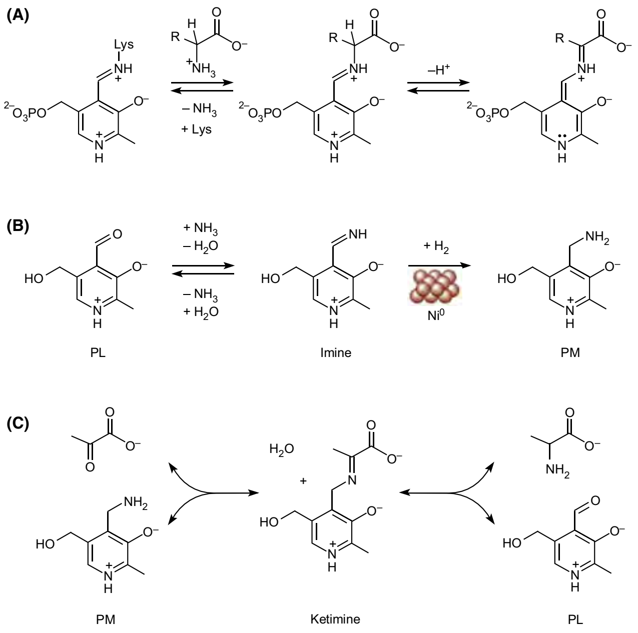


Fig. 10. Reactions of PL. (A) The intermediates in PL catalyzed enzymatic reactions with amino acids, redrawn from Ref. [52]. (B) Proposal for reductive amination of PL to PM with ammonium and H_2 over Ni^0 (see text). (C) Proposal for reversible PM-mediated transamination half reaction to alanine, with ketimine hydrolysis instead of reaction with a second 2-oxoacid regenerating PL, analogous to the mechanism of PL-premediated transaminations [41].

racemizations, decarboxylations, transaldolations, and *S*-adenosyl methionine-dependent radical reactions [52,64]. Underlying its catalytic proficiency, many pyridoxal-dependent enzymes sequester the cofactor via aldimine formation with the side chain amino group of lysine, which is then displaced by the substrate amino moiety via transamination [52,65] (Fig. 10). It is possible that PL can catalyze a broader spectrum of reactions under protometabolic conditions than just transaminations.

Though the reaction mechanism of PL-promoted reductive amination is unknown, the reactions of carbonyls with ammonia and reactions of PL (Fig. 10A) are well-studied and can provide a guide. Imine formation from aldehydes or ketones and ammonia is fast and freely reversible in water. Under alkaline conditions, the equilibrium in the reaction of NH_4^+ and pyruvate lies far on the side of the oxoacid, with imine reduction being irreversible even with the mild reductant $NaBH_3CN$ [41]. In the present study, at 100 °C pH 11 and 5 bar H_2, the midpoint potential of the H_2 oxidation reaction $H_2 \rightarrow 2e^- + 2H^+$ is ca. −820 mV, strongly reducing conditions, such that the reductive amination of pyridoxal over Ni (Fig. 5) might proceed as sketched in Fig. 10B, with PM condensing with pyruvate to form the aldimine [41], followed by hydrolysis releasing alanine and regenerating PL (Fig. 10C). This reaction sequence could account for the low PM concentration relative to alanine (Fig. 7A). Imine reduction (Fig. 10B) also takes place during the enzymatic reductive amination reaction of α-ketoglutarate with NH_4^+ and NADH in the glutamate dehydrogenase reaction mechanism [66].

Our findings are consistent with the proposal that core biochemistry arose from reactions of H_2, CO_2, and NH_3 with the help of solid-state catalysts in serpentinizing hydrothermal systems [21,46]. The path from protometabolic reactions to cells entails a transition from solid state to soluble catalysts, whereby the latter could be metal ions or cofactors at first, followed by enzymes [10]. Reductive amination of PL to PM represents such an intermediate state in the development of prebiotic N incorporation before the origins of enzymes (Fig. 11). While soluble metal ions alone can catalyze transamination reactions, as can PL alone [40], net N incorporation is required for the accumulation of nitrogenous compounds prior to the origin of enzymes. Pyridoxamine synthesized on solid-state

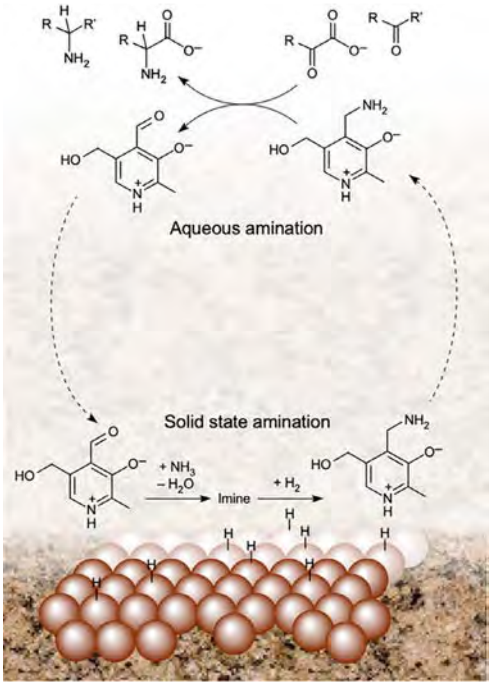


Fig. 11. An evolutionary intermediate. Reductive amination of PL on solid-state catalyst surfaces generates a soluble aminating reagent that could participate in amino acid synthesis from α-ketoacid or any number of PM-catalyzed reactions [52], marking a transition from solid state [36] to soluble catalysis in early biochemical evolution.

catalysts is a diffusible aminating agent. In primordial metabolic evolution, PM could have freed N-incorporating reactions from physical contact with solid-state catalysts, acting as a shuttle that allowed surface metabolism (reductive amination) to become soluble metabolism (transamination) using organic cofactors as catalysts. While amino acids synthesized by reductive amination on Ni^0 [36] could serve a similar function in transferring syntheses to the aqueous phase, PM can participate in a broad spectrum of reactions [52], which could have accelerated early biochemical evolution.

Materials and methods

Reaction

Reactions contained 20 mM each of pyruvate, pyridoxal hydrochloride (Merck, Sigma-Aldrich, Darmstadt, Germany) or pyridoxamine (Merck Millipore, Billerica, Massachusetts, USA), and ammonium chloride, dissolved in distilled water in Falcon tubes. We used the nonphosphorylated forms of the cofactors (PL and PM, respectively) because of their better solubility in water relative to PLP and PMP. The pH was adjusted to 11 through the addition of 1 M KOH. Nickel nanopowder (Sigma-Aldrich, St. Louis, Missouri, USA) was weighed out in an anaerobic glovebox (GS 79821; GS Glovebox System, Malsch, Germany). Samples contained 1 mmol of nano nickel per mL reaction volume. Samples (3 mL glass vials) were prepared in the glove box, placed in glass sample holders, and closed with corresponding lids (VWR International, Darmstadt, Germany), which were punctured to permit gas exchange before placing in the reactor (Berghof BR-300 with BTC-3000 temperature controller). The reactor was filled with 5-bar hydrogen (99.999%; Air Liquide, Paris, France). After completion, reactors were depressurized and glass vial contents (metal powder and supernatant), were transferred to 2 mL Eppendorf tubes and centrifuged for 20 min at 16 060 g (Biofuge Fresco, Heraeus, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Supernatants were analyzed by NMR.

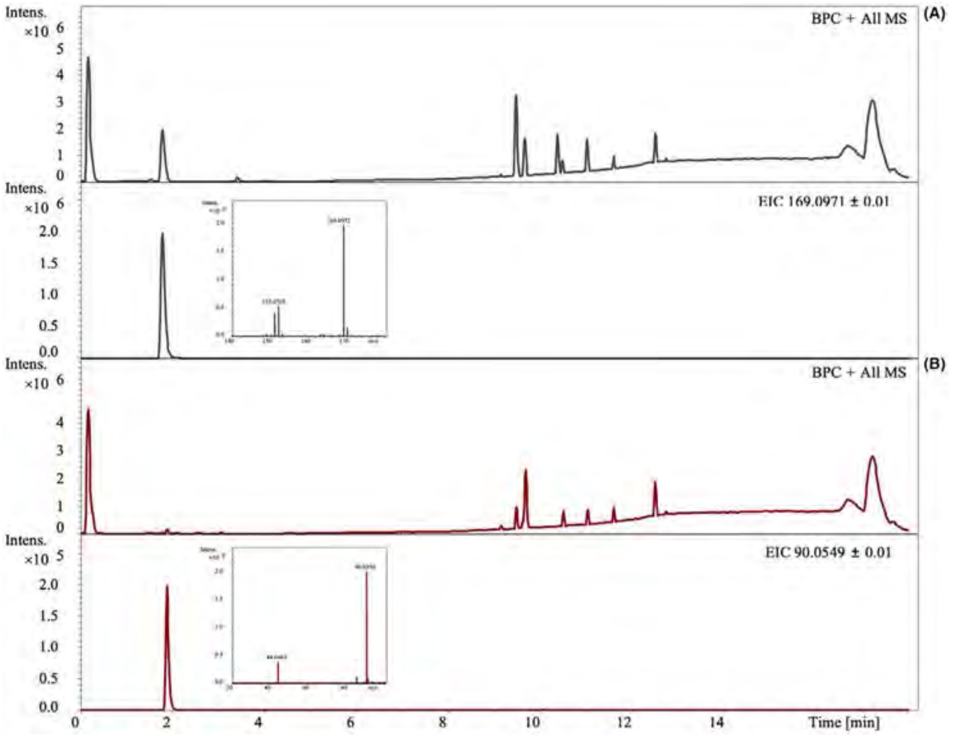


Fig. 12. HPLC-MS analysis of standard solutions of pyridoxamine and alanine. Base peak chromatogram (BPC) and extracted ion chromatogram (EIC) of 50 mmol standard solutions of pyridoxamine (A) and alanine (B). The insets show the mass spectra for pyridoxamine *m/z* = 169.0971 and alanine *m/z* = 90.0549, respectively.

Product identification

After centrifugation, 600 μL of each sample was transferred to an NMR tube (VWR International). DSS (2,2-dimethyl-2-silapentane-5-sulfonate) was added to a final concentration of 1 mM as a reference for calibration. NMR spectra were measured on a Bruker Avance III – 600 MHz spectrometer (Bruker Corporation, Billerica, Massachusetts, USA) by the Center for Molecular and Structural Analytics at Heinrich Heine University Düsseldorf. Spectra were analyzed using CHENOMX NMR SUITE version 9.02 software (Chenomx Inc., Edmonton, Alberta, Canada). Accumulation of PM and alanine identified by NMR was confirmed in selected probes by mass spectrometry (Figs 12 and 13).

LC–MS analysis of pyridoxamine

Pyridoxamine was identified using the Dionex UltiMate 3000 UPLC system (Thermo Scientific, Germering, Germany) coupled to a maXis 4G (Bruker Daltonics, Bremen, Germany) quadrupole-time-of-flight (Q-TOF) mass spectrometer connected to an electrospray (ESI) ion source. Sample volumes of 10 μL were applied to a 3 mm by 150 mm C18 XSelect® HSS T3 column (2.5 μm particle size, 100 Å pore diameter; Waters, Drinagh, Wexford, Ireland) and separated using a binary gradient with a flow rate of 0.4 mL·min^{-1}. Mobile phase A was water + 0.1% formic acid, and mobile phase B was methanol + 0.1% formic acid. Starting with 8% B, a linear gradient to 95% B was applied from 2.5 to 10 min, followed by 95% B for additional 5 min and return to 8% B within 1 min. The system was equilibrated with 8% B for another 2 min prior to the next injection. The MS (positive-ion mode) was run at 3.5 kV capillary voltage, 1 bar nebulizer pressure, 8 L·min^{-1} dry gas flow, and dry temperature of 200 °C. Data acquisition was performed with COMPASS HYSTAR software (version 5.5) (Bruker). Pyridoxamine was quantified from full-scan MS data (mass range

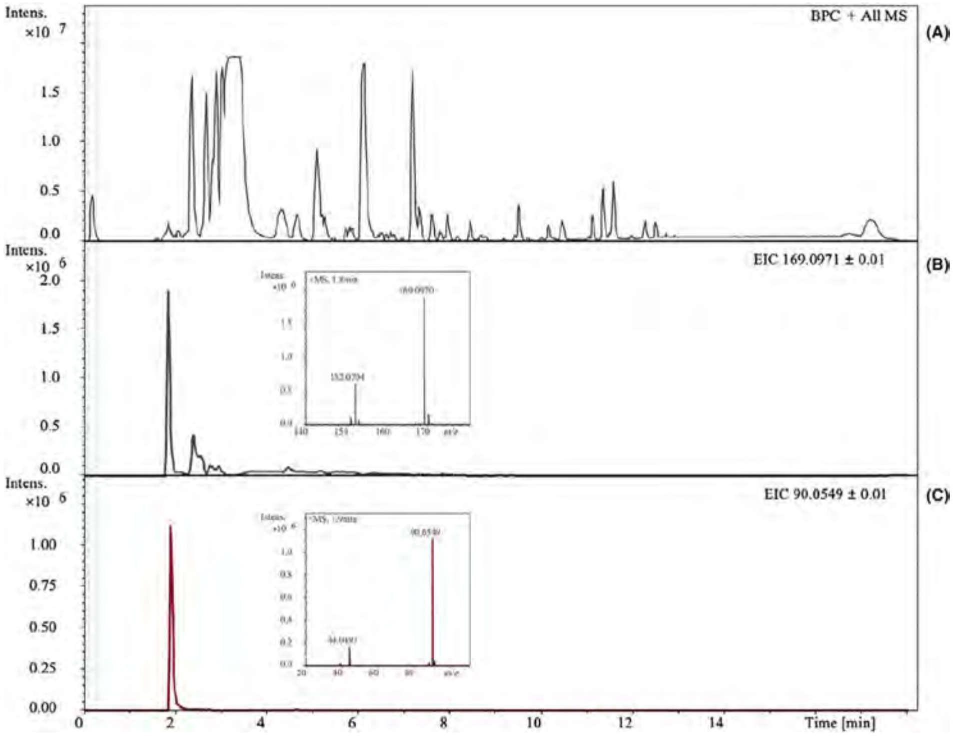


Fig. 13. HPLC-MS analysis of the effect of pH and addition of pyridoxal on the nickel-catalyzed reductive amination of pyruvate. Reaction was performed at 5 bar H_2 and 80 °C over 18 h. Searching for the EICs of 169.0971 *m/z* of pyridoxamine and 90.0549 of alanine in the base peak chromatogram (BPC) (A) revealed clear signals for both educts in the reaction solution. The insets show the mass spectra for pyridoxamine *m/z* = 169.0971 (B) and alanine *m/z* = 90.0549 (C), respectively.

50–1000 *m*/*z*) using the DATAANALYSIS (version 4.2) software (Bruker).

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

MB, MLS, and WFM designed the research. MLS, MBu, CGG, and SM performed experiments. MB, MLS, LS, SM, JM, and WFM interpreted and analyzed the data. MB, MLS, and WFM visualized the data. MB, MLS, MBu, SM, LS, and WFM wrote the paper. MB, MLS, LS, CGG, JM, and WFM edited the paper.

Peer review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/febs.17357.

Data availability statement

The data that support the findings of this study are available in Figs 2–6 and the Supporting Information of this article.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Raw data Fig. 2.
Table S2. Raw data Fig. 3.
Table S3. Raw data Fig. 4.
Table S4. Raw data Fig. 5.
Table S5. Raw data Fig. 6.
Table S6. Raw data Fig. 7A.
Table S7. Raw data Fig. 7B.
Table S8. Raw data Fig. 8.
Table S9. Raw data Fig. 9.

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