

## Iron Catalysis at the Origin of Life

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### Abstract

Iron–sulphur proteins are ancient and drive fundamental processes in cells, notably electron transfer and CO<sub>2</sub> fixation. Iron–sulphur minerals with equivalent structures could have played a key role in the origin of life. However, the ‘iron–sulphur world’ hypothesis has had a mixed reception, with questions raised especially about the feasibility of a pyrites-pulled reverse Krebs cycle. Phylogenetics suggests that the earliest cells drove carbon and energy metabolism via the acetyl CoA pathway, which is also replete in Fe(Ni)S proteins. Deep differences between bacteria and archaea in this pathway obscure the ancestral state. These differences make sense if early cells depended on natural proton gradients in alkaline hydrothermal vents. If so, the acetyl CoA pathway diverged with the origins of active ion pumping, and ancestral CO<sub>2</sub> fixation might have been equivalent to methanogens, which depend on a membrane-bound NiFe hydrogenase, energy converting hydrogenase. This uses the proton-motive force to reduce ferredoxin, thence CO<sub>2</sub>. The mechanism suggests

that pH could modulate reduction potential at the active site of the enzyme, facilitating the difficult reduction of CO<sub>2</sub> by H<sub>2</sub>. This mechanism could be generalised under abiotic conditions so that steep pH differences across semi-conducting Fe(Ni)S barriers drives not just the first steps of CO<sub>2</sub> fixation to C1 and C2 organics such as CO, CH<sub>3</sub>SH and CH<sub>3</sub>COSH, but a series of similar carbonylation and hydrogenation reactions to form longer chain carboxylic acids such as pyruvate, oxaloacetate and  $\alpha$ -ketoglutarate, as in the incomplete reverse Krebs cycle found in methanogens. We suggest that the closure of a complete reverse Krebs cycle, by regenerating acetyl CoA directly, displaced the acetyl CoA pathway from many modern groups. A later reliance on acetyl CoA and ATP eliminated the need for the proton-motive force to drive most steps of the reverse Krebs cycle. © 2017 The Authors IUBMB Life published by Wiley Periodicals, Inc. on behalf of International Union of Biochemistry and Molecular Biology, 00(0):000–000, 2017

**Keywords:** origin of life; iron–sulphur minerals; alkaline hydrothermal vent; incomplete reverse Krebs cycle; ferredoxin; acetyl CoA pathway; proton gradients

**Abbreviations:** CODH/ACS, carbon monoxide dehydrogenase/acetyl CoA synthase; Ech, Energy-converting hydrogenase; LUCA, Last Universal Common Ancestor

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### A Primordial Iron–Sulphur World?

Two articles published in 1966 had an enduring impact on ideas about the origin of life. The first, by Eck and Dayhoff, attempted to reconstruct the evolutionary history of ferredoxin on the basis of its amino acid sequence (1). Eck and Dayhoff proposed that ferredoxin, with its simple FeS cofactors, was formed through the doubling of a shorter protein, containing only the simplest amino acids, which had itself developed from a repeating sequence of only four amino acids. They argued that ferredoxin had been incorporated into metabolism even before complex proteins and the complete modern genetic code existed. That same year, Evans et al. showed that the Krebs cycle can operate in reverse, driving a ferredoxin-dependent carbon-reduction cycle in a photosynthetic bacterium (2). This pathway remained controversial for two decades, but was eventually accepted as an alternative to the

Calvin–Benson cycle as a route for CO<sub>2</sub> fixation. The reverse Krebs cycle is autocatalytic—from oxaloacetate, a single spin of the reverse Krebs cycle generates two oxaloacetates (the second from acetyl CoA, after the splitting of citrate). This finding fed ideas on autocatalytic cycles and hypercycles as a means of driving stable growth at the origin of life, dating back to Eigen in 1971 (3).

It was against this background that Wächtershäuser proposed his radical conception of a pyrites-pulled reverse Krebs cycle in 1990 (4). In dismissing a heterotrophic origin of life in primordial soup, Wächtershäuser attempted to reconstruct the first metabolic cycles by retrodiction, on the basis that the reverse Krebs cycle was the archaic ancestor of the oxidative cycle, was common in bacteria and apparently some archaea, and (as he described it), was strictly chemoautotrophic; it was not necessary to invoke photoautotrophy. Wächtershäuser was aware that, unlike the facile chemistry proceeding from electrical discharges or UV radiation acting on reduced gases such as methane and ammonia, the reduction of CO<sub>2</sub> by H<sub>2</sub> was not easy (5). Despite being exergonic overall under strictly anoxic conditions, the first steps to produce CO, HCOO<sup>−</sup> or CH<sub>2</sub>O (formaldehyde) are strongly endergonic, and inhibit the synthesis of organic molecules from H<sub>2</sub> and CO<sub>2</sub> (6). Autotrophic cells that live from this reaction alone must in principle use some form of coupling to lower the kinetic barrier to reaction, and Wächtershäuser proposed pyrites pulling as the coupling mechanism, in which the oxidation of FeS to pyrites (FeS<sub>2</sub>) is coupled to the reduction of CO<sub>2</sub> by H<sub>2</sub> (6):



While some studies have supported this mechanism, at least to form simple but reactive organics such as CH<sub>3</sub>SH (methane thiol) (7), most successful studies testing the hypothesis, including those from Wächtershäuser himself, start with the more reactive substrate CO rather than CO<sub>2</sub> (8,9), and therefore technically avoid pyrites pulling. Ironically, parallel work on primordial soup was beginning to take account of geochemical evidence which suggested that the Earth's atmosphere was never rich in reduced gases such as CH<sub>4</sub> and NH<sub>3</sub>, but was instead comparatively oxidising, composed mainly of CO<sub>2</sub> and N<sub>2</sub> (10–12). Electrical discharges through such relatively oxidising atmospheres did not readily form amino acids; but inclusion of high concentrations of CO did facilitate organic chemistry (13). Accordingly, both Wächtershäuser and his opponents who favoured 'soup chemistry' called on the reactivity of CO (8,9,13). The synthesis of 'activated acetate' (methyl thioacetate) by Huber and Wächtershäuser in 1996 was achieved under an atmosphere of 1 Bar of CO, which is geologically highly implausible, and occurred readily in the absence of FeS: NiSO<sub>4</sub> was also effective as a catalyst, despite being incapable of pyrites pulling.

## Problems With Primordial Metabolic Cycles

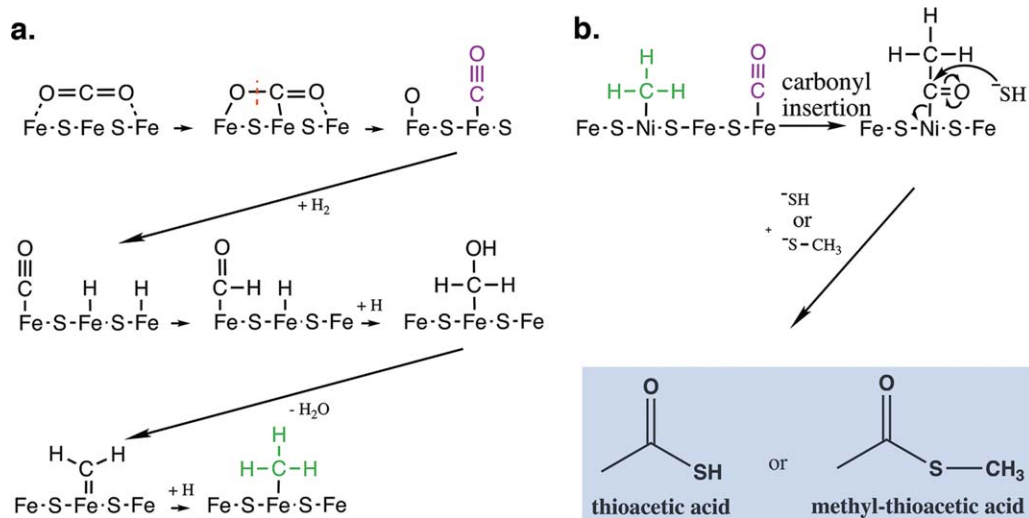
Most discussion of prebiotic chemistry has been framed in terms of specificity and yield—synthetic chemists tend to see the problem in terms of achieving high yields of specific products, and dislike chemistry that produces low yields of mixed products (14). Biologists are more inclined to the opposite view, arguing instead that selection will favour higher yields of specific products, hence the earliest prebiotic chemistry should have produced low yields of mixed products. Particular catalysts such as metal ions or minerals favour some products over others, and increase yields somewhat, hinting at the beginnings of geochemical 'pathways' (15). Chelation by simple organics such as amino acids, then short non-coded polypeptides and ultimately genetically encoded proteins, each favour increasing yields of more specific products (15,16). As a rule of thumb, cells (using enzymes) lower the kinetic barriers to thermodynamically favoured reactions. This applies equally to the tardy reaction between H<sub>2</sub> and CO<sub>2</sub>—methanogens and acetogens that depend on the acetyl CoA pathway gain all the carbon and energy they need to grow from this reaction alone (17–19). Because it barely occurs as prebiotic chemistry, the reactants remain far from equilibrium, an opportunity waiting to be exploited.

From this point of view, the fact that Wächtershäuser's hypothetical pyrites-pulled Krebs cycle has never been shown to work is not unexpected. But there is a deeper problem with the conception of primordial metabolic cycles, as pointed out by Orgel (20), which applies specifically to the idea of cycles rather than linear pathways. The problem is that: if abiotic reactions occur at low yield, and the product of one reaction forms the substrate of the next reaction, then the more reaction steps there are, the more catastrophic the decline in yield. That much is true for any metabolic pathway, and must presumably favour the shortest pathways of carbon fixation. But it becomes far more of a serious problem for metabolic cycles, in part because they usually comprise many steps [the pyrites-pulled reverse Krebs cycle as depicted by Wächtershäuser has 16 steps (4)], but especially because the substrate concentration for the first step depends wholly on the yield of the final step. This is surely unworkable as abiotic chemistry—Orgel denounced it as 'if pigs could fly' hypothetical chemistry (20).

This last issue, incidentally, is not solved by a recent study suggesting that Krebs cycle intermediates are favoured by sulphate radical chemistry (21): this study showed a decomposition of longer chain Krebs cycle intermediates to form simpler molecules such as pyruvate, and not a proto-metabolic turning of the cycle. It therefore suggests that Krebs cycle intermediates are thermodynamically metastable (like many amino acids) but does not solve the problem of declining yield at each step of a full cycle.

## The Acetyl CoA Pathway Is Ancient

In fact the complete reverse Krebs cycle is found only in bacteria, and so despite the appeal of FeS catalysis in CO<sub>2</sub> fixation,



**FIG 1**

(a) Fe-catalysed reduction of  $\text{CO}_2$  into surface-bound CO (purple) via the cleavage of a C–O bond, followed by subsequent hydrogenations and a dehydration yielding surface-bound methyl group (green). (b) Carbonylation of a Ni-bound methyl group by Fe-bound CO, followed by elution through nucleophilic attack by a sulphhydryl or methyl-sulphhydryl ion yielding thioacetic acid or methyl-thioacetic acid, respectively. This abiotic mechanism is analogous to the carbon monoxide dehydrogenase/acetyl CoA synthase (CODH/ACS) enzymatic mechanism, where coenzyme A acts as the eluting nucleophile.

its deep antiquity is not sustained by phylogenetics (unless it was lost from all archaea) (22–25). Since the discovery of the reverse Krebs cycle in 1966, another four pathways of  $\text{CO}_2$  fixation have been described, giving six in total to date (25). Of these, only the acetyl CoA pathway (or Wood/Ljungdahl pathway) is found in both bacteria and archaea (22–25); hence likely dates back to their common ancestor, the last universal common ancestor of life (LUCA), which arguably might have lived in submarine hydrothermal vents (see below) (26).

Other factors are consistent with the early evolution of the acetyl CoA pathway. It is a short, linear pathway, which avoids the problems discussed above (27). Unlike other pathways of  $\text{CO}_2$  fixation, it is exergonic overall, meaning that there is no net input of ATP to drive the pathway; on the contrary, the reaction of  $\text{H}_2$  with  $\text{CO}_2$  provides all the energy required for growth (27). Finally, it is replete in Fe(Ni)S cofactors, to the extent that the pathway has been said to have ‘rocky roots’ (27). The three groups of cells that rely on the acetyl CoA pathway for carbon fixation (methanogens, acetogens and sulphate-reducers) have notably more FeS proteins in their genomes than other prokaryotes (28). The key enzyme, the carbon monoxide dehydrogenase/acetyl CoA synthase (CODH/ACS), is an unusual hydrogenase with three  $\text{Fe}_4\text{S}_4$  clusters, two of which bridge via sulphide to nickel (Ni-S- $\text{Fe}_4\text{S}_4$ ) (29). This enzyme catalyses the formation of acetyl CoA, the hub of metabolism, from CO bound to iron and a  $\text{CH}_3^-$  group bound to nickel (29).

Similar abiotic reactions have been proposed to take place on Fe(Ni)S surfaces, via Fischer–Tropsch-type hydrogenations and Koch-type carbonyl transfers (30,31), as shown in Figure 1. This reaction mechanism also depicts the first difficult step, the reduction of  $\text{CO}_2$  to CO on an iron-sulphide surface (32), which we discuss later in relation to pH modulation of reduction potential.

## Deep Differences Between Bacteria And Archaea

While these factors are all consistent with the primordial origins of the acetyl CoA pathway, there are deep differences in methyl synthesis between bacteria and archaea, which make it difficult to discern the evolution of the pathway (33–35). One possibility is that reactive methyl groups (perhaps in the form of  $\text{CH}_3\text{SH}$ , methane thiol) were so plentiful in Hadean hydrothermal systems that there was no requirement for genetically encoded methyl synthesis in the earliest stages of evolution (33). If that were the case, then the true substrate for the origin of life was not  $\text{CO}_2$ , as in most autotrophs, but  $\text{CH}_3\text{SH}$ . To date, little abiotic  $\text{CH}_3\text{SH}$  has been detected in modern hydrothermal systems (36), but it could be consumed by cells lower in the crust, or rapidly oxidised under modern aerobic conditions. If so, the concentration of  $\text{CH}_3\text{SH}$  could have been far greater in anoxic Hadean vents.

An alternative possibility is that  $\text{H}_2$  reduced  $\text{CO}_2$  to both CO and to  $\text{CH}_3^-$  at the vent-ocean interface, through a process similar to that which occurs in methanogens (34). The logic here relates to the process of flavin-based electron bifurcation, which is similar in concept, but differs in many details, between methanogens and acetogens (archaea and bacteria, respectively) (37–39). Electron bifurcation accomplishes the endergonic reduction of a low-potential ferredoxin by coupling it to the exergonic reduction of a heterodisulphide (in methanogens) (38) or  $\text{NAD}^+$  (in acetogens) (39). The specific steps are discussed in detail elsewhere (33,34,37–39) and we will not do so here. The salient point is that, in both groups, electron bifurcation achieves two outcomes, albeit via distinct pathways: (i) the difficult reduction of  $\text{CO}_2$  by  $\text{H}_2$  to form

reactive methyl groups and (ii) the generation of an electrochemical ion gradient via a membrane-bound pump, Mtr in the case of methanogens, and Rnf or energy converting hydrogenase (Ech) in the case of acetogens (37).

Critically, for electron bifurcation to continue operating, the exergonic reduction products (thiols or NADH) must be reoxidised, and this is achieved by the excretion of either methane (in methanogens) or acetate (in acetogens) (37). So electron bifurcation generates an electrochemical membrane potential (which is then used to drive both ATP synthesis and CO<sub>2</sub> fixation, as discussed below), but it does not directly generate biomass. This is a key point, worth reiterating. While electron bifurcation achieves the difficult reduction of ferredoxin, all that is actually conserved is an electrochemical ion gradient across a membrane. In other words, electron bifurcation drives active ion pumping (34).

## Alkaline Hydrothermal Vents

The fact that electron bifurcation generates electrochemical ion gradients allows us to structure the problem of its origin in a specific environment: alkaline hydrothermal vents. These vents have many properties that make them conducive to the origins of life, which have been developed in detail by Russell and coworkers over two decades (40–44). We will not discuss these details here. Suffice to say that, in anoxic Hadean oceans, alkaline vents should have formed labyrinths of micropores with thin, catalytic walls containing Fe(Ni)S minerals, through which hydrothermal fluids and ocean waters percolated (40–44). Like modern vents, the hydrothermal fluids should have been warm (70–90°C), rich in H<sub>2</sub> (15–200 mM) and strongly alkaline (about pH 11), whereas in the Hadean, the ocean waters were probably mildly acidic (pH 6) and saturated in CO<sub>2</sub> (40–44). Within vents, laminar flow of hydrothermal fluids and ocean waters through interconnected micropores should have generated proton gradients across the thin semi-conducting Fe(Ni)S walls, with CO<sub>2</sub> in acidic ocean waters in pores off from the main hydrothermal flow, and H<sub>2</sub> in alkaline hydrothermal fluids in more actively venting regions. In short: these vents should have provided everything needed to drive the acetyl CoA pathway: H<sub>2</sub>, CO<sub>2</sub> and natural proton gradients (up to 5 pH units) across thin, semi-conducting barriers containing Fe(Ni)S minerals, with structures similar to the FeS clusters in ferredoxin or Ech. There would have been no need to actively pump ions via electron bifurcation if hydrothermal flow provided equivalent proton gradients across natural Fe(Ni)S barriers (34,45,46). No need to actively generate proton gradients if the vents provided them for free.

Both methanogens and acetogens need electrochemical ion gradients to grow. Acetogens use them to generate ATP via the ATP synthase, and then use ATP and NADH to drive CO<sub>2</sub> fixation (37,39). As the ATP synthase is a sophisticated protein, a rotating nanomotor, it is unlikely to have been primordial (47). In contrast, methanogens stand out: they use the proton-motive membrane-bound NiFe hydrogenase Ech to

drive the reduction of ferredoxin directly (38). This is then used to reduce CO<sub>2</sub>, to form a methyl group bound to a cofactor, as well as CO, which then react together to form acetyl CoA on the Ni-S-Fe<sub>4</sub>S<sub>4</sub> clusters of CODH/ACS (29). The entire path of CO<sub>2</sub> fixation in methanogens is driven by the proton gradient [likely converted from a Na<sup>+</sup> gradient, generated by Mtr, via an obligate Na<sup>+</sup>/H<sup>+</sup> antiporter (48)] and does not require ATP (37,38). While the proteins involved today are moderately complex, all the critical electron transfers are achieved by the Fe(Ni)S cofactors, with structures similar to Fe(Ni)S minerals found in hydrothermal systems (27). In principle, the fact that alkaline vents contain H<sub>2</sub>, CO<sub>2</sub> and natural proton gradients across thin semi-conducting Fe(Ni)S barriers means that they could drive prebiotic CO<sub>2</sub> fixation via a mechanism analogous to methanogens (34). If so, that was the ancestral pathway of carbon fixation, and the detailed differences in electron bifurcation and methyl synthesis between methanogens and acetogens could be ascribed to the independent evolution of active pumping in the two groups, as proposed in detail elsewhere (34,45,46).

## How Proton Gradients Could Drive CO<sub>2</sub> Fixation

Why does the reduction of ferredoxin via Ech depend on the proton-motive force? The answer is as yet unknown, but cannot relate to reverse electron flow [as originally proposed (49)] as these methanogens do not possess an electron-transport chain (37,38). A more pleasing possibility is that pH modulates reduction potential at the active site of the enzyme. The flux of protons through Ech from the relatively acidic exterior could lower the pH at the active site of the enzyme, which should facilitate reductions that depend on protons, including CO<sub>2</sub> as well as some ferredoxins (50). As Wächtershäuser pointed out in his proposal for pyrites pulling, H<sub>2</sub> is not sufficiently electropositive to reduce CO<sub>2</sub> to CO, formate or formaldehyde (4,5), even though the later steps to methanol and methane are strongly exergonic (6). This is the reason that electron bifurcation works: the endergonic initial steps are coupled to the exergonic final steps, so that the final steps pull through the first steps (37). But that is not the mechanism of Ech (37). Something else is going on.

We propose that pH modulates the reduction potential of H<sub>2</sub> and CO<sub>2</sub> (34,51). When protons are involved in a reduction (for example balancing charges), the Nernst equation shows that the reduction potential falls by approximately –59 mV per pH unit (52). At pH 0, the reduction potential of H<sub>2</sub> is 0 (as defined by the hydrogen electrode). At pH 7, it is –414 mV; and at pH 11, it falls to –650 mV, strongly reducing. The reason is simple: if H<sub>2</sub> gives up its electrons under alkaline conditions, the remaining H<sup>+</sup> ions will react swiftly with OH<sup>–</sup> to form water, thermodynamically a highly favoured reaction. Conversely, CO<sub>2</sub> is more easily reduced under acidic conditions. At pH 7, the mid-point reduction potential of the CO<sub>2</sub>/CH<sub>2</sub>O couple is about –580 mV (34,51), but at pH 6 it rises to

–520 mV. Again the reason is simple: CO<sub>2</sub> will pick up the four electrons (to form CH<sub>2</sub>O) more easily if protons are readily available to balance the charges. Plainly they are more readily available in acid conditions. Much the same applies to some FeS minerals such as mackinawite, which can be protonated under mild acidic conditions (below pH 7.5) but deprotonate under alkaline conditions (53). At least some FeS clusters, in certain ferredoxins, seem to follow similar behaviour (50). If so, ferredoxin is easier to reduce under mildly acidic conditions, and in the context of the active site of Ech should be most readily reduced in the vicinity of proton channels that lower the pH locally. Conversely, in the more alkaline interior of the cell, ferredoxin should deprotonate and become more reducing—more able to reduce CO<sub>2</sub> to CO and beyond.

These principles could have significant connotations for electrochemistry at the origin of life. While the discussion above relates to the active site of Ech, the same principles should hold in an abiotic context, given a structure that is capable of separating two phases of different pH. This is precisely what alkaline vents do provide. While H<sub>2</sub> cannot reduce CO<sub>2</sub> to CH<sub>2</sub>O at pH 7 (because the reduction potentials of the H<sub>2</sub>/H<sup>+</sup> and CO<sub>2</sub>/CH<sub>2</sub>O couples are –414 mV and –580 mV, respectively), in alkaline vents H<sub>2</sub> is dissolved in alkaline solution at pH 11 or 11.5, giving it a reduction potential as low as –680 mV, whereas CO<sub>2</sub> is dissolved in ocean waters at a pH of around 5.5 to 6, giving it a reduction potential of about –500 mV. So long as the two pH phases are separate [which is aided by laminar flow in vents (54)], and as long as they are separated by a semi-conducting barrier capable of transferring electrons from one pH phase to the other, then H<sub>2</sub> in the alkaline phase should reduce CO<sub>2</sub> in the acidic phase via transfer of electrons across the semi-conducting barrier. Not only are Fe(Ni)S minerals semi-conducting (55) but they can also be protonated under mildly acidic conditions (53), making the acid-facing surfaces more electronegative, hence drawing electrons across the barrier. While such a process would necessarily generate an electrical charge on the barrier (56), this should be dissipated by the physical mixing of hydrothermal fluids and ocean waters elsewhere in the vent. In sum: natural pH gradients across thin, semi-conducting catalytic Fe(Ni)S barriers should facilitate the reduction of CO<sub>2</sub> by H<sub>2</sub> to form CO and more highly reduced organics such as –CH<sub>3</sub> groups, as depicted in Figure 1, through a mechanism analogous to Ech in methanogens.

## Analogous Reductions In The Incomplete Reverse Krebs Cycle

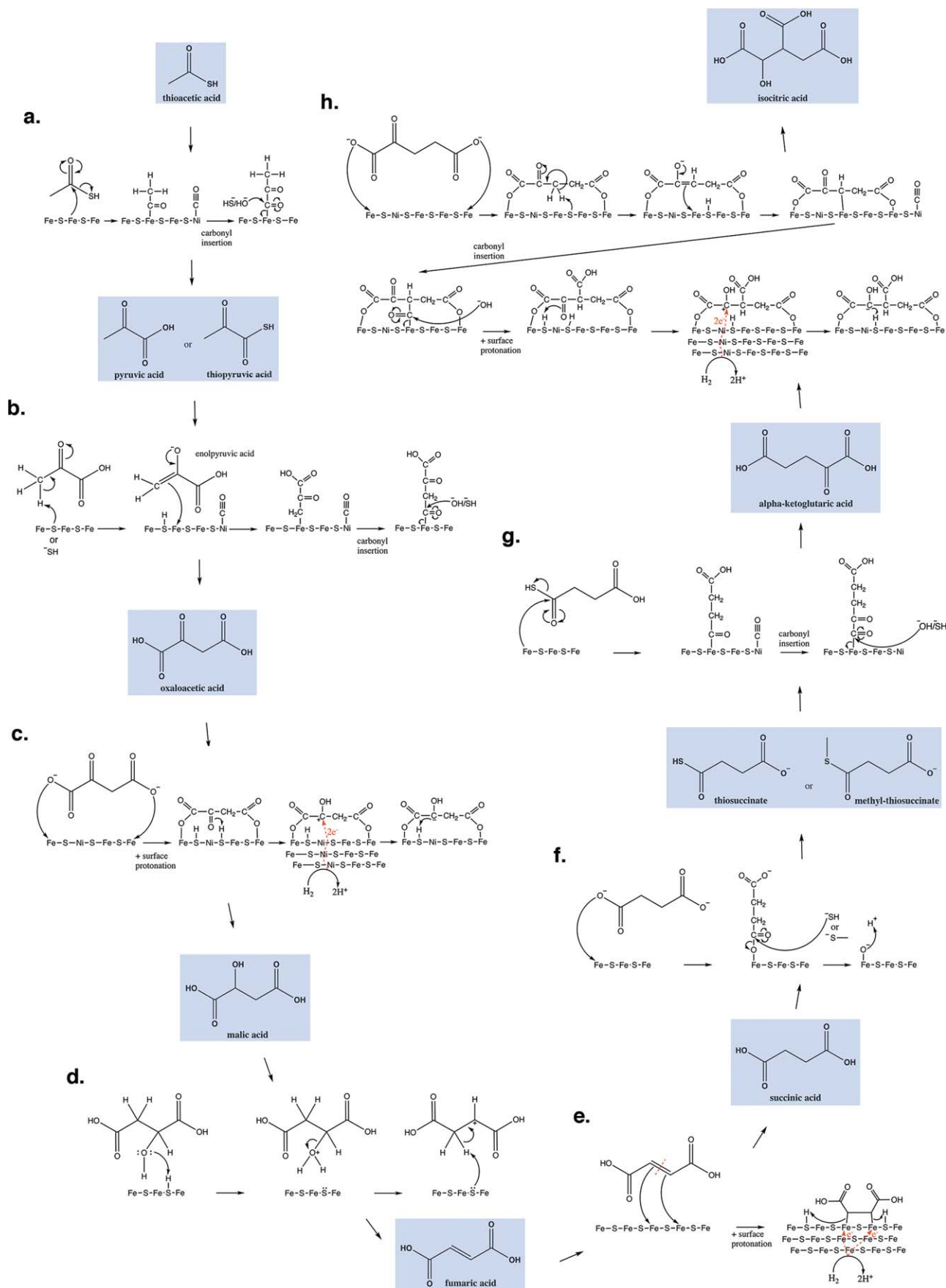
Reducing CO<sub>2</sub> to reactive thioesters such as acetyl CoA, or abiotic equivalents such as thioacetate or methyl thioester is an important first step, but is only the first step of metabolism. Amino acids and nucleotides are produced from carboxylic acids with 3-6 carbons—Krebs cycle intermediates—and producing these requires a series of further carbonylation and hydrogenation reactions. Considering this in terms of prebiotic

chemistry, Martin and Russell have previously suggested that the acetyl CoA pathway could feed into the reverse incomplete Krebs cycle, as in methanogens (42).

The incomplete reverse Krebs cycle has two advantages over the complete cycle. First, given high H<sub>2</sub> concentrations and natural proton gradients transecting semi-conducting Fe(Ni)S barriers, the short, linear acetyl CoA pathway should provide a continuous supply of reactive thioesters—far more than would be attained through a complete turn of the cycle, with its calamitous decline in yield at each step. Second, we might expect that the high concentration of thioesters could drive at least the next couple of steps, to pyruvate or oxaloacetate, both of which are important precursors for amino acid biosynthesis. These in turn are likely to chelate Fe(Ni)S minerals, enhancing their catalytic properties [by mimicking the active site of the enzyme and increasing the catalytic surface area (57–59)], driving faster CO<sub>2</sub> reduction—a valuable positive feedback (60). As catalytic properties improve, the yield of individual steps should increase, ultimately extending the pathway—still linear—through to C6 tri-carboxylic acids, such as isocitrate.

While this mechanism is pleasing in itself, we note that the carbonylations and hydrogenations of the reverse incomplete Krebs cycle are closely analogous to those in the acetyl CoA pathway itself. The idea that there are two branches of the acetyl CoA pathway, one which generates CO and the other a methyl group, is chemically misleading. As shown in Figure 1, the formation of thioesters is best seen as the carbonylation of a methyl group, which is formed through the partial reduction of CO<sub>2</sub> on an Fe(Ni)S surface by Fischer–Tropsch and Koch-type reactions. In Figure 2, we depict the reverse incomplete Krebs cycle as a series of analogous carbonylations and hydrogenations on an Fe(Ni)S surface. For simplicity, we show CO bound to the surface, but we are not calling for a 1 Bar atmosphere of CO, or even for high concentrations in hydrothermal vents—we merely assume the reduction of CO<sub>2</sub> to CO on the Fe(Ni)S surface, as shown in Figure 1. From thioacetate, in Figure 2, we depict the carboxylic acids of the reverse Krebs cycle as being successively carbonylated by CO, and then hydrogenated by electrons from H<sub>2</sub>, which are transferred across a thin, semi-conducting Fe(Ni)S barrier as described above.

We stress that each carbonylation reaction should be facilitated by the pH gradient across a barrier, as discussed above, to form CO from CO<sub>2</sub>; and each hydrogenation reaction should likewise be aided by the pH difference, with protons deriving either from the acidic ocean waters or from adjoining –S– groups in the Fe(Ni)S barrier, which are protonated below pH 7.5. This is admittedly hypothetical chemistry, and some steps might not be facile, but it is not of the ‘if pigs could fly’ type (20), as there is no requirement for a complete cycle with declining yield at each step. On the contrary, each step is driven independently by the high concentration of H<sub>2</sub> and proton gradients across semi-conducting barriers, both of which are sustained by hydrothermal flow. These predictions are experimentally testable and we are currently doing so in a microfluidic reactor. Others (62) have achieved equivalent reductions of CO<sub>2</sub> to pyruvate, using direct



electrochemical potential rather than  $H_2$  and pH modulation of reduction potential, as discussed here.

## Collapse to the Complete Reverse Krebs Cycle

The steps discussed above concern abiotic chemistry, driven by the far-from-equilibrium conditions and topological structure of pores in alkaline hydrothermal vents, based on a deep congruence with the carbon and energy metabolism of methanogens. We propose that the ancestral metabolism of the first cells was similar to methanogens (34), and the early divergence of bacteria and archaea arose from the independent origins of electron bifurcation and active pumping in the methanogens and acetogens (34,45,46). We conclude with some thoughts on the origins of the complete reverse Krebs cycle in bacteria—which obviously do not suffer from the abiotic problem of declining yield.

So what is the advantage of a complete reductive Krebs cycle, given that the modern cycle requires an investment of ATP at several steps? One factor might be thermodynamic. The rate of flux through a linear metabolic pathway depends on the concentration of end products relative to substrates; an accumulation of end-products inhibits flux, as does a low concentration of substrates. In the case of the incomplete reverse Krebs cycle, an accumulation of citrate would slow flux through the pathway. But if the citrate were split into oxaloacetate and acetyl CoA, the effect would be to remove the end product and replenish the substrates, providing a thermodynamic drive and balancing availability of precursors for the synthesis of amino acids, fatty acids, sugars and nucleotides needed for growth (3). As noted earlier, the reverse Krebs cycle is autocatalytic, providing two oxaloacetates for each turn of the cycle, which promotes stable growth. The input of acetyl CoA depends stoichiometrically on the spinning of the cycle itself, meaning there is no longer a need for input from the acetyl CoA pathway.

Other factors might have contributed to displacing the acetyl CoA pathway from most bacteria. C1 analytical chemistry is notoriously difficult, and we wonder whether cells also struggle to react two C1 molecules to form acetyl CoA. It could be simpler for enzymes to recognize substrates if the C1 carbons are

attached to longer chain carboxylic acids, rather than other C1 molecules. The acetyl CoA pathway is unique among the six known pathways of carbon fixation in dealing solely with C1 chemistry (25). Another advantage of the reverse Krebs cycle is that the acetyl CoA pathway combines carbon and energy metabolism through the same pathway, whereas the reverse Krebs cycle has to derive the necessary reducing power and ATP from alternative sources (42), notably phototrophy as originally reported by Evans et al. in 1966 (2).  $H_2$  and  $CO_2$  can then be used solely for carbon fixation, rather than for ATP synthesis too, enabling growth at lower partial pressures of  $H_2$  or in its absence (24,61). Given either phototrophy or relatively high concentrations of anaerobic electron acceptors for a respiratory chain, ATP synthesis ceases to be rate limiting. And with abundant ATP, there would no longer be any need for a proton-motive force to power the chemistry proposed in Figure 2, freeing membrane surface for other purposes, including faster ATP synthesis. That could explain why most steps of the reverse Krebs cycle no longer depend on the proton-motive force.

## Conclusions

Ever since its discovery in 1966, the reverse Krebs cycle has been proposed as an ancient pathway of  $CO_2$  fixation (2), potentially even prebiotic (4). However, the conception of primordial metabolic cycles was challenged by Orgel on the basis that a steep decline in yield at each step would preclude non-enzymatically catalysed flux through the full cycle (20). We see this as a valid criticism. The reverse Krebs cycle is also phylogenetically restricted almost entirely to bacteria, and so is unlikely to date back to the common ancestor of bacteria and archaea (LUCA) (22–25). The short, linear acetyl CoA pathway, which uses Fe(Ni)S proteins and electrochemical ion gradients across membranes to drive both carbon and energy metabolism from  $H_2$  and  $CO_2$ , solves many of these problems. This pathway is found in both methanogenic archaea and acetogenic bacteria, but deep differences in the mechanism of electron bifurcation and methyl synthesis obscure the ancestral pathway of  $CO_2$  fixation (33–35). We have proposed elsewhere that these differences arose with the origin of active pumping (45,46), and that ancestral carbon fixation in the first cells resembled that of methanogens (34), which use electrochemical

FIG 2

Primordial incomplete reverse Krebs cycle facilitated by pH gradients across Fe(Ni)S semipermeable barriers. (a) Carbonylation of a surface-bound acetyl group (from thioacetic acid), and elution from the mineral surface by a hydroxide or sulphhydryl ion yielding pyruvic acid or thiopyruvic acid, respectively. To simplify, the non-thiolated version of the products is shown for the remaining reactions. (b) Adsorption of pyruvic acid through its enolate form onto the mineral surface followed by carbonylation and elution to form oxaloacetic acid. (c) Reversible adsorption of oxaloacetate followed by hydrogenation of its keto group by two electrons (from  $H_2$  oxidation by  $Ni^{2+}$  in the alkaline phase) and  $H^+$  ions from surface mineral protonation yielding malic acid. Nickel atoms channel electrons from  $H_2$  catalysing a two-electron reduction. (d) Acid–base catalysed dehydration of malic acid by the mineral surface yielding fumaric acid. (e) Adsorption of fumaric acid through  $\pi$  bond cleavage followed by hydrogenation of its secondary carbons (with electrons from  $H_2$  oxidation in the alkaline phase and  $H^+$  ions from surface mineral protonation) yielding free succinic acid. Iron atoms channel electrons from  $H_2$  catalysing two one-electron reductions (f) Thiolation or methyl-thiolation of succinic acid yielding thiosuccinate or methyl-thiosuccinate. The collateral hydroxylation of the catalyst surface can be readily removed by dehydration at low pH. (g) Thiosuccinate carbonylation yielding  $\alpha$ -ketoglutaric acid. (h) Isocitric acid synthesis by carbonylation of its 3' carbon and Ni-mediated keto group hydrogenation by a mechanism equivalent to reaction (c).

ion gradients to drive ferredoxin reduction via the membrane-bound NiFe hydrogenase Ech.

These factors map onto the topology of pores in submarine alkaline hydrothermal vents, in which natural proton gradients across Fe(Ni)S barriers could drive the reduction of CO<sub>2</sub> by H<sub>2</sub>. We suggest that the initial reduction of CO<sub>2</sub> to a methyl group proceeded via a Fischer Tropsch-type mechanism on the acid side of an Fe(Ni)S surface, followed by carbonylation via a Koch-type mechanism. These endergonic reactions ought to be facilitated by pH differences between phases, which modulate the reduction potential of H<sub>2</sub>, CO<sub>2</sub> and Fe(Ni)S minerals such as mackinawite (34,51,53). Similar hydrogenations and carbonylations, facilitated by natural proton gradients across Fe(Ni)S barriers, could generate longer chain carboxylic acids (up to 6 carbons) via a prebiotic equivalent to the reverse, incomplete Krebs cycle in methanogens. Finally, after the evolution of cells with genes and proteins, closure of the complete reverse Krebs cycle should displace the acetyl CoA pathway from bacteria and archaea, except under anoxic conditions and at high partial pressures of H<sub>2</sub>. This framework helps explain the origins of autotrophic CO<sub>2</sub> fixation in alkaline vents, the early divergence of bacteria and archaea, and the later evolution of the reductive Krebs cycle in bacteria.

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