Native metals, electron bifurcation, and CO_2 reduction in early biochemical evolution

Filipa L Sousa[1], Martina Preiner[2] and William F Martin[2,3]

Molecular hydrogen is an ancient source of energy and electrons. Anaerobic autotrophs that harness the H_2/CO_2 redox couple harbour ancient biochemical traits that trace back to the universal common ancestor. Aspects of their physiology, including the abundance of transition metals, radical reaction mechanisms, and their main exergonic bioenergetic reactions, forge links between ancient microbes and geochemical reactions at hydrothermal vents. The midpoint potential of H_2 however requires anaerobes that reduce CO_2 with H_2 to use flavin based electron bifurcation — a mechanism to conserve energy as low potential reduced ferredoxins via soluble proteins — for CO_2 fixation. This presents a paradox. At the onset of biochemical evolution, before there were proteins, how was CO_2 reduced using H_2? FeS minerals alone are probably not the solution, because biological CO_2 reduction is a two electron reaction. Physiology can provide clues. Some acetogens and some methanogens can grow using native iron (Fe^0) instead of H_2 as the electron donor. In the laboratory, Fe^0 efficiently reduces CO_2 to acetate and methanol. Hydrothermal vents harbour awaruite, Ni_3Fe, a natural compound of native metals. Native metals might have been the precursors of electron bifurcation in biochemical evolution.

Addresses
[1] Division of Archaea Biology and Ecogenomics, Department of Ecogenomics and Systems Biology, University of Vienna, Althanstrasse 14 UZA I, 1090 Vienna, Austria
[2] Institute of Molecular Evolution, Heinrich-Heine-University, Universitätsstr. 1, 40225 Düsseldorf, Germany
[3] Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, 2780-157 Oeiras, Portugal

Corresponding author: Sousa, Filipa L (filipa.sousa@univie.ac.at)

Introduction

The only thing we know for sure about life's origin some 3.95 billion years ago [1] is that energy was required. Without energy release, no chemical reactions can take place that could ultimately lead to complex chemicals, metabolism or primitive ecosystems [2•]. Two sources of energy at origins are mainly discussed: ultraviolet (UV) light emitted from the sun [3,4] and chemical energy at hydrothermal vents [3,4]. Although UV light can be conveniently integrated into elegant laboratory syntheses of organic molecules at low temperatures and pressures [5,6], it does not connect to the microbial world (life) because no known form of microbial physiology is powered by UV light. From the biological standpoint, chemical energy at hydrothermal vents, in particular the H_2/CO_2 redox couple, is interesting as a source of energy at origins. Why?

First, many forms of microbes use H_2 as a source of chemical energy for ATP synthesis in conjunction with a suitable electron acceptor such as CO_2 [7,8], and H_2-dependent autotrophs provided the initial primary production that supported the first heterotrophic metabolisms [9]. Second, the Earth's crust has been generating large amounts of H_2 since there was liquid water, through a process called serpentinization [10]. In addition, biologists have long held that anaerobic autotrophs that reduce CO_2 using electrons from H_2 harbour the most ancient forms of metabolism [11–13]. H_2 dependent anaerobic autotrophs are furthermore rich in transition metal catalysts such as Fe and Ni [13], traits long regarded as ancient, and hydrogenases that extract the electron pair from H_2 to provide reduction equivalents and energy that drive metabolism forward [14].

Among the kinds of carbon and energy metabolism known among modern microbes, the acetyl-CoA pathway (or Wood–Ljungdahl pathway) as it is used in acetogens (bacteria) and methanogens (archaea) appears to be the most ancient [12]. It is the only exergonic pathway of biological CO_2 fixation known [12], all others require energy input in the form of ATP. The intermediate product of the pathway is a thioester, reactive compounds that have long been thought to play an important role in early chemical evolution [15]. In bacteria, the acetyl-CoA pathway generates acetyl phosphate from H_2, CO_2 and phosphate (Figure 1). Acetyl phosphate is an excellent source of phosphorylation potential with a free energy of hydrolysis of −43 kJ/mol, 30% better than ATP. Even to

Figure 1
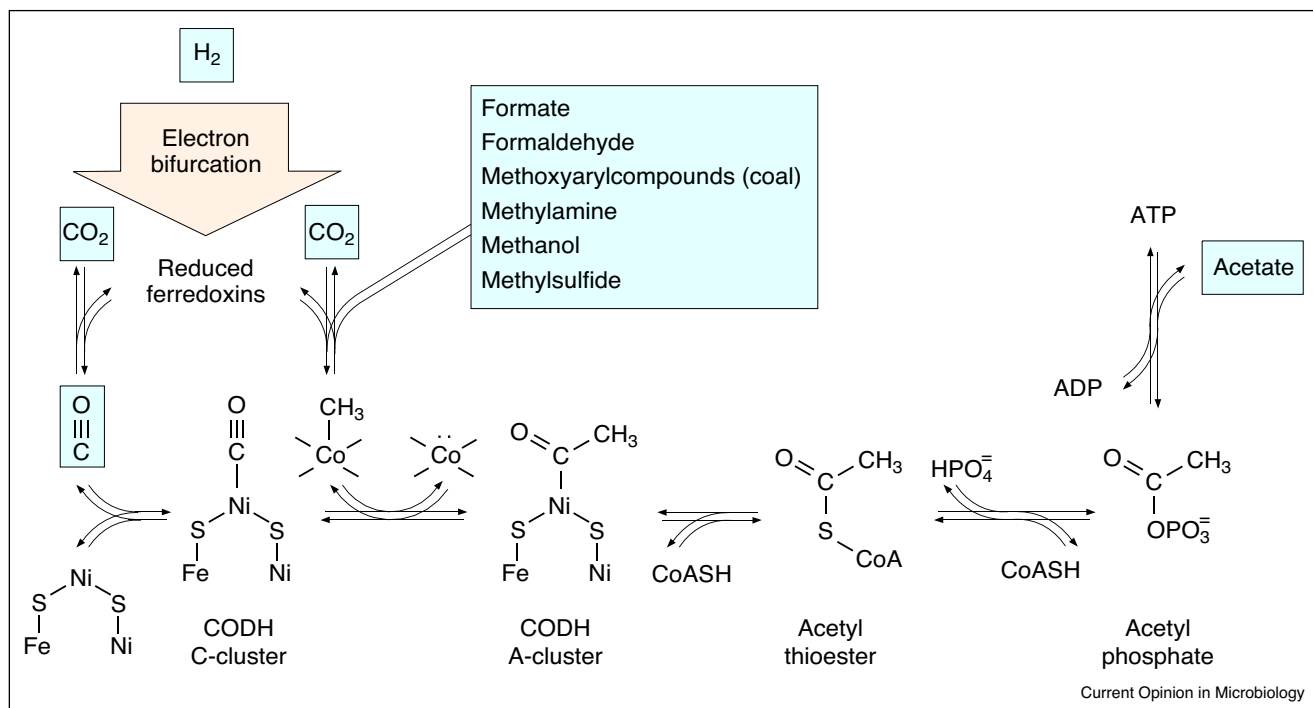
Scheme of energy conservation from ferredoxin to ATP in the acetyl CoA pathway. Redrawn after [12] incorporating intermediates in CO generation [13] and of substrate level phosphorylation [11]. Methanogenic growth on methoxy groups from coal was recently reported by [38•]. The beige arrow indicates the requirement for electron bifurcation in the synthesis of low potential reduced ferredoxins with electrons from H_2 [7]. Insights into the mechanisms of electron bifurcation were recently revealed by the structures of two bifurcating enzymes [25•,26•]. Substrates and endproducts of the reversible reaction sequence are boxed in blue. The synthesis of the methyl group from H_2 and CO_2 entails energy investment, such that net synthesis of acyl phosphate or ATP from H_2 requires chemiosmotic coupling [6–8].

the level of the energy rich thioester the reaction is exergonic:

$$2CO_2 + 4H_2 + CoASH \rightarrow CH_3COSCoA + 3H_2O$$
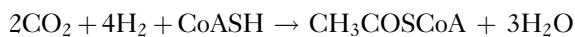
$$\Delta G_o' = -59 \, \text{kJ/mol} \, [12].$$

Microbial genomes also point to the antiquity of anaerobic autotroph physiology. A recent study identified 355 ancient gene families that, based on their phylogenies, provided insights into the physiology and habitat of the last universal common ancestor, LUCA [16]. LUCA lived in a hot environment rich in gasses (H_2, CO_2, N_2, CO) and metals, a habitat very similar to hydrothermal vents, which existed on the early Earth [10]. It used the acetyl-CoA pathway [16], its metabolism was rich in FeS and thioester dependent reactions, which are enriched in the ancient ATP-independent core of metabolism [17••]. It harboured a diversified small molecule chemistry [18], many radical-based reactions that are dependent upon S-adenosyl methionine (SAM), which can form spontaneously *in vitro* [19•], and it harboured features found in microbes that today still inhabit ancient geochemical niches [20,21].

The problem with H_2 and FeS

Despite its chemical simplicity, its abundance in ancient environments, and the clear tendency of H_2-dependent autotrophs to branch deeply in phylogenetic trees [16], H_2 has a rather severe Achilles' heel as a source of electrons for CO_2 reduction at origins. Its midpoint potential is unfavourable for CO_2 reduction beyond the near-equilibrium reaction with formate [22]. That is why microbes that reduce CO_2 with H_2 employ flavin based electron bifurcation [23], a biochemical mechanism that generates reduced ferredoxins (Fd^-) with a midpoint potential on the order of –500 mV from H_2 with a more positive midpoint potential of only –414 mV [7]. Electrons from H_2 have to flow energetically uphill to low potential Fd. That might appear to violate the second law of thermodynamics, but electron bifurcation obeys the law in that one electron from H_2 is transferred energetically downhill to a high potential acceptor like NAD^+ or heterodisulfide CoB–S–S–CoM [24], while the other is transferred uphill to Fd so that the overall energetics of the reaction are favourable [8].

Why is that problematic? The problem with H_2 in an early evolution context is that flavin based electron bifurcation

is an elaborate physiological process that requires sophisticated proteins [25•,26•] working in concert with other proteins [7] as an energy metabolic pathway to reduce Fd (for reducing CO_2). This presents a familiar chicken-and-egg type paradox, namely how was CO_2 reduced with H_2 before there were proteins to catalyze electron bifurcation? One might counter that if early CO_2 fixation took place at hydrothermal vents, then electron bifurcation was not needed, because there was plenty of natural FeS around that could do the job of Fd^- when it comes to fixing CO_2.

But FeS minerals do not really solve the problem, because FeS has its own, different issues. FeS clusters in proteins are one electron donors, with iron undergoing Fe^{2+} to Fe^{3+} valence changes. The steps of biological CO_2 reduction in autotrophs are always two electron reactions [12]. In biology, the electrons from Fd^- are donated to C in CO_2 via metals that readily undergo two electron reactions, such as Ni, Mo, or W atoms coordinated in proteins or cofactors (Figure 2), or electron pairs are donated via hydride transfer from organic cofactors like NAD(P)H [12].

Huber and Wächtershäuser [27] obtained excellent yields (40 mol%) of the thioester methyl thioacetate from CH_3SH and CO using FeS, Ni^{2+} and Fe^{2+} salts, emulating the central anabolic reaction of the acetyl CoA pathway, but no one has reported genuine success involving FeS or other Fe^{2+} species as a reductant for CO_2 in an early evolution context, electrochemical experiments where external voltage is applied aside [28]. Could it be that in the beginning, CO_2 was not reduced directly by H_2 at all? What does nature say?

Geochemical CO_2 reduction

Modern hydrothermal systems provide a check for CO_2 reduction in that CH_4 (ca. 1 mM) [29] and other small organic compounds including formate are present in hydrothermal effluent [30–32]. Those small organic compounds are not synthesized where vent effluent discharges into the ocean on the sea floor, rather, the organics are apparently made deep in the crust, as organics in terrestrial vents attest [33]. CO_2 reduction in the modern crust is still not well understood in its details, although it is thought to stem from the same geochemical process that generates H_2 in hydrothermal effluent: serpentinization [30–32].

During serpentinization, H_2 synthesis stems from the reduction of water in hydrothermal systems via the oxidation of Fe^{2+} that is present in the Earth's crust in vast amounts as iron magnesium silicates [10]. Fresh iron silicates for serpentinization are continuously supplied anew at spreading zones such as the mid-Atlantic ridge, where crust emerges as magma that subsequently cools, ultimately being recycled back into the mantle at subduction zones [34]. The isotope signatures of CH_4 emergent from hydrothermal systems are distinct from that of marine CO_2 [29,32], the reasons for which are unclear. The rates of methane synthesis in laboratory scale serpentinization reactions so far are generally very slow [35•]. Thus, carbon is being reduced in serpentinizing systems, by a yet unidentified mechanism.

The process of serpentinization has been going on for the last 4.2 billion years, since there was liquid water on Earth [10]. Both its basic ingredients — water, Fe(II)-rich rocks,

Figure 2
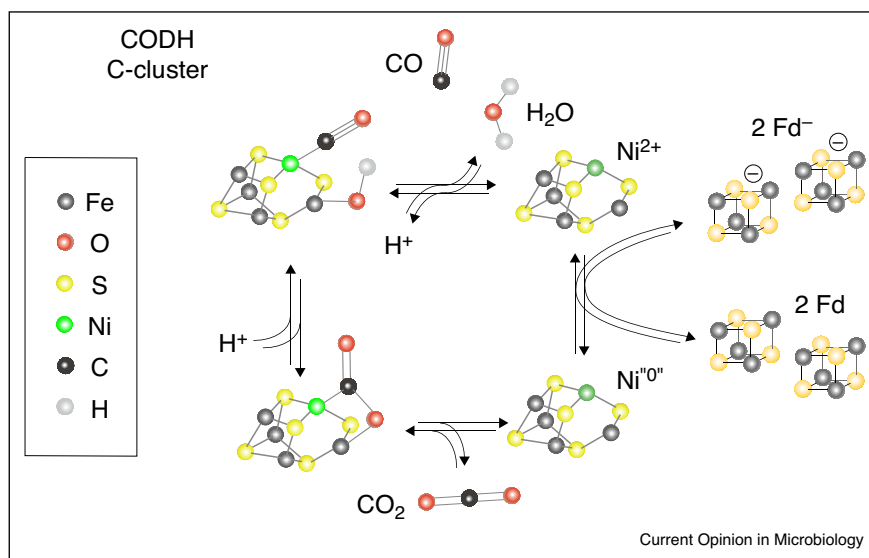
Reaction mechanism of CO_2 reduction in bifunctional CO dehydrogenase acetyl-CoA synthase (CODH/ACS) synthase in the acetyl-CoA pathway, proposed by Ragsdale [13], modified from [13]. C-cluster refers to the NiFeS cluster at the active site of the CODH enzyme [13].

There is broad agreement among geochemists and planetary researchers that the early Earth was molten at some point, by the moon forming impact roughly 4.4 billion years ago, at the latest [10,33]. On the molten Earth (>1500 K), carbon that had been brought to the freshly accreted planet was converted to CO_2, almost all of which was outgassed into the atmosphere, a small fraction being retained in magma oceans [10,33]. By about 4.4 Ga the magma oceans had cooled [33], by about 4.2 Ga there was liquid water on Earth [10], some outgassed from accretion and some delivered later by comets, and by around 4 Ga, perhaps as late as 3.7 Ga, the late heavy bombardment had come to an end [33]. By 3.95 Ga a carbon isotopic signature compatible with that produced by the acetyl-CoA pathway appeared [1].

As it relates to the source of energy for CO_2 reduction, the relevant sequence of events is this: magma oceans in the molten phase oxidized Earth's early carbon to atmospheric CO_2 and small amounts of mantle CO_2. Because it cooled from magma, the primordial crust was depleted in water, consisted mostly of iron magnesium silicates with very low water content [10,55]. As the crust cooled, water condensed to surface oceans. Gravity pulled water into cracks in the solid crust, creating convective currents — the process of serpentinization set in. Serpentinization drew very CO_2-rich water into the crust where serpentinization took place, such that H_2 was synthesized in an otherwise H_2-free environment and at sites where CO_2 existed in hydrothermal downcurrent water and as bound CO_2 (carbonates) in a generally dry crust. The primordial interaction between H_2 and CO_2 thus probably took place deep in the crust, not at sites where hydrothermal vent effluent reached the ocean floor in contrast to earlier views [6]; H_2 and CO_2 first interacted in the presence of vast amounts of dry rock, and at temperatures likely exceeding 100 °C.

and heat for convection — and the chemical reaction are simple, as such the process appears to be occurring on the Saturn moon Enceladus as well [36•]. Serpentinization is a spontaneous process that releases chemical energy, a notable property that it shares with microbial energy metabolism. If serpentinization releases chemical energy, where does the energy that serpentinization releases come from? A look at early Earth history is instructive: the energy comes from the molten state of the early Earth (magma oxidizes carbon to CO_2) and subsequent rock–water interactions, which then generate H_2 in the presence of CO_2 (Box 1).

Metagenomics tells us that approximately half of today's biomass lives in the crust in rocky, H_2 rich environments [37] and that substantial components of the modern subsurface biomass lives from the H_2/CO_2 redox couple as acetogens and methanogens [38•], which fuel subsurface primary production. Today, microbes in the crust can also grow from organic carbon deposits such as coal [39••], but that was not an option 4 billion years ago. Another main difference between today's crust and the primordial crust is that about half the water on Earth (i.e., roughly the volume of the ocean) is bound in the modern crust (and mantle), brought there by submarine hydrothermal activity [34]. The flipside of that coin is that the primordial oceans were twice as deep as today's [34], meaning that land for warm little ponds was probably in short supply.

Whence electrons, if not H_2 or FeS?
Serpentinization alters the rocks that host hydrothermal activity. A very notable component of hydrothermally altered rocks is the mineral awaruite. Awaruite is an intermetallic compound with the formula Ni_3Fe (or $Ni_{2-3}Fe$): native transition metals with oxidation state zero. It is a normal constituent of serpentinizing hydrothermal systems [35•,40], formed there naturally during serpentinization under conditions where high H_2 activities of $\sim$200 mmol/kg [41] reduce the divalent metal ions.

Almost 20 years ago Horita and Berndt [42] reported that Ni_3Fe could catalyze the synthesis of methane in mmol/kg amounts from H_2 and CO_2 [42] in simulated hydrothermal conditions (200–400 °C, ca. 50 MPa) although it cannot be excluded that awaruite was the reductant rather than the catalyst, at least in part. They also reported that after 1–2 weeks at lower temperature (200 °C) carbon compounds of oxidation state intermediate between CO_2 and CH_4 were obtained in amounts similar to or exceeding those observed for CH_4. Guan et al. [43] showed that Fe^0 in the presence of salts will reduce CO_2 to CH_4, C_3H_8, CH_3OH and C_2H_5OH in the 10–70 μM range at room temperature. He et al. [44] reported reduction of CO_2 to formate and acetate in the 1–10 mM range using nanoparticular Fe^0 at 80–200 °C. Moreover, Muchowska et al. [45••] recently showed native iron to accelerate and promote reactions of the reverse citric acid cycle (Figure 3). The message is that native metals can efficiently reduce CO_2. That is not the case for either H_2 alone or for FeS minerals. The native metals are interesting.

Awaruite is today synthesized where H_2 is produced and where organic compounds are being made. Are native metals involved in geochemical CO_2 reduction, and were they involved in primordial CO_2 reduction? Considering the mechanism proposed by Steve Ragsdale [13] for acetyl-CoA synthesis in acetogens (Figure 2), the active Ni species for CO_2 reduction in the (4 billion year old) biochemical reaction is formally Ni^0. The electrons are delivered to the enzyme one at a time via FeS clusters in Fd^-, but are delivered to carbon as a pair. The Fe^{2+} species in FeS clusters perform one electron chemistry, but the two electron carbon reduction reaction is performed by the transiently native metal. This might be a clue about ancient life.

Microbes always have the last word
What do anaerobic microbes say about native metals? Basically they say 'yes, please', and they are the source of much corrosion to things made of steel, as recently surveyed by Enning and Garrelfs [46]. Early reports showed that methanogens grow from Fe^0 and CO_2 [47], but questions remained whether the growth was really from iron or just from H_2 generated by interaction of iron with water. Dinh et al. [48] then showed that methanogens grew rapidly on native iron as an electron

Figure 3
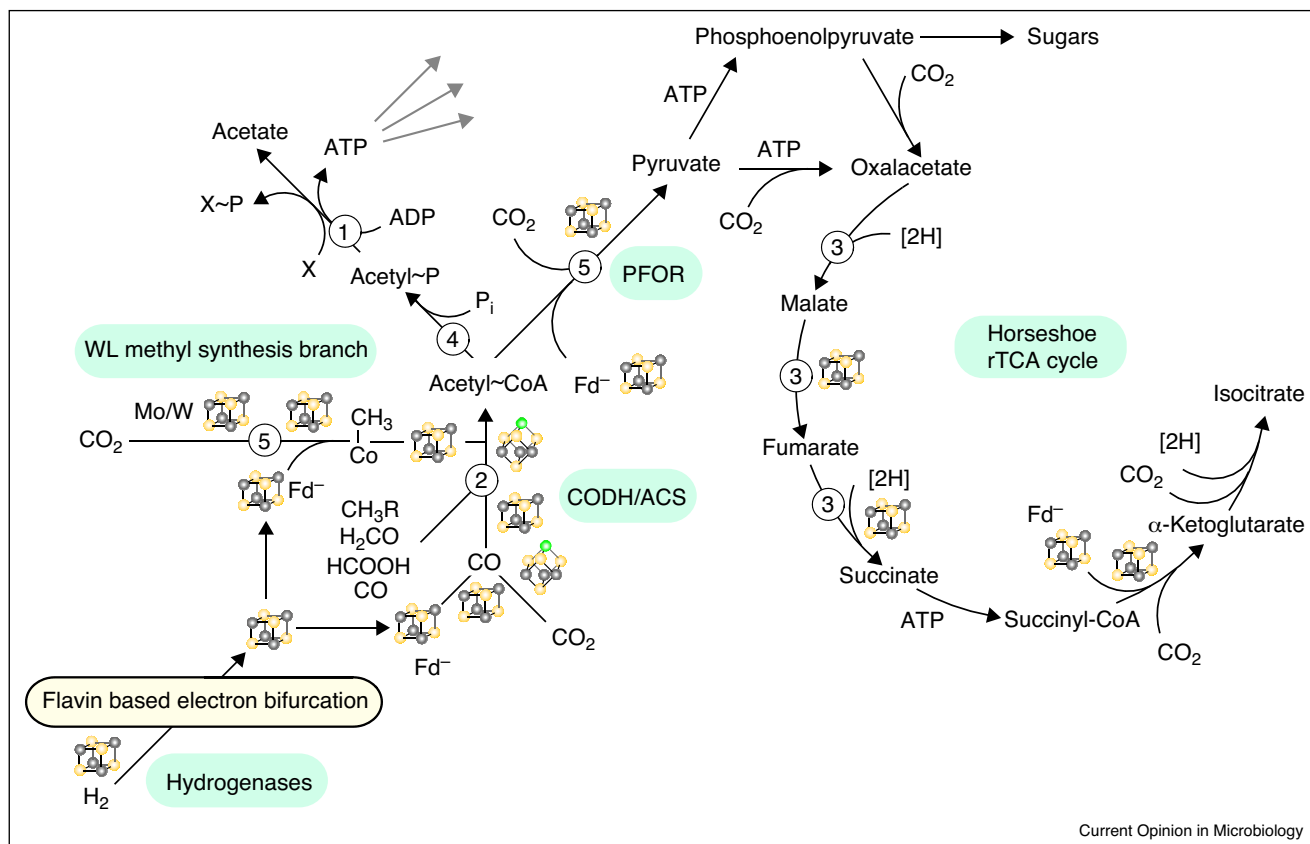
The acetyl-CoA pathway and the incomplete reverse citric acid cycle as the core carbon end energy metabolism in some modern microbes and the first microbes, modified from Ref. [6] to include the energetic impediment imposed by flavin based electron bifurcation in ferredoxin reduction with electrons from H_2 [7] and updated to include new information. Participation of FeS or FeNiS clusters is indicated using database information for the corresponding enzymes indicated in Fuchs [12]. Note the paucity of ATP-dependent steps and the acyl phosphate/ATP-generating steps, indicated with ATP next to the reaction (see also Ref. [17••]). Circled numbers at reactions indicate that the non-enzymatic laboratory reaction has been reported as follows. (1) Kitani *et al.* reported that ATP is readily generated from acetyl phosphate and ADP using only Fe^{3+} [57] or Fe^{2+} [58] as catalysts. (2) Wächtershäuser and Huber reported the divalent metal ion-catalyzed synthesis of acetyl thioesters from CH_3SH and CO [26•]. (3) Muchowska et al. showed these steps to proceed spontaneously *in vitro* using native metal and metal ion catalysts [45••]. (4) Weber [56] reported the synthesis of pyrophosphate from an acetylthioester and P_i, whereby the presence of acetyl phosphate was inferred but not directly shown [6]. (5) Varma *et al.* [54••] recently demonstrated synthesis of metal bound methyl groups and synthesis of pyruvate from CO_2 with native metals as the source of electrons. The free energy of hydrolysis for acetyl phosphate (–43 kJ/mol) is greater than that for ATP (–31 kJ/mol) [6]. In the context of early biochemical evolution, reactions that are today coupled to ATP hydrolysis would be thermodynamically even more favourable if coupled to acetyl phosphate (or other acyl phosphate) hydrolysis, an argument in favour of acetyl phosphate as a primitive energy currency [6,59]. The involvement of reductants other than Fd^- in the enzymatic reaction is indicated with [2H]. *Abbreviations*: CODH/ACS, carbon monoxide dehydrogenase/acetyl-CoA synthase; WL, Wood–Ljungdahl; PFOR, pyruvate ferredoxin oxidoreductase.

source, suggesting that H_2 was not involved as an intermediate. More recently, Tan *et al.* [49] showed that *Methanosarcina barkeri* will grow on Fe^0 as an electron source, but only when deprived of standard electron donors such as CH_3OH or H_2.

The molecular mechanisms of microbial electron extraction from Fe^0 are so far elusive. Lohner *et al.* [50] reported Fe^0 oxidation by methanogens in the presence of externally applied voltage. The results suggested that methanogens can access electrons from Fe^0 via routes that do not involve H_2. More recent results by Deutzmann and colleagues using applied potentials suggest that

methanogens might acquire electrons from Fe^0 via extracellular enzymes that oxidize the native metal to standard electron sources such as H_2 or formate [51,52]. There are many reports about anaerobic growth on iron, many involving methanogens or sulfate reducers [46]. But acetogens?

Kato *et al.* [53•] recently isolated acetogens from the genus *Sporomusa* (firmicutes) that grow on native iron without externally applied potentials. Most acetogens that Kato *et al.* [53•] tested do not grow on iron as the electron source, indicating the presence of genetically specified mechanisms to access electrons from Fe^0, in line

with the conclusions of Dinh *et al.* [48] for sulfate reducers and methanogens. For sulfate reducers, the terminal acceptor of electrons from Fe^0 can be a sulfur compound or CO_2, for acetogens and methanogens, the terminal acceptor is CO_2. The proteins, cofactors and mechanisms involved in Fe^0 oxidation are still unknown [46,52,53•]. It is possible that non-enzymatic reactions of Fe^0 with CO_2 such as those generating formate, methanol, and acetate in the laboratory [43,44] play a role in microbial growth on iron. Very recent work reports the non-enzymatic synthesis of pyruvate from aqueous CO_2 and Fe^0 under mild hydrothermal conditions [54••].

Conclusions

As usual, nature leaves us with observations and questions. Is the oxidation of native iron (and other metals) an ancient trait, preserved from the very earliest phases of biological CO_2 reduction and is it prevalent in hydrothermal vents, where Ni_3Fe is still made today? The two electron iron oxidation reaction $Fe^0 \rightarrow Fe^{2+} + 2e^-$ has a midpoint potential of $E_o' = -470$ mV [53•], more negative than that for hydrogen $H_2 \rightarrow 2H^+ + 2e^-$, with $E_o' = -410$ mV. Might anaerobic autotrophs that oxidize Fe^0 short circuit flavin based electron bifurcation to generate their low potential reduced ferredoxins? If so, they still would have to direct two electron (Fe^0) to one electron (FeS clusters in Fd^-) reactions. The physiological reactions by which microbes access Fe^0 and other native metals as electron sources might uncover hints about early life, possibly even probing a phase of physiological evolution before there was genetically encoded electron bifurcation.

Acknowledgements

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