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Life as a Guide to its Own Origins

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Abstract

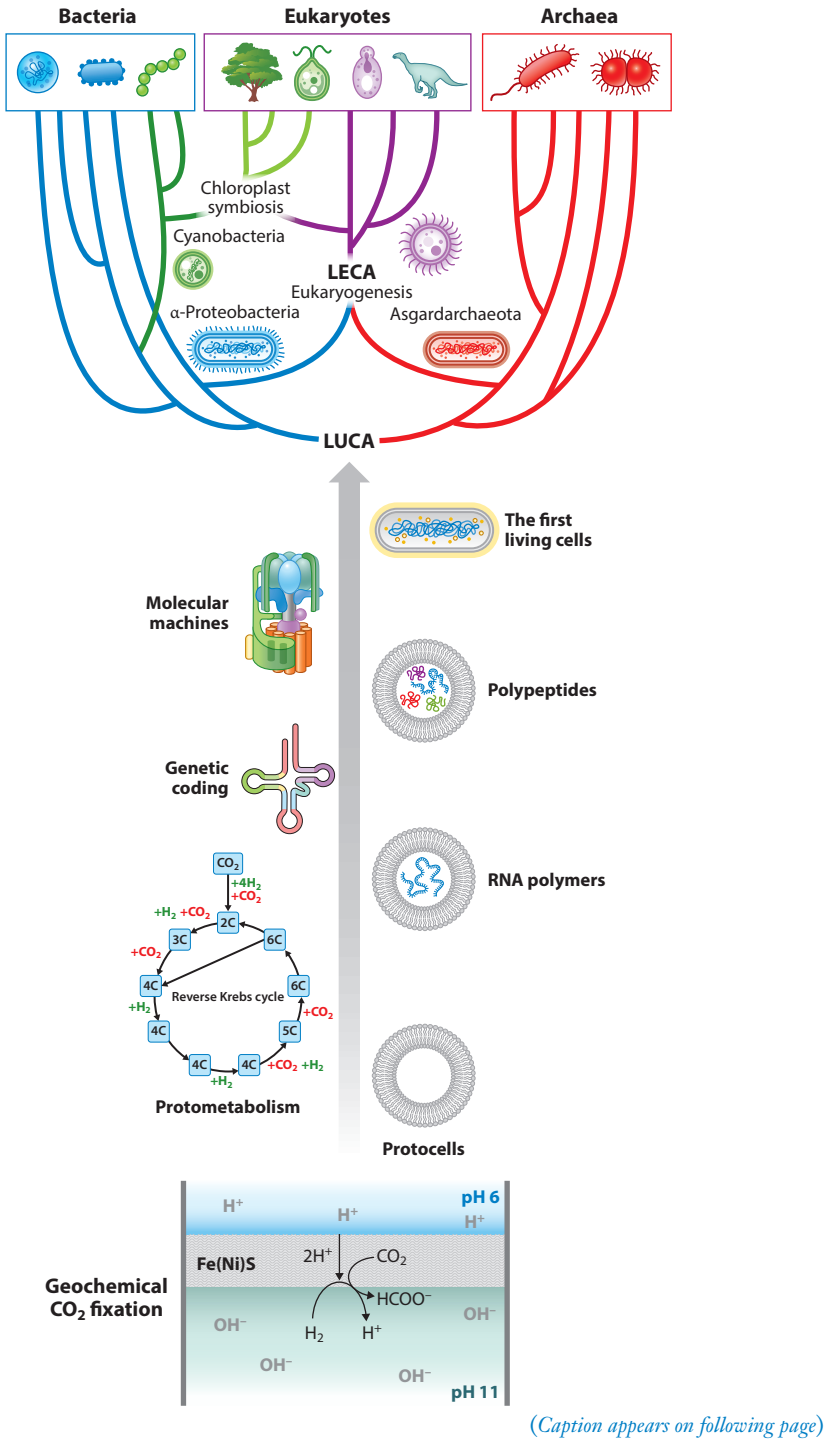
The origin of life entails a continuum from simple prebiotic chemistry to cells with genes and molecular machines. Using life as a guide to this continuum, we consider how selection could promote increased complexity before the emergence of genes. Structured, far-from-equilibrium environments such as hydrothermal systems drive the reaction between CO_2 and H_2 to form organics that self-organize into protocells. CO_2 fixation within protocells generates a reaction network with a topology that prefigures the universal core of metabolism. Positive feedback loops amplify flux through this network, giving a metabolic heredity that promotes growth. Patterns in the genetic code show that genes and proteins arose through direct biophysical interactions between amino acids and nucleotides in this protometabolic network. Random genetic sequences template nonrandom peptides, producing selectable function in growing protocells. This context-dependent emergence of information gives rise seamlessly to an autotrophic last universal common ancestor.

INTRODUCTION

How life arose has long been considered a question in prebiotic chemistry, but there is clearly an extended continuum from the simplest prebiotic chemistry to the emergence of genes and molecular machines. The sheer length of this continuum suggests that only some form of selection could facilitate the sustained increase in complexity (**Figure 1**), but selection on what and how? A full explanation for the origin of life needs to link the steps from one end of this continuum to the other in a logical, testable, and selectable order. Strangely, the use of life itself as a guide to how selection might work has been relatively neglected, given that one end of the spectrum is life itself. Biology is clear that selection can operate on entities other than genes, notably cells (Godfrey-Smith 2011). Our own recent work is beginning to explore how the universal core of metabolism could have been selected in autotrophic protocells, driving the emergence of genes and biological information rather than the other way around (Halpern et al. 2023, Harrison et al. 2022, Nunes Palmeira et al. 2022, West et al. 2017). This review draws on experimental and theoretical work over the last decade that suggests life can act as a guide to the full origin-of-life continuum, although some significant gaps remain.

What do we know about the end point? Phylogenetics indicates that the last universal common ancestor (LUCA) was probably chemolithotrophic and obligately chemiosmotic, likely living off gases such as H_2 and CO_2 (Braakman & Smith 2012, Crapitto et al. 2022, Lane & Martin 2012, Martin & Russell 2003, Sousa et al. 2013, Stetter 2006, Weiss et al. 2016). There are deep differences between bacteria and archaea but even deeper similarities: LUCA had the universal genetic code, transcription, translation, DNA, RNA, proteins, and ribosomes (Berkemer & McGlynn 2021, Crapitto et al. 2022, Koonin 2003, Moody et al. 2022). LUCA's core autotrophic metabolism arguably predated the genes that encode these pathways (Wimmer et al. 2021), as the chemistry and reaction mechanisms are more deeply conserved than the genes themselves (Harrison & Lane 2018, Martin 2020). LUCA was most likely cellular, with lipid bilayer membranes, although the lipid composition and protein content of these membranes remain ambiguous (Coleman et al. 2019, Sojo 2015, Villanueva et al. 2021). Nonetheless, LUCA, as an end point, was recognizably prokaryotic, with a cellular structure, complete genetic code, molecular machines such as the ribosome, and membrane-integral proteins including the ATP synthase (Lane et al. 2010, Martin et al. 2014, Mulkidjanian et al. 2009).

There is no necessary direct link between LUCA and the early prebiotic chemistry giving rise to that level of sophistication, but Occam's razor stipulates that we should at least entertain the possibility. If so, then the starting point would be a spontaneous protometabolism, beginning with gases such as CO_2 and H_2 (Lane & Martin 2012, Lane et al. 2010, Martin & Russell 2007, Martin et al. 2014, Russell & Hall 1997, Russell & Martin 2004). That is not unreasonable, because rock–water interactions such as serpentinization in the submarine crust can produce H_2-rich hydrothermal systems on a global scale, with individual systems persisting over hundreds of thousands of years (Arndt & Nisbet 2012; Russell & Arndt 2005; Russell et al. 2014; Sleep 2010, 2018; Westall et al. 2018). These hydrothermal systems are internally structured, containing labyrinths of interconnected cell-like pores that frustrate the mixing of alkaline hydrothermal fluids with ocean waters saturated in CO_2 (Martin & Russell 2003, 2007; Martin et al. 2008; Nitschke & Russell 2009; Russell & Hall 1997; Russell et al. 1989, 1994). Critically, in the Hadean era, 4 billion years ago, there may have been as much as 1 bar of atmospheric CO_2, making the early oceans mildly acidic (pH 5–6) (Arndt & Nisbet 2012, Russell & Arndt 2005, Sleep 2018). The difference in pH between acidic oceans and alkaline fluids could therefore sustain steep pH gradients across thin inorganic barriers in vents, forming a natural proton-motive force (PMF) that could, in principle, drive work (Barge et al. 2019, Branscomb & Russell 2013, Lane & Martin



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Tree of life, emphasizing the long evolutionary distance from geochemical CO_2 fixation to LUCA. Note that LUCA is the common ancestor of the two prokaryotic domains, bacteria and archaea, and that eukaryotes derive from an endosymbiosis between an archaeal host cell and a bacterial endosymbiont. This means that the properties of eukaryotes have no phylogenetic relevance to the origin of life. The deepest branching bacteria and archaea are autotrophic, growing from CO_2 and electron donors such as H_2. Abbreviations: LECA, last eukaryotic common ancestor; LUCA, last universal common ancestor. Figure adapted from images created with BioRender.com.

2012, Lane et al. 2010, Martin et al. 2014, Nitschke & Russell 2009, Russell & Hall 1997). An important distinction in the chemistry of Hadean and modern hydrothermal systems relates to oxygen, which was essentially absent before the Great Oxidation Event approximately 2.4 billion years ago (Arndt & Nisbet 2012). In the absence of oxygen, the early oceans were metal rich, containing micromolar concentrations of metal ions such as Fe^{2+} and Ni^{2+} (Poulton & Canfeld 2011), which precipitate as sulfides and hydroxides in hydrothermal systems, giving early vents catalytic walls (Nitschke & Russell 2009, Nitschke et al. 2013, Russell 2018, Russell & Martin 2004, Russell et al. 1994). Second, in the absence of oxygen, the reaction between H_2 and CO_2 to form organic molecules is thermodynamically favored (Amend & McCollom 2009, Amend et al. 2013, Barge et al. 2018, Russell et al. 2014). Thus, alkaline hydrothermal vents can be seen as continually replenished (far-from-equilibrium) electrochemical flow reactors that catalyze the thermodynamically favored reaction between CO_2 and H_2 to drive an autotrophic protometabolism (Russell & Hall 1997, Russell et al. 2014).

The idea that life began with an autotrophic protometabolism dates back decades (Hartman 1975; Morowitz et al. 2000; Russell & Hall 1997; Smith & Morowitz 2016; Wachtershauser 1988, 1990) and has been most fully developed in alkaline hydrothermal systems (Martin 2020, Martin & Russell 2007, Wimmer et al. 2021, Xavier & Kauffman 2022, Xavier et al. 2020). From the point of view of life as a guide to its own origins, autotrophic protometabolism solves one of the critical steps in the origin-of-life continuum: the problem of how genes came to encode the multiple steps of metabolic pathways, which amounts to the origin of information in biology. The problem is this: If the first genes encoded enzymes or ribozymes that catalyzed single reaction steps, as they do today, then introducing genes one at a time would have little or no benefit so long as the rest of the pathway was missing. The combinatorial problem of adding all the genes for all the steps of all biochemical pathways at once is akin to Fred Hoyle's tornado in a junkyard assembling a jumbo jet (Chandra Wickramasinghe & Hoyle 1981). The usual answer, of building biochemical pathways step by step from one end or the other (Horowitz 1945, Lazcano & Miller 1999, Noda-Garcia et al. 2018, Tawfik 2020) lowers those combinatorial odds but explicitly requires that all the intermediates should be stable and useful, which is certainly not the case for pathways such as purine synthesis (Harrison & Lane 2018, Ralser 2018). That also begs the question: To what kind of entity are they useful? In fact, the solution is simple: If protometabolism occurs spontaneously in some propitious, far-from-equilibrium environment, then the first genetically encoded catalysts had to do no more than promote flux through this network. This could be achieved simply, for example, by facilitating CO_2 fixation, which increases the concentration of metabolic precursors and so steepens the driving force for flux through the whole network.

While on paper this solution seems simple, the reality is more challenging. Until recently, little of the requisite CO_2 chemistry had been demonstrated in the lab. This has changed dramatically in the last decade; large sections of intermediary metabolism have now been accomplished under reasonable prebiotic conditions (Muchowska et al. 2019, 2020; Preiner et al. 2020; Ralser 2018). But that is still far from demonstrating flux through the entire network. Even if flux through the entire network is achieved, it seems likely that only trace amounts of products such as nucleotides

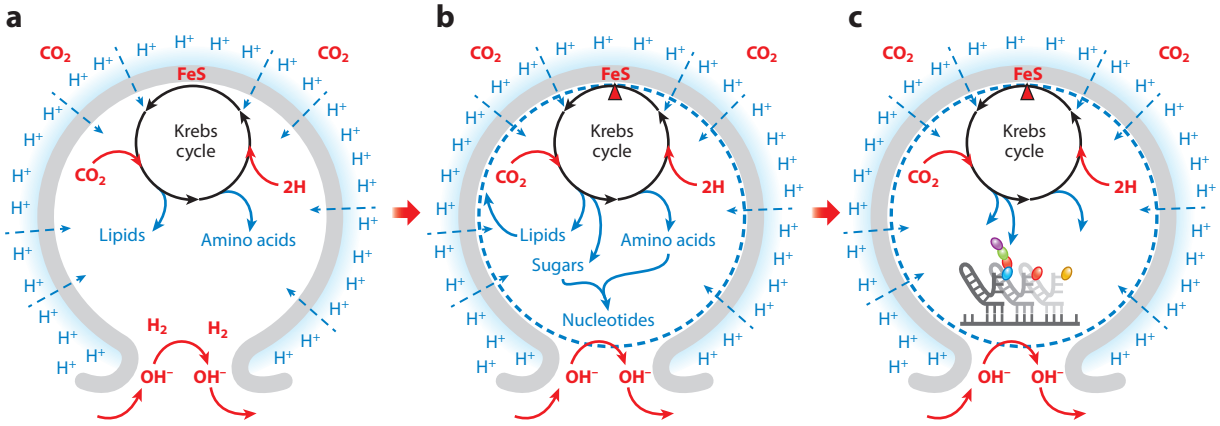


Figure 2

Stages of protocell evolution up to an RNA world. (*a*) A pore in a hydrothermal vent with an inorganic barrier containing FeS minerals (*gray*), separating alkaline hydrothermal fluids inside from acidic ocean waters outside. Straight blue arrows show the influx of protons across the barrier, driving CO_2 fixation via a reverse (incomplete) Krebs cycle to form lipids and amino acids. (*b*) Formation of a fatty acid bilayer membrane lining a hydrothermal pore (*dashed blue line*) enables CO_2 fixation within the protocell using [4Fe–4S] clusters associated with the membrane (*red triangle*). Steepening of the metabolic disequilibrium within protocells drives greater flux right through to nucleotide synthesis. (*c*) Polymerization of nucleotides gives rise to an RNA world and templated peptide formation within protocells.

could be synthesized, given the large number of steps from CO_2 fixation to, say, ATP synthesis as the precursor for polymerization of both RNA and peptides (using life as a guide). It would then be necessary for protometabolism to improve over time—to bootstrap itself up to enhanced flux via positive feedback loops or autocatalysis before the emergence of polymers or true genetic information. In other words, there would have to be selection before genes. If so, then the entities under selection and the mechanisms of selection need to be explicitly defined and tested.

In this review, we use life as a guide to its own origins. We consider the necessary and sufficient steps from CO_2 fixation to the emergence of genetic information (**Figure 2**). That is to say, while we cannot exclude the possible contribution of factors such as extraneous catalysts, peptides, or RNA (which might sporadically enhance flux), if we cannot propose a mechanism through which selection could operate generation after generation, then we neglect these as unnecessary or insufficient steps. We posit that life was cellular from an early stage, as all known autotrophic cells power CO_2 fixation using electrical membrane potential linked to transition metal clusters in enzymes such as the energy-converting hydrogenase (Ech). As noted, labyrinths of cell-like pores form spontaneously in alkaline hydrothermal vents, offering a pleasing template for the first protocells (Martin & Russell 2003, Nitschke & Russell 2009, Russell & Hall 1997, Sojo et al. 2016). In chemolithotrophic prokaryotes, CO_2 fixation generates not only the 1–2-carbon intermediates of the acetyl coenzyme A (CoA) pathway but also longer chain carboxylic acids via pathways such as the reverse incomplete Krebs cycle (Camprubi et al. 2017, Fuchs 2011, Lane 2022, Martin & Russell 2007, Smith & Morowitz 2004). Sugars, amino acids, fatty acids, isoprenoids, and nucleotides are all synthesized from these 1–5-carbon carboxylic acid precursors (Lane 2022, Martin & Russell 2007, Smith & Morowitz 2004). This means that CO_2 fixation beyond acetyl CoA is necessary, so conditions would have to favor multiple steps of CO_2 fixation above competing processes that could help generate acetyl CoA, such as methane oxidation. We posit that polymerization of RNA and templated peptides arose within protocells via a spontaneous autotrophic protometabolism with a network topology that prefigured the universal core of metabolism.

Patterns in the genetic code correspond to these autotrophic origins, indicating that direct interactions between amino acids and their cognate nucleotides framed the emergence of information. Using these precepts, we span the entire origin-of-life continuum from CO_2 fixation to the emergence of genes and molecular machines.

A GEOLOGICALLY SUSTAINED PROTON-MOTIVE FORCE DRIVES CARBON DIOXIDE FIXATION

Several years before proposing his chemiosmotic hypothesis, Peter Mitchell (Mitchell 1959, p. 437) observed that the organism and the environment are "equivalent phases between which dynamic contact is maintained by the membranes that separate and link them."

At the origin of life, before the advent of cells, Mitchell's emphasis on dynamic contact between phases separated by membranes is penetrating. Membranes frustrate the mixing of two phases, enabling steep differences in pH, ion concentrations, or redox potentials. Since the late 1980s, Mike Russell and colleagues (Martin & Russell 2003, 2007; Nitschke & Russell 2009; Russell 2018; Russell & Hall 1997; Russell et al. 1989, 1994) have called attention to the microporous labyrinths of alkaline hydrothermal systems, in which thin inorganic barriers containing catalytic transition metals [Fe(Ni)S minerals] frustrate the mixing of two phases: strongly alkaline, highly reduced hydrothermal fluids containing hydrogen gas and mildly acidic, more oxidized ocean waters, saturated in CO_2. The overall topology of hydrothermal pores is strikingly similar to cells, with potentially 3–4 pH units difference between the inside and outside (**Figure 2**). This is equivalent in both magnitude and polarity to prokaryotic cells (Lane et al. 2010, Nitschke & Russell 2009). Living cells maintain this electrochemical H^+ difference by actively pumping H^+ ions across the membrane to generate a PMF that powers work within the cell (Buckel & Thauer 2013, Mitchell 1966, Mitchell & Moyle 1967). In vents, the continuous hydrothermal flow, combined with ocean convection, can maintain steep pH differences across thin inorganic barriers. This natural PMF can, in principle, power work, as H^+ ions continuously traverse the barrier down a concentration gradient (Branscomb & Russell 2013, Nitschke & Russell 2009, Russell & Hall 1997). Respiration does not generate the geochemical PMF; hydrothermal flow does.

This topological structure is conserved universally across life. The membrane is far more than a bag that contains the constituents of a cell: it is an active barrier, energized by steep differences in ion concentration. All autotrophic cells are obligately chemiosmotic, meaning that they couple the influx of ions from the outside phase to work done on the inside (Branscomb & Russell 2013, Lane & Martin 2012, Lane et al. 2010, Nitschke & Russell 2009). What kind of work? Sophisticated ion-tight membranes and the proteins that power chemiosmotic coupling such as the ATP synthase are clearly the product of genes and natural selection. Strictly homologous prebiotic equivalents of the ATP synthase or ion pumps are hard to conceive. But there is no need for them. The PMF powers much more than ATP synthesis. The most essential prebiotic possibility is CO_2 fixation. For example, methanogenic archaea drive CO_2 fixation using a membrane-bound, proton-motive Ech (Buckel & Thauer 2013, Schoelmerich & Müller 2020, Thauer et al. 2008). This relatively simple protein contains four transition metal clusters, including three [4Fe–4S] clusters, similar to greigite in their unit-cell structure (Yu et al. 2018), two of which have a reduction potential that is modulated by pH (Kurkin et al. 2002). The flux of protons through an associated membrane channel offers a simple biophysical mechanism by which the presence or absence of protons in the proximity of these [4Fe–4S] clusters drives a redox switch (Lane 2017, Vasiliadou et al. 2019). Importantly, it is easy to envisage prebiotic equivalents to this mechanism (**Figure 3**).

The redox switch depends on the involvement of protons in redox reactions. Both the oxidation of H_2 and the reduction of CO_2 hinge on the availability of H^+ ions but in opposite directions,

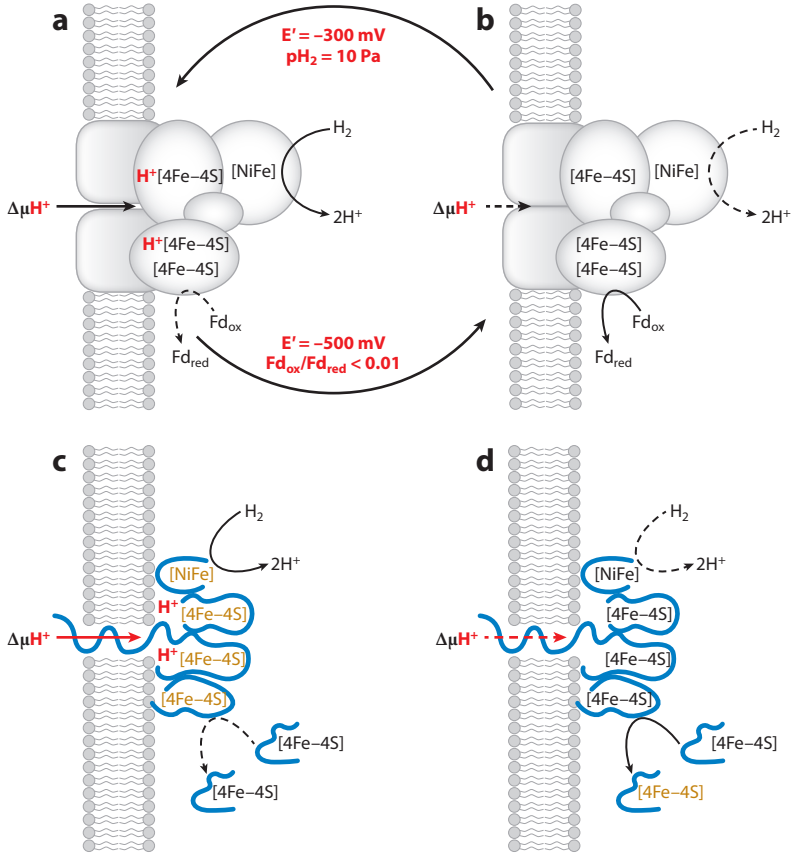


Figure 3

Possible redox-switch mechanisms of the Ech. (*a*) Protons enter through membrane channels, down an electrochemical gradient ($\Delta\mu H^+$), and bind to adjacent [4Fe–4S] clusters, raising their redox potential so that they can be reduced by H_2 at approximately –300 mV but cannot reduce Fd at –500 mV. (*b*) The pore closes, protons detach from [4Fe–4S] clusters, and the redox potential falls low enough to reduce ferredoxin. (*c*) A potential prebiotic (nongenetically encoded) Ech peptide. A simple transmembrane helix binds water and allows protons to cross via Grotthus transfer. Cysteine-containing peptides bind [4Fe–4S] clusters that bind protons, raising their redox potential as in panel *a*, enabling them to be reduced by H_2. (*d*) Natural oscillations in proton flux mean that protons periodically detach from [4Fe–4S] clusters, lowering their redox potential sufficiently to reduce a proto-ferredoxin peptide that can diffuse into the cytosol. Brown text indicates that the clusters are reduced; black text indicates oxidized clusters. Abbreviations: Ech, energy-converting hydrogenase; Fd, ferredoxin; ox, oxidized; pH_2, partial pressure of hydrogen gas; red, reduced. Panels *a* and *b* adapted from Vasiliadou et al. (2019) (CC BY 4.0).

hence the requirement for two phases separated and linked by a membrane (Lane 2014). H_2 is more reducing under alkaline conditions (as in alkaline hydrothermal systems) by –59 mV per pH unit, according to the Nernst equation (Boyd et al. 2020, Lane 2014, Vasiliadou et al. 2019). The transfer of electrons from H_2 leaves behind protons, which immediately react with OH^- ions in an alkaline environment, an exothermic neutralization. Conversely, CO_2 accepts electrons more readily in acidic conditions, where protons balance the charge, facilitating further electron transfer, here by +59 mV per pH unit. The redox potential of [4Fe–4S] clusters and FeS mineral surfaces can also be pH dependent (Wolthers et al. 2003), presumably because protons bind to

lone electron pairs on sulfur (Chakrabarti & Pal 1997). In terms of topology, the upshot is that protonated [4Fe–4S] clusters should accept electrons from H_2 when close to the membrane inside protocells, whereas deprotonated clusters in the cytosol switch to a lower redox potential that is able to reduce CO_2. This arrangement depends on cell structure: The inside must be relatively alkaline versus the outside, and the continuous influx of protons across the membrane enables the reduction of FeS clusters and CO_2 close to the membrane.

Until recently, these ideas made sense in theory but had little experimental support. While higher pressure (~100 bars) and mineral catalysts are capable of reducing CO_2 to carboxylic acids including formate, acetate, and pyruvate in the absence of pH gradients (Preiner et al. 2020), work from Hudson and colleagues (Hudson et al. 2020) demonstrated for the first time that pH differences across Fe(Ni)S barriers are indeed capable of driving CO_2 reduction to formate at pressures as low as 1.5 bars. Importantly, Hudson et al. showed that the electrons came from H_2, whereas H^+ and CO_2 derived from the acid phase; the Fe(Ni)S barrier, pH difference, and H_2 gas were all required for CO_2 reduction (Hudson et al. 2020). Laminar flow in microfluidic chips, combined with the rapid transfer of protons across freshly precipitated Fe(Ni)S barriers [probably via the Grotthuss mechanism (Agmon 1995), as H^+ transfer across the barrier is ~2 million–fold faster than OH^- transfer] can maintain pH differences of 3–4 pH units across distances of less than 100 nm for extended periods (Vasiliadou et al. 2019). Optimizing microfluidic catalysts and flux rates could drive the synthesis of longer chain carboxylic acids (He et al. 2021) and amino acids (Barge et al. 2019), both of which are also predicted by theoretical thermodynamics under these conditions (Amend & McCollom 2009, Amend et al. 2013).

Longer chain carboxylic acids—fatty acids—as well as long-chain alcohols and hydrocarbons have in fact already been formed at higher temperatures, albeit interestingly only in steel reactors (McCollom et al. 1999), probably reflecting catalysis by carbide bonds (Martin 2019, Xu et al. 2014). The higher temperatures (175°C) were needed to disproportionate formate or oxalate, which were added for simplicity in place of H_2 and CO_2 (McCollom et al. 1999). In principle the same chemistry could work at lower temperatures, starting from H_2 and CO_2 at higher pressure rather than carboxylic acids. In both cases, hydrothermal Fischer–Tropsch-type synthesis produced long-chain lipid compounds ranging from C_2 to $>C_{35}$ in length, with a peak distribution around C_{10}–C_{15}, not dissimilar to fatty-acid chain length in modern membranes (McCollom et al. 1999). While the mechanistic chemistry of Fischer–Tropsch-type synthesis is reminiscent of the enzyme carbon-monoxide dehydrogenase (Cody et al. 2004), little work has yet been done on the synthesis of fatty acids from acetyl subunits, as occurs in life itself.

Taking all this into consideration, we conclude that steep pH differences across Fe(Ni)S barriers can drive CO_2 fixation to form carboxylic acids including reverse Krebs cycle intermediates (acetate and pyruvate), as well as potentially longer-chain fatty acids. If this hypothesis is correct, then these amphiphiles should form protocells bounded by bilayer membranes within the hydrothermal pores, as depicted in **Figure 2**.

AUTOTROPHIC PROTOCELLS CAPABLE OF MEMBRANE HEREDITY FORM SPONTANEOUSLY

The literature long suggested that fatty-acid vesicles could not form under oceanic hydrothermal conditions, but these results were mostly based on single (usually C_{10}) fatty acids or simple mixtures of only two or three amphiphiles (Maurer et al. 2018, Milshteyn et al. 2018, Monnard et al. 2002). Using more complex mixtures of six C_{10}–C_{15} fatty acids and six fatty alcohols or isoprenols, we have shown that mixed amphiphiles can readily self-assemble into vesicles with bilayer membranes under a wide range of conditions (Jordan et al. 2019a,b). These included, importantly,

pH 7–13, modern ocean salinity, warm temperatures (70°C), and modern oceanic concentrations of the divalent cations Ca^{2+} and Mg^{2+}—all equivalent to the submarine alkaline hydrothermal conditions discussed in the previous section. The specific amphiphiles selected were chosen from the peak spectrum of lipids generated by hydrothermal Fischer–Tropsch-type synthesis (McCollom et al. 1999), making them a reasonable prebiotic assortment. Because they were all single-chain amphiphiles, lacking either ester or ether bonds to glycerol phosphate headgroups, stable vesicles did not form in acidic conditions, which instead protonate the carboxylate headgroups to form lipid droplets (Monnard et al. 2002).

The lack of phospholipid headgroups is important for several reasons. Single-chain amphiphiles are simpler to form, and are therefore likely to dominate in any prebiotic environment (Morowitz et al. 1988). They should also be better at enabling a natural PMF to power work. The reason relates to flux. For a geologically sustained PMF to power work inside protocells, protons must enter the protocell and then leave again, otherwise the gradient collapses (Sojo et al. 2014). Modern cells have pumps linked to respiration that actively pump protons out, but in a monomeric world before the existence of genes or pumps, hydrothermal flow had to eliminate protons from inside protocells for any pH difference to persist. Fatty-acid flip-flop allows protons to cross fatty acid bilayers orders of magnitude faster than they can cross phospholipid membranes (Barile et al. 2016, Brunaldi et al. 2005). Mathematical modelling shows that fatty-acid bilayers are sufficiently leaky to protons for a continuous influx and efflux of H^+ ions to be maintained by hydrothermal flow, whereas cells composed of phospholipid membranes cannot be sustained by a natural PMF in the absence of H^+ pumps (Sojo et al. 2014). This requirement for a proton-leaky membrane could explain why LUCA did not have a modern phospholipid membrane: If LUCA lived off a geochemically sustained PMF in hydrothermal systems, then a modern phospholipid membrane would have been a disadvantage, as it would have stopped them from tapping the natural PMF. If so, then bacteria and archaea, which descended from LUCA, developed their phospholipid membranes independently as they learned to generate their own PMF (Sojo et al. 2014, 2016).

Be that as it may, for protocells to be powered by a natural PMF, they would need to form within alkaline pores subject to a continuous influx of protons from across the barrier. We and others have shown that protocells composed of mixed amphiphiles do bind to mineral surfaces (Hanczyc et al. 2007, Jordan et al. 2019a, Sahai et al. 2017). Once inside a protocell, H^+ ions need to be neutralized by OH^- ions from hydrothermal flow or the driving force collapses, and the membrane disaggregates. (Note that the exit of H^+ ions is formally equivalent to the entry of OH^- ions.) The aqueous interior of protocells would therefore necessarily have an intermediate pH range, more amenable to biochemistry as we know it. In short, protocells should form from mixed amphiphiles, specifically inside alkaline pores, and the continuous influxes of H^+ ions across adjacent inorganic barriers and OH^- ions from hydrothermal flow could in principle power work (Sojo et al. 2014).

We have already indicated that the most plausible work that could be achieved in a monomer world would be CO_2 fixation. The conversion of the gases CO_2, H_2, and NH_3 into reverse Krebs cycle intermediates, longer-chain fatty acids, and amino acids within protocells would in effect drive the autotrophic growth of the protocell. For these organic molecules to be formed within the protocell, rather than in the acidic space outside, the influx of protons across the fatty-acid bilayer membrane would need to protonate FeS nanocrystals associated with the membrane, not the external barrier. West et al. (2017) modeled possible interactions between intracellular amino acids and FeS nanocrystals that could associate with the membrane. Coordination of growing FeS nanocrystals by cysteine was assumed to hinder nanocrystal growth, thereby generating a larger number of small nanocrystals that could power more CO_2 fixation, and so more amino acid synthesis, giving a positive feedback loop: The more amino acids formed, the more they provided

feedback on CO_2 fixation. The modelling showed that this positive feedback loop could indeed drive autotrophic protocell growth. Protocell division was modeled simply on the basis of surface area-to-volume constraints, and experiments show that fatty-acid protocells do grow and divide readily (Hanczyc & Szostak 2004, Jordan et al. 2019b, Zhu & Szostak 2009). If indeed small FeS nanocrystals do associate with the membrane, which grows in area as more fatty acids are formed by CO_2 fixation, then daughter cells physically inherit a membrane already studded with amino-acid-coordinated FeS nanocrystals capable of driving growth: a rudimentary form of membrane heredity (West et al. 2017).

Several experimentally testable predictions follow directly from this modelling: Amino acids such as cysteine should interact with Fe^{3+} and HS^- to form FeS nanocrystals; these FeS structures should be capable of redox cycling, reducing CO_2 to form organic molecules and being reduced themselves by H_2; their redox potential should be pH dependent; they should associate with the membrane; and it should be possible to synthesize cysteine under relevant prebiotic conditions. In fact, experimental validation goes beyond these predictions. Rather than forming FeS nanocrystals, the interaction of micromolar concentrations of monomeric cysteine with Fe^{3+} and HS^- at alkaline pH directly forms a variety of biological FeS clusters, including [2Fe–2S] clusters and [4Fe–4S] clusters with structures exactly equivalent to those in ferredoxin and Ech (Jordan et al. 2021). These [4Fe–4S] clusters are redox active, with a midpoint redox potential of approximately –500 mV (versus standard hydrogen electrode), in the correct range for reducing CO_2 (though CO_2 fixation has yet to be demonstrated). The redox potential of cysteine-coordinated [4Fe–4S] clusters is pH dependent, roughly following the Nernst equation. In addition, our recent work (SA Harrison, H Rammu, M Schalkwijk, N Lane, unpublished data) shows that it is possible to synthesize cysteine directly from O-phosphoserine (and to synthesize glycine and serine directly from CO_2 following the biological pathway).

The only prediction yet to be experimentally validated is how cysteine-coordinated [4Fe–4S] clusters might associate with the membrane. Given that [4Fe–4S] clusters have a net charge of +2 and the surface of a fatty-acid bilayer membrane has a net negative charge, [4Fe–4S] clusters could in principle associate with the membrane, but preliminary work using isothermal titration calorimetry showed no indication of membrane association. Other studies (using size-exclusion chromatography and fluorescence microscopy) are ongoing. Nonetheless, the observations that protocells with bilayer membranes composed of single-chain amphiphiles spontaneously self-assemble under alkaline hydrothermal conditions, that these bind to mineral surfaces, and that cysteine-coordinated [4Fe–4S] clusters with redox potentials equivalent to ferredoxin form spontaneously at micromolar concentrations are all consistent with the hypothesis that protocells could promote CO_2 fixation and their own autotrophic growth in far-from-equilibrium hydrothermal systems. Using life as a guide, the first few steps from prebiotic chemistry to an autotrophic LUCA all fall into place: the formation of simple protocells, the machinery for driving CO_2 fixation, and a simple means of selection that enables protocells to inherit this machinery before the emergence of genes.

PROTOMETABOLISM OCCURS SPONTANEOUSLY FROM CARBON DIOXIDE RIGHT UP TO NUCLEOBASES

If CO_2 fixation inside protocells is indeed feasible, then the next question is, Could nucleotides form under these conditions, ultimately giving rise to an RNA world within autotrophic protocells? If the hypothesis is correct, then nucleotides should by synthesized from H_2, CO_2, and NH_3 following the universally conserved pathways of core metabolism in the absence of genetically encoded catalysts (Harrison & Lane 2018). A decade ago, such a claim had little support.

Since then, however, large parts of core metabolism have been demonstrated under realistic prebiotic conditions (**Figure 4**), albeit not always starting from CO_2 fixation (but with metabolic intermediates instead). Specifically, the acetyl CoA pathway and much of the reverse Krebs cycle have been reconstituted under mild aqueous conditions, using metal ions as catalysts and H_2 or reducing equivalents (such as Fe^0) as electron donors (Huber & Wächtershäuser 1997; Keller et al. 2017; Muchowska et al. 2017, 2019; Preiner et al. 2020; Varma et al. 2018). A number of amino acids have been synthesized by reductive amination from Krebs cycle intermediates, including glycine, alanine, and glutamate (Barge et al. 2019, Huber & Wächtershäuser 2003, Kitadai et al. 2019, Muchowska et al. 2019). Our own work shows that the simple cofactor pyridoxamine can transfer NH_3 and hydrogen in the absence of enzymes to form a number of amino acids, including aspartate and alanine (Harrison et al. 2023). Most of gluconeogenesis, glycolysis, and the pentose phosphate pathway have also been demonstrated in the absence of enzymes (Camprubi et al. 2022, Keller et al. 2014, Messner et al. 2017, Piedrafita et al. 2021). More recently, the nucleobase uracil has successfully been synthesized following the metabolic pathway (Yi et al. 2022). We have accomplished a one-pot synthesis of both orotate and uracil using metal-ion catalysts (S.A. Harrison, T. Harries, S. Pinna, N. Lane, unpublished data). The yield of uracil was optimized by mild hydrothermal conditions (90°C, pH 7–9, modern ocean salinity, and 100 bars pressure).

Much prebiotic chemistry remains incomplete when using life as a guide, including rationalizing a set of conditions that can optimize flux through all these pathways simultaneously, which is necessary if metabolism really did have an autotrophic emergence. But the elephant in the room is purine nucleotide synthesis. The metabolic pathway for purine synthesis contains ∼12 steps, ∼6 of which require adenosine triphosphate (ATP) (Kappock et al. 2000). To our knowledge nobody has yet succeeded in synthesizing purine nucleotides using this pathway, though we and others are trying to do so. While the requirement for ATP might seem to be a paradox (ATP being a purine nucleotide) this is not necessarily the case, as simpler phosphorylating agents such as acetyl phosphate might be able to drive these steps in place of ATP. Acetyl phosphate retains a central place in intermediary metabolism as the fulcrum between thioester and phosphate metabolism. It has been synthesized from thioacetate under mildly hydrothermal conditions (Whicher et al. 2018), and in the presence of Fe^{3+} ions, it is capable of substrate-level phosphorylation of adenosine diphosphate to ATP at 15–20% yield (Pinna et al. 2022). Curiously, acetyl phosphate failed to phosphorylate other nucleoside diphosphates under equivalent conditions, implying that ATP might be the universal energy currency in part because it can be formed through prebiotic chemistry (Pinna et al. 2022). Nonetheless, even if acetyl phosphate is able to substitute for ATP in purine synthesis, and even if it is possible to synthesize ATP and guanosine triphosphate (GTP) via the biological route in the absence of enzymes, the number of steps and the instability of many intermediates means it is unlikely that more than trace amounts of product could be formed.

This leads to another dilemma. It is hardly possible to conceive of an RNA world in autotrophic protocells if they could not somehow amplify nucleotide synthesis. Yet without genes, how could they bootstrap themselves up to a higher throughput of purine nucleotides? Again, positive feedbacks might solve this problem, and two feedback loops potentially involve purine synthesis. The first is the use of GTP-derived cofactors in CO_2 fixation—folates and pterins in bacteria and archaea, respectively—that are related in structure (Braakman & Smith 2012, Maden 2000). Increasing the rate of purine nucleotide synthesis could then promote CO_2 fixation, raising the concentration of early metabolic intermediates linked to CO_2 fixation such as glycine (Braakman & Smith 2012), the starting point for purine synthesis, closing the positive feedback loop. The second feedback loop involves ATP itself, which could potentially displace acetyl phosphate or other equivalent phosphorylating agents, increasing the throughput of ATP synthesis. This is not unreasonable given that acetyl phosphate tends to acetylate amine groups, including amino acids

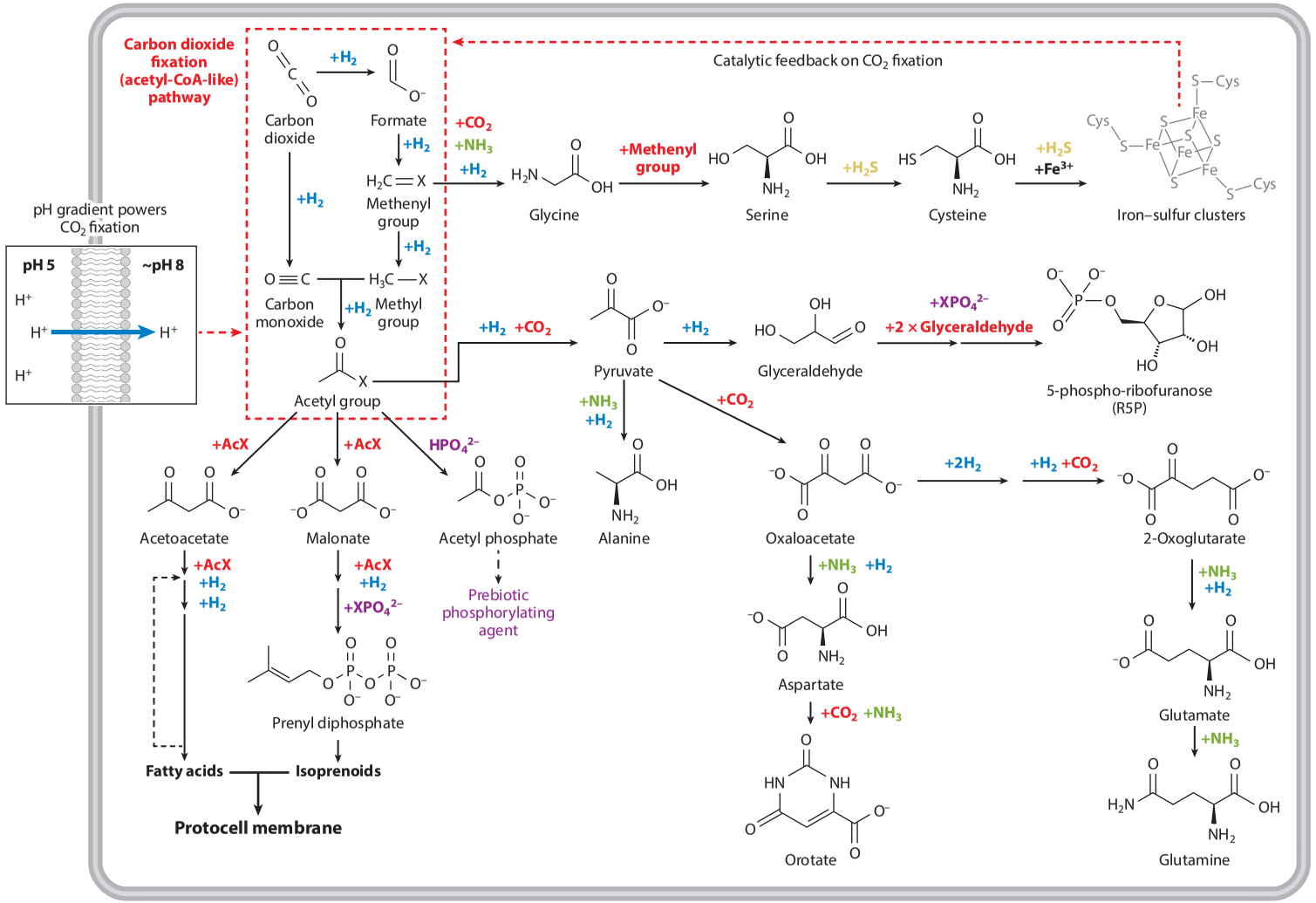


Figure 4

Protometabolism from CO_2 fixation driven by a natural PMF across a fatty-acid bilayer membrane (*blue arrow*). The universally conserved core of metabolism is strikingly dependent on CO_2 (*red*) and hydrogen (H_2 or reducing equivalents, *blue*) as well as ammonia (*green*), phosphate (*purple*), and sulfide (*yellow*). This network extends up to the formation of lipid species required for protocell growth, iron sulfur catalysts to feedback on CO_2 fixation, and the key amino acids and early nucleotide species. Many reactions in this network have been achieved in laboratory contexts. Abbreviations: CoA, coenzyme A; PMF, proton-motive force.

(di Sabato & Jencks 1961, Whicher et al. 2018), potentially interfering with efficient purine synthesis. The use of ATP might also promote the decoration of ribose-5-phosphate with a 1′ pyrophosphate group, to give phosphoribosyl pyrophosphate, on which the purine ring is built.

These positive feedback loops are not the only ones possible. Many cofactors derive from purine nucleotides, including NAD (nicotinamide adenine dinucleotide), FAD (flavin adenine dinucleotide), and CoA. These tend to catalyze general processes, such as hydride or acetyl group transfer, and catalyze the same reactions in the form of naked cofactors in the absence of the enzyme (Kirschning 2021, Raffaelli 2011). Modelling multiple feedback loops in autotrophic protocells shows that nucleotide catalysis can indeed increase flux through the entire protometabolic network (Nunes Palmeira et al. 2022). Catalysis of specific pathways (rather than general processes) is not helpful, because increased flux down one pathway comes at the cost of flux down other pathways, which unbalances protocell growth. Autocatalysis of nucleotide synthesis—for example, by ATP catalyzing purine nucleotide synthesis—does lead to the accumulation of nucleotides but can promote protocell growth only when nucleotides catalyze CO_2 fixation as well (or promiscuously enhance all branches of metabolism equally). Because cell division is tied to growth in the model, catalytic nucleotides tend not to accumulate within protocells, as their number halves at each cell division (Nunes Palmeira et al. 2022).

Overall, this work shows that positive feedback loops can, in principle, promote the autotrophic growth of protocells. This equates to an extended form of heredity and selection, because the daughter cells directly inherit nucleotides that promote flux through the entire protometabolic network, thereby regenerating nucleotides that are passed on to their own daughter cells (Nunes Palmeira et al. 2022). Two features are required for this system to operate: Metabolic flux must be possible through the entire network, and flux must be increased by steepening the driving force, which is to say, the conversion of CO_2 into metabolic precursors. An environmental disequilibrium (of H_2 and CO_2) is therefore converted into a metabolic disequilibrium, where higher concentrations of metabolic precursors push flux through the network. But critically, while offering a simple form of heredity, this system is not evolvable. It can extend the protometabolic network, and get better at generating nucleotides, but the entire system is determined by thermodynamics and kinetics and will therefore always regenerate the same topological network from the same starting point (Nunes Palmeira et al. 2022). To evolve any further than that needs genes, i.e., selection for sequences.

PATTERNS IN THE GENETIC CODE POINT TO THE EMERGENCE OF INFORMATION

Everything described so far refers to a monomer world, lacking genetic information or any polymeric molecular machines, a world defined by deterministic chemistry in a specific environment and with a particular starting point. Let us allow that nucleotides can polymerize. This has been demonstrated in many environments, including wet–dry cycles (Hassenkam et al. 2020) and the surface of rock glasses (Jerome et al. 2022), but not yet in the aqueous interior of protocells or equivalent conditions. Failure to polymerize nucleotides into random sequences of RNA under these conditions poses a serious challenge to the hypothesis outlined here, but the use of nucleoside triphosphates combined with simple monomeric equivalents of the RNA polymerase (divalent metal ions coordinated by amino acids such as aspartate) is underexplored. Successful polymerization would reinforce the metabolic pathways discussed above: The first peptides and RNA molecules had to fish amino acids and nucleotides from solution as they polymerized, lowering the concentration of the terminal monomers and so pulling greater flux through their biosynthetic pathways. This architecture is self-reinforcing.

Assuming it is possible, RNA polymerization in protocells offers a unique context for the emergence of genetic information. Ambiguous patterns in the genetic code have long been known—a code within the codons that points to both biosynthetic and stereochemical associations between amino acids and their cognate codons or anticodons (Copley et al. 2005, Taylor & Coates 1989). The first letter of the codon is associated with the biosynthetic precursor of the amino acid encoded (Copley et al. 2005, Wong 1975, Wong et al. 2016). The second letter of the anticodon is associated with the hydrophobicity (or perhaps more generally, the partition energy) of the amino acid encoded (Caldararo & di Giulio 2022; Taylor & Coates 1989; Woese 1965, 1968a). The third letter of the anticodon is frequently redundant (degenerate), but when not so, there is a loose association with the complexity of the amino acid encoded (Baumann & Oro 1993, Taylor & Coates 1989). What these patterns signify has long defied interpretation, but they are not consistent with the idea of a frozen accident (Woese 1969).

Reinterpreting these patterns from the point of view of autotrophic protocells is illuminating (Harrison et al. 2022). There is a clear relationship between the first letter of the codon and the distance from CO_2 fixation, by way of the universally conserved metabolic network (**Figure 5**). This relationship is consistent with the idea of positive feedback loops involving purine nucleotide synthesis. The amino acids that are closest to CO_2 fixation are generally encoded by G at the first position of the codon; those slightly more distant from CO_2 fixation are encoded by A at that first position. The amino acids encoded by the pyrimidine nucleotides C and U at the first position are the most distant from CO_2 fixation. While the number of steps gives only a rough indication of the likelihood of them occurring (as it does not take kinetic or thermodynamic factors into account), there seems little doubt that coding is structured around an autotrophic protometabolism (Harrison et al. 2022). This structure implies that purine nucleotides dominated early protometabolism and that the metabolic network expanded over time, gradually incorporating more complex amino acids and a fuller genetic code (Harrison et al. 2022).

Structuring the genetic code around these groups of amino acids (defined by the base at the first position of the codon) sheds light on hydrophobicity relationships too: The correlation between the hydrophobicity of an amino acid and its cognate base in the anticodon is stronger in the earlier amino acids incorporated into the code (those encoded by G and A at the first position of the codon) (Harrison et al. 2022). While hydrophobicity is an opaque term and may well be a proxy for more subtle factors such as the partition energy of binding interactions (Caldararo & di Giulio 2022), this pattern clearly indicates specific biophysical interactions between amino acids and bases. Our own molecular-dynamic simulations and nuclear magnetic resonance studies broadly indicate that these nonrandom biophysical associations exist and that the displayed preferences often emulate the assignments of the anticodon middle base (Halpern et al. 2023). These associations allow us to correctly predict half of the second-base anticodon assignments, though it is notable that both methods predict hydrophilic interactions better than hydrophobic associations. In any case, as Carl Woese (Woese 1965, 1968ba,b, 1969) anticipated in the 1960s, these biophysical relationships are weak and statistical but nonetheless real and pervasive across the code.

We also find a simple stereochemical relationship between the third base of the codon and the size of the amino acid encoded, specifically in those cases where the third position is not redundant (Harrison et al. 2022). Interestingly, the patterns of third-position redundancy are themselves nonrandom and depend on the identity of the base at the first or second positions of the codon (Harrison et al. 2022). For example:

- If there is a bulky G at the second position of the anticodon, then the third position is always redundant.

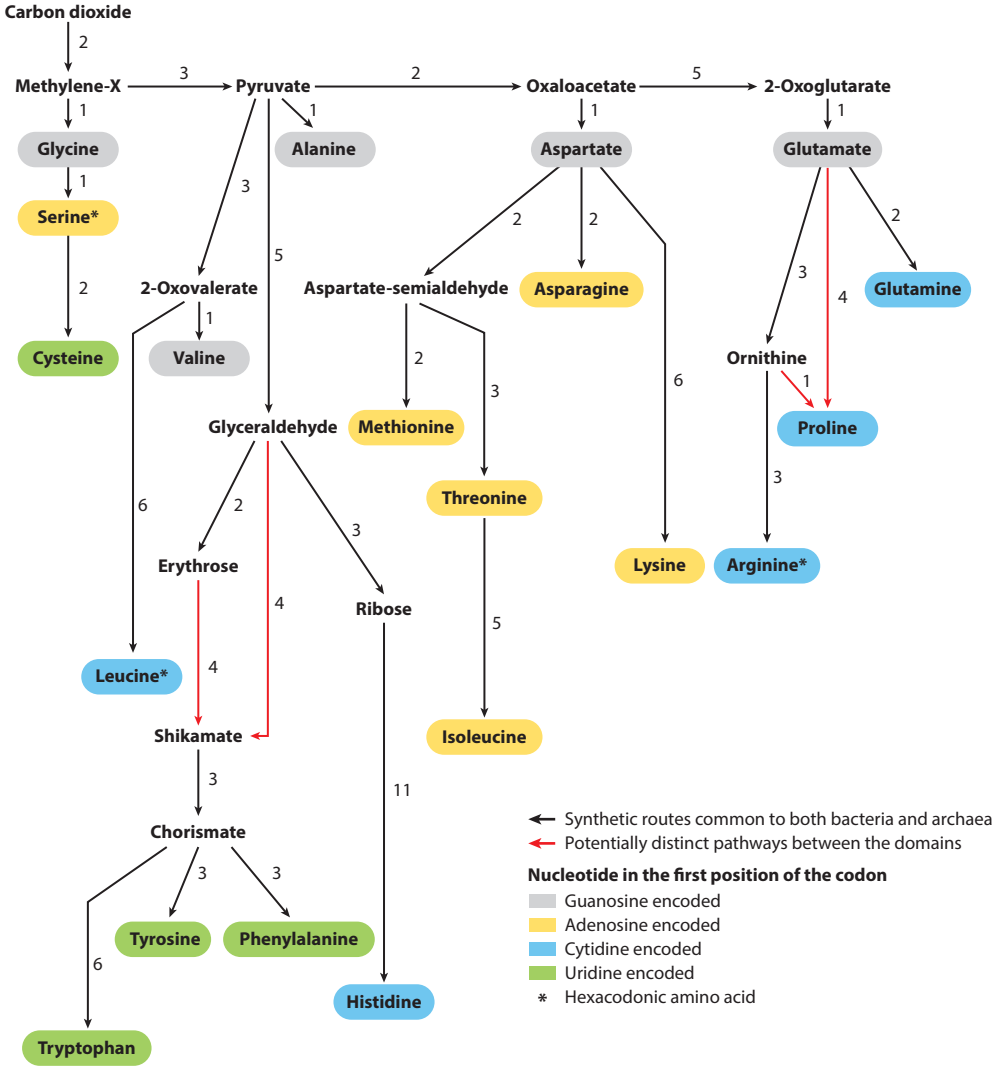


Figure 5

The structure of protometabolism, illustrating how the autotrophic biosynthesis of amino acids links to the genetic code. Amino acids are colored according to the nucleotide specified at their first codon position. Black arrows indicate synthetic routes common to both bacteria and archaea; red arrows indicate potentially distinct pathways between the domains. Arrows are labeled with the number of reaction steps between the precursor and the amino acid, with their length scaled accordingly. A clear sequential, though overlapping, emergence can be observed, with earlier amino acids encoded by G and A compared with later species. Cysteine is an interesting outlier: Its tendency to self-react during adenylation might explain the apparent delay in incorporating it into the code (Harrison et al. 2022). Figure adapted from Harrison et al. (2022) (CC BY 4.0).

- If there is a small U at the second position, then the third position is never redundant.
- If there is a C or a G at the first position of the codon (which form three hydrogen bonds via standard Watson–Crick pairing), then codons tend to exhibit third position redundancy.

Taken together, these patterns suggest that amino acids bind to short aptamers of RNA, perhaps in a binding pocket that incorporates the anticodon sequence.

None of that matters if the binding of amino acids does not facilitate their polymerization into a templated peptide. Before we consider how this templating might work, we should reflect on the larger picture. Allowing that amino acids do bind to RNA sequences in a nonrandom manner and that this binding facilitates their polymerization into short peptides (Müller et al. 2022, Mullins et al. 1984, Shimizu 1995, Tamura & Schimmel 2003), then the amino-acid sequence of those peptides is specified at least loosely by the RNA sequence. In other words, because even random RNA sequences template peptides nonrandomly, then there is a platform for generating the same function repeatedly (Halpern et al. 2023). If these functions benefit the cell in any way, for example, by influencing growth rate, then selection can take place with even the most rudimentary copying. The standard base-pairing mechanisms are another purely biophysical phenomenon, so copying RNA sequences is not magic but statistically predictable. Natural selection therefore emerges spontaneously on RNA sequences resembling genes, bypassing any need to evolve code-enforcing translational machinery first. In the context of autotrophic protocells, there is no problem with the emergence of information in biology.

EVOLUTION OF THE FIRST GENETICALLY ENCODED MOLECULAR MACHINES

What kind of functions could the first short RNA molecules and peptides assume in the context of autotrophically growing protocells? Selection for growth rate and heritability stand out. While selection can reflect persistence, as well as faster growth (Black et al. 2020), persistence alone cannot facilitate an increase in complexity over time. In the same way, cells that grow quickly but cannot pass on faithful copies of the genetic sequences that enabled them to do so cannot evolve greater complexity over time either.

Because faster growth rates must be sustained by faster flux through the entire protometabolic network, selection would need to enhance protometabolism. The proto-Ech depicted in **Figure 3** shows how simple peptides could work. Cysteine-containing peptides as short as the trimer glutathione bind to [4Fe–4S] clusters (Bonfio et al. 2017). We have shown that a small cysteine-containing peptide based on ferredoxin chelates only [4Fe–4S] clusters and is capable of far more robust electron transfer than cysteine-coordinated clusters (H Rammu, A Marechal, M Roessler, N Lane, unpublished data). This selectivity is based on physical chemistry rather than genetic specification: Peptides containing cysteine simply tend to coordinate [4Fe–4S] clusters. Likewise, hydrophobic peptides would be expected to partition to the membrane, again for biophysical reasons. Any membrane-spanning helix is likely to trap some water, facilitating proton transfer across the membrane down a concentration gradient, as happens in Ech (Mühlbauer et al. 2021). Interactions between peptides could readily fabricate a proto-Ech, in which protons are funneled onto [4Fe–4S] clusters, driving CO_2 fixation (**Figure 3**). Much the same behavior would be anticipated for peptides binding to nucleotide cofactors: Specific peptide maquettes that bind to nucleotides would be expected to mimic conserved motifs in modern enzymes (Chu & Zhang 2020, Narunsky et al. 2020). This area is bursting with experimental potential.

Similar principles should apply to copying RNA. The RNA polymerase is not a ribozyme but a Mg^{2+}-dependent protein. The Mg^{2+} ion is usually coordinated by aspartate residues in a characteristic DXDXD motif. While aspartate alone should coordinate Mg^{2+} in solution, short peptides containing aspartate residues would be expected to mimic the active site of the enzyme more robustly and with a wider amino-acid context (for example, modifying the charge environment), making them more functional. Copying RNA allows the template to be inherited by daughter cells. The fidelity of Watson–Crick base pairing may be modest (albeit better than the interactions between amino acids and nucleotides) but should generate at least approximate copies that

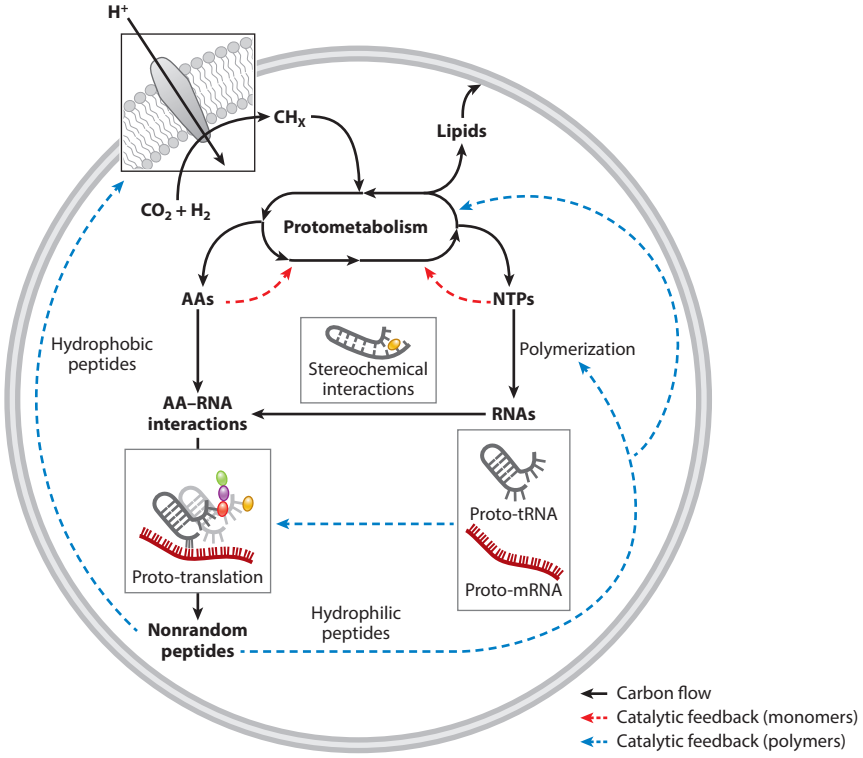


Figure 6

The beginnings of an RNA–peptide world in autotrophic protocells. CO_2 fixation is facilitated by a proto-Ech (as in **Figure 3*c***) which drives protometabolism, generating amino acids and NTPs. These can catalyze positive feedback loops on CO_2 fixation and protometabolism (*red dashed lines*) or polymerize into RNA and peptides. Amino acids bind to RNA via the stereochemical interactions implicit in the genetic code. Random RNA sequences can therefore template nonrandom peptides, which have function in growing protocells. In particular, peptides can catalyze CO_2 fixation (as a proto-Ech) or nucleotide polymerization (as a proto–RNA polymerase) or bind to cofactors and facilitate protometabolic flux (*blue dotted lines*). None of these interactions require a full metabolic code but can take place on the basis of biophysical interactions alone. Abbreviations: AA, amino acid; Ech, energy-converting hydrogenase; mRNA, messenger RNA; NTP, nucleoside triphosphate; tRNA, transfer RNA.

are substantially better than random. Likewise, the weak and statistical nature of amino acid–base interactions (Halpern et al. 2023) would limit the replicability of specific peptides, but still, a range of peptides with similar sequences (and so functions) should form. Even the simplest copying and translation mechanisms could in principle promote protocell growth and evolution (**Figure 6**), but such templating, based on weak binding interactions, is unlikely to fashion more sophisticated molecular machines that depend on precise coding.

So what would produce such sophisticated machines? A clue might lie in the binding pockets of RNA aptamers mentioned in the previous section. Short RNA aptamers containing a few Watson–Crick pairings (potentially a hairpin loop), as well as an anticodon, look a lot like a proto–transfer RNA (tRNA). But of course, amino acids do not bind to the anticodon in tRNA; they are first adenylated before binding to the CCA acceptor stem. So how could early biophysical interactions involving the anticodon shine through into the modern genetic code? One possibility would be a hairpin loop, with two free ends forming a binding pocket; we imagine the two ends dangling

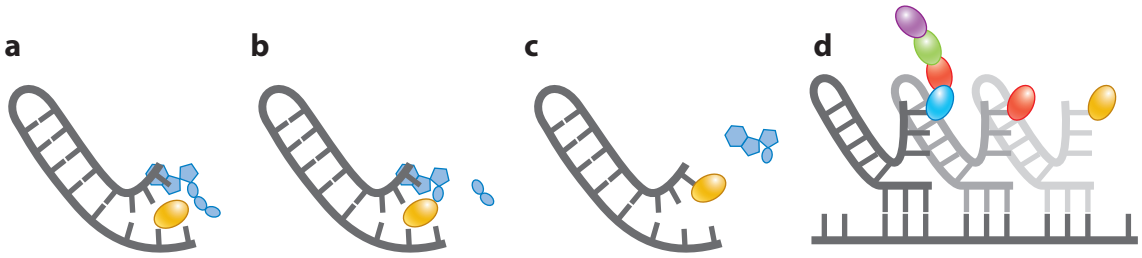


Figure 7

A possible mechanism for amino acid binding and adenylation on a proto-tRNA. (*a*) An amino acid (*yellow oval*) binding to its cognate anticodon on a short proto-tRNA with a hairpin loop. An ATP (*blue*) stacks onto the terminal A of the CCA acceptor stem adjacent to the anticodon. (*b*) Nucleophilic attack of the carboxylate oxygen on the α-phosphate releases the pyrophosphate tail (*blue ovals*), which adenylates the amino acid. (*c*) Transfer of the amino acid from the adenosine to the 2′ ribose of the terminal A on the CCA acceptor stem results in an amino-acylated tRNA. (*d*) A possible primordial mechanism of translation, in which the flexible hinge of the proto-tRNA allows binding of the anticodon to a codon on an adjacent proto-mRNA (where the reading frame is also determined by stereochemical interactions), enabling the synthesis of short peptide sequences specified by the RNA sequence. Figure adapted from Halpern et al. (2023) (CC BY 4.0).

close to each other, flexibly hinged. One end has the anticodon, which binds its cognate amino acid; the other end is the CCA acceptor stem (Shimizu 1995). The A is critical here. Adenosine can form stacks through Pi-bond interactions between purine rings (Brown et al. 2015, Whicher et al. 2018). We suggest that an ATP stacks onto the terminal A of the acceptor stem and dangles its triphosphate tail in the vicinity of the amino acid loosely bound onto the adjacent anticodon. The carboxylate residue of the amino acid is weakly nucleophilic, the phosphorus of the nearby α-phosphate is weakly electrophilic, and pyrophosphate is a strongly exergonic leaving group that facilitates the nucleophilic attack. The amino acid is left covalently bound to the α-phosphate of the stacked adenosine monophosphate (AMP)—it is adenylated and dangles next to the 2′ OH of the ribose of the adenosine on the CCA acceptor stem. Transfer to the 2′ OH releases the AMP. We now have an amino-acylated proto-tRNA with the flexibly hinged anticodon free to bind to an adjacent proto–messenger RNA (mRNA). If amino-acylated proto-tRNAs bound adjacently, their amino acids would be suitably positioned for polymerization (**Figure 7**).

We readily admit that this is speculation, albeit grounded in known biology and at least partially supported by preliminary molecular dynamic simulations. It does not ask an RNA world to do any more than is universally conserved at the heart of biology in the translation machinery, and it can explain the observed patterns in the genetic code, which is very difficult if amino acids never associated directly with the anticodon (in other words, if the CCA acceptor stem and anticodon had always been far apart on the tRNA adaptor molecule). Nor does it ask for extended RNA sequences to provide function; short aptamers of 12–15 bases would be sufficient to form a single hairpin loop as depicted in **Figure 7**. While much work remains to define the reading frame, start codon, sequential tRNA binding (procession), and the emerging distinction between mRNA, tRNA, and ribosomal RNA, this simple scenario dissipates some of the mystery: It has the potential to ramp up selection to the level of ribosomal translation and error minimization, opening the gateway to the evolution of genetically specified molecular machines.

CONCLUSIONS

We have outlined a possible continuum to explain the origin of life, beginning with simple prebiotic chemistry in a structured far-from-equilibrium environment and ending with the emergence of molecular machines. Some aspects of this hypothesis have been tested experimentally

or through computational modelling and shown to be viable; whether the remaining gaps can be bridged and the hypothesis as a whole can be demonstrated as realistic is the focus of ongoing work. The central conception here, which links CO_2 fixation driven by a geochemical PMF to the emergence of genes and information within protocells, is a spontaneous protometabolism that prefigures the topology of metabolism. It might seem troubling, even uncanny, that metabolism should recapitulate protometabolism so exactly—that the same spontaneous interconversions between molecules along pathways should "just happen." Why does this network exist and not some other network? The answer is simple. This is the network that is favored from a starting point of H_2 and CO_2, under Earth-like conditions. It is exactly what we would expect to see. The forms of selection outlined here necessarily amplify flux through the same thermodynamically favored protometabolic reactions to genetically encoded systems based on exactly the same chemistry—including the same biophysical interactions between amino acids and RNA—preconfiguring the genetic code to a considerable degree. If life did not begin with CO_2 under the environmental conditions presented here, that should have given rise to a different protometabolic network. The first genes should then have amplified that network instead, though there may of course be overlap. This is a prediction of any metabolism-first hypothesis, and it is correspondingly difficult to explain by any other hypothesis. If the ideas explored here are correct, that does not mean that life is an uncanny guide to its own origin: Life on earth is an elaboration of the spontaneous chemistry that should happen on any wet, rocky planet.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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