

The Origin of Life in Alkaline Hydrothermal Vents

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Abstract

Over the last 70 years, prebiotic chemists have been very successful in synthesizing the molecules of life, from amino acids to nucleotides. Yet there is strikingly little resemblance between much of this chemistry and the metabolic pathways of cells, in terms of substrates, catalysts, and synthetic pathways. In contrast, alkaline hydrothermal vents offer conditions similar to those harnessed by modern autotrophs, but there has been limited experimental evidence that such conditions could drive prebiotic chemistry. In the Hadean, in the absence of oxygen, alkaline vents are proposed to have acted as electrochemical flow reactors, in which alkaline fluids saturated in H₂ mixed with relatively acidic ocean waters rich in CO₂, through a labyrinth of interconnected micropores with thin inorganic walls containing catalytic Fe(Ni)S minerals. The difference in pH across these thin barriers produced natural proton gradients with equivalent magnitude and polarity to the proton-motive force required for carbon fixation in extant bacteria and archaea. How such gradients could have powered carbon reduction or energy flux before the advent of organic protocells with genes and proteins is unknown. Work over the last decade suggests several possible hypotheses that are currently being tested in laboratory experiments, field observations, and phylogenetic reconstructions of ancestral metabolism. We analyze the perplexing differences in carbon and energy metabolism in methanogenic archaea and acetogenic bacteria to propose a possible ancestral mechanism of CO₂ reduction in alkaline hydrothermal vents. Based on this mechanism, we show that the evolution of active ion pumping could have driven the deep divergence of bacteria and archaea. Key Words: Origin of life—Alkaline hydrothermal vent—Chemiosmotic coupling—Proton gradients—Methanogens—Acetogens—CO₂ reduction. *Astrobiology* 16, 181–197.

1. The Carbon Paradox

The most likely substance to be assimilated is CO₂, not only because CO₂ without doubt has been the dominating carbon compound of primordial atmospheres, but because the assimilation of CO₂ still is the common denominator of all living matter on the earth. An important result of modern biological chemistry is the conception that the accretion of organic matter is based upon the assimilation of CO₂, by means of hydrogen donors, and this dependence on assimilation of CO₂ may be a common inheritance of all living matter.

—Victor Goldschmidt, 1952

ONE YEAR BEFORE publication of the famous Miller-Urey experiment (Miller, 1953), the distinguished father of geochemistry Victor Goldschmidt laid out a very different view of the conditions required for the origin of life (Goldschmidt, 1952). In fact Goldschmidt had died in 1947; his short essay on geochemical aspects of the origin of complex organic molecules had been written in 1945 and was eventually published by his friend Norman Pirie in *New Biology*.

Goldschmidt (1952) had short shrift with the ideas of A.I. Oparin (1938), dismissing his proposed atmosphere of carbon monoxide and gaseous hydrocarbons as “a very unusual and not very promising composition.” He acknowledged that methane was a feasible product of inorganic processes, but he was clear that “the main carbon compound of the atmosphere was without doubt carbon dioxide,” which “throughout geological history has been exhaled from the interior of the earth.”

Goldschmidt went on to suggest that the free reticular planes of minerals could have catalyzed the reduction of carbon dioxide to organic molecules. These clearly reasoned reflections were lost in the furor that greeted the Miller-Urey experiment, which produced amino acids by means of electrical discharges in a reducing atmosphere composed of hydrogen, methane, and ammonia (Miller, 1953). If science is about experiment, then Miller’s spectacular experimental findings, which made it onto the cover of *Time* magazine in 1953, were right to trump the informed yet untested opinions of geochemists. Nonetheless, later evidence confirmed Goldschmidt’s view that the early atmosphere was

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not strongly reducing but was probably weakly oxidizing (Trail *et al.*, 2011). Miller successfully repeated many of his own experiments in an atmosphere of CO (Miyakawa *et al.*, 2002; Cleaves *et al.*, 2008); but this gas, as observed by Goldschmidt (1952), was even less likely than methane to accumulate in the atmosphere.

So there is a paradox. CO₂ is the molecule assimilated by practically all autotrophic cells and is the dominant volatile form of carbon, yet the great body of prebiotic chemistry has disregarded CO₂ in favor of more reactive but less probable precursors, from CO to cyanide (Oro' *et al.*, 1959; Hulett *et al.*, 1971; Hennet *et al.*, 1992; Huber and Wächtershäuser, 1997; Saladino *et al.*, 2012). The successes have been impressive, including the synthesis of activated nucleotides (Powner *et al.*, 2009). Nonetheless, no known form of life uses cyanide, formamide, or cyanamide as substrates for carbon (or nitrogen) fixation (Martin *et al.*, 2014), and the concentrations used in these successful experiments were orders of magnitude greater than could be expected in any natural environment (McCollom, 2013a). Nor does any known form of life use UV radiation or lightning as a source of energy. While it is feasible that the earliest prebiotic chemistry was later overwritten by evolved metabolic pathways encoded by genes and catalyzed by enzymes, the more parsimonious interpretation—that from the very start life assimilated CO₂—has received relatively scant attention.

Parsimony is abetted by logic. de Duve (2005) pointed out that the “only scientifically plausible” explanation for the advent of biological catalysts, whether enzymes or ribozymes, is selection. The first biological catalysts were presumably selected because they enhanced processes that occurred spontaneously, driven by natural disequilibria and catalyzed by inorganic mineral catalysts. If so, then selection imposes a link between metabolism and geochemical flux—some geochemical environment must have driven the spontaneous reduction of CO₂. For selection to convert any geochemical flux into a genetically encoded metabolism, each intermediate step must be modified from the previous step: there must be mechanistic continuity. It follows that catalysts, whether abiotic or biological, must act on the same molecules in the same environment, and that the physical catalysts (*e.g.*, transition metals) should become incorporated in enzymes or ribozymes. Selection plainly cannot modify a geochemical process operating elsewhere. This means that the geochemical reduction of CO₂ by H₂ has to occur in the same environment as the later biochemical reduction—the substrates, catalysts, and reaction mechanisms should remain essentially unchanged.

This perspective casts doubt on some proposed mechanisms such as pyrites pulling, in which the reduction of CO₂ by H₂S is coupled to the oxidation of ferrous sulfide (FeS) to iron pyrites (FeS₂). No known life grows by pyrites pulling, implying a lack of continuity between geochemistry and biochemistry in this case (White *et al.*, 2015). In their experiments, Huber and Wächtershäuser (1997) were obliged to use high concentrations of CO to make their proposed chemistry work. Indeed, Wächtershäuser even declared the direct reduction of CO₂ by H₂ to be impossible (Wächtershäuser, 1988) because the reduction potential of H₂ is not sufficiently low, even though methanogens and acetogens gain all their carbon and energy from this reaction alone (Fuchs, 2011). Wächtershäuser actually celebrated this lack

of continuity, entitling one paper “Life as we don't know it” (Wächtershäuser, 2000).

What has been largely missing from experimental work on the origin of life over the last 70 years is much insight from life itself (Martin *et al.*, 2014). Prebiotic chemistry has been approached from the intellectual tradition of synthetic chemistry, and the apotheosis is the “one-pot synthesis” (Patel *et al.*, 2015). But cells are not simply a pot of chemicals; they have a structure in space. Biochemistry is vectorial (Mitchell, 1957, 1961, 1966), meaning that reactions have a direction in space, frequently in relation to structures such as membranes. The fixation of CO₂ is typically powered by electrochemical differences in ion concentration across the plasma membrane and by membrane-bound transition metal catalysts capable of electron transfer. This complexity has been overlooked for good reason: the proton-motive force used by more or less all cells to drive carbon and energy metabolism depends on sophisticated ion-tight phospholipid membranes and on protein nanomachines with moving parts (Sojo *et al.*, 2014). Plainly these arose in a world of genes and proteins and, hence, can have little to say about prebiotic chemistry. Even so, de Duve's (2005) axiom still holds: both membranes and membrane proteins are predicted to have arisen by selection in an environment in which spontaneous vectorial ion flux drove CO₂ reduction. That might sound like a tall order, were it not for the fact that precisely such an environment was discovered in the year 2000 (Kelley *et al.*, 2001, 2005), having been predicted a decade earlier (Russell *et al.*, 1989, 1993, 1994)—deep-sea alkaline hydrothermal vents.

2. Vectorial Chemistry in Alkaline Vents

Alkaline hydrothermal vents are not volcanic systems and have nothing directly to do with magma. Rather, they are produced by a chemical reaction between water and ultramafic rocks characteristic of the upper mantle (Sleep *et al.*, 2004; Russell and Arndt, 2005; Russell *et al.*, 2010). These are composed of ultramafic (high Fe²⁺ and Mg²⁺ and low silicate content) minerals such as olivine ((Fe,Mg)₂SiO₄). Water hydroxylates olivine, oxidizing Fe²⁺ to Fe³⁺ and thereby metamorphosing the mineral into serpentinite (Mg₃Si₂O₅(OH)₄), hence the term *serpentinization* (Sleep *et al.*, 2004; Kelley *et al.*, 2005; Bach *et al.*, 2006; Konn *et al.*, 2009). The process of serpentinization typically occurs in ultramafic rocks within the oceanic crust. Water can percolate down to depths of 5–7 km beneath the seafloor (Macdonald and Fyfe, 1985; Fyfe, 1995). Serpentinization releases H₂ and OH[−] ions into the surrounding fluids, making them strongly alkaline (pH 9–11) and highly reducing (Russell *et al.*, 2010). The reaction is exothermic, so these alkaline hydrothermal fluids are typically warm (70–90°C) rather than hot (Kelley *et al.*, 2001; Früh-Green *et al.*, 2003; McCollom and Bach, 2009), although they can reach up to 116°C (Seyfried *et al.*, 2015); being warm, the buoyant fluids rise back up to the seafloor, where they react with seawater to precipitate into alkaline hydrothermal vents (Ludwig *et al.*, 2006; Tivey, 2007).

Today, mantle rocks mostly come into contact with ocean waters close to the mid-ocean spreading centers; the best known deep-sea alkaline vent field, Lost City, lies about 20 km off the Mid-Atlantic Ridge (Kelley *et al.*, 2001, 2005).

In the Hadean and early Archean, 4 billion years ago, the crust and mantle were less differentiated (Foley *et al.*, 2003; Zahnle *et al.*, 2007). As attested by the relative abundance of komatiite lavas from the Archean, much of the seafloor of early Earth is likely to have serpentinized (Sleep *et al.*, 2004; Russell and Arndt, 2005; Jaffrés *et al.*, 2007; Shields and Kasting, 2007; Arndt and Nisbet, 2012), making alkaline hydrothermal systems potentially nearly continuous in distribution. Such vents can be very large—up to 60 m in height at Lost City—and geologically stable, persisting for periods of at least 100,000 years (Früh-Green *et al.*, 2003; Ludwig *et al.*, 2005), compared with a few decades for volcanic systems such as black smokers, which, being magma-hosted, are far more aggressive: strongly acidic, reaching temperatures of 450°C, and with flux rates several orders of magnitude faster (Lowell *et al.*, 1995; Kelley *et al.*, 2002).

Modern serpentine-hosted systems such as Lost City or Strýtan in Iceland (Geptner *et al.*, 2002) can have very contrasting mineralogy, but the hydrothermal fluids themselves are consistent in being warm, enriched in millimolar concentrations of H_2 , and strongly alkaline (Martin *et al.*, 2008). Likewise, alkaline vents are usually highly porous, lacking the central chimney characteristic of black smokers (Kelley *et al.*, 2005). Hydrothermal fluids instead percolate through labyrinths of interconnected micropores with diameters of tens to hundreds of micrometers, reminiscent of a

mineralized sponge (Geptner *et al.*, 2002; Kelley *et al.*, 2005). Warm (70–90°C) alkaline hydrothermal fluids and cool (1–5°C) ocean waters mix within the microporous labyrinth, producing steep gradients of heat, pH, and reduction potential across thin inorganic barriers (Russell, 2003; Martin and Russell, 2007; Martin *et al.*, 2008). Heat gradients drive convection currents and thermal diffusion, which can concentrate organics over many orders of magnitude by thermophoresis (Duhr and Braun, 2006; Baaske *et al.*, 2007; Mast and Braun, 2010; Mast *et al.*, 2013; Herschy *et al.*, 2014). The redox and pH gradients give rise to vectorial chemistry that is remarkably similar in both magnitude and polarity to modern autotrophic cells (Fig. 1). Specifically, individual pores could have differences in pH of 3 units across the bounding thin-walled inorganic barriers, giving a proton-motive force of around 200 mV, with the inside alkaline and strongly reducing and the outside relatively acidic and oxidizing (Russell *et al.*, 1989, 1993, 1994; Russell, 2003; Martin and Russell, 2007; Martin *et al.*, 2008). This structure is precisely analogous to autotrophic cells (Fig. 1) (Nitschke and Russell, 2009; Lane *et al.*, 2010; Lane and Martin, 2012).

The barriers themselves, however, are far from analogous to cell membranes. Modern phospholipid membranes are about 5 nm in diameter, whereas the inorganic walls of vent pores are typically several micrometers thick (Russell *et al.*,

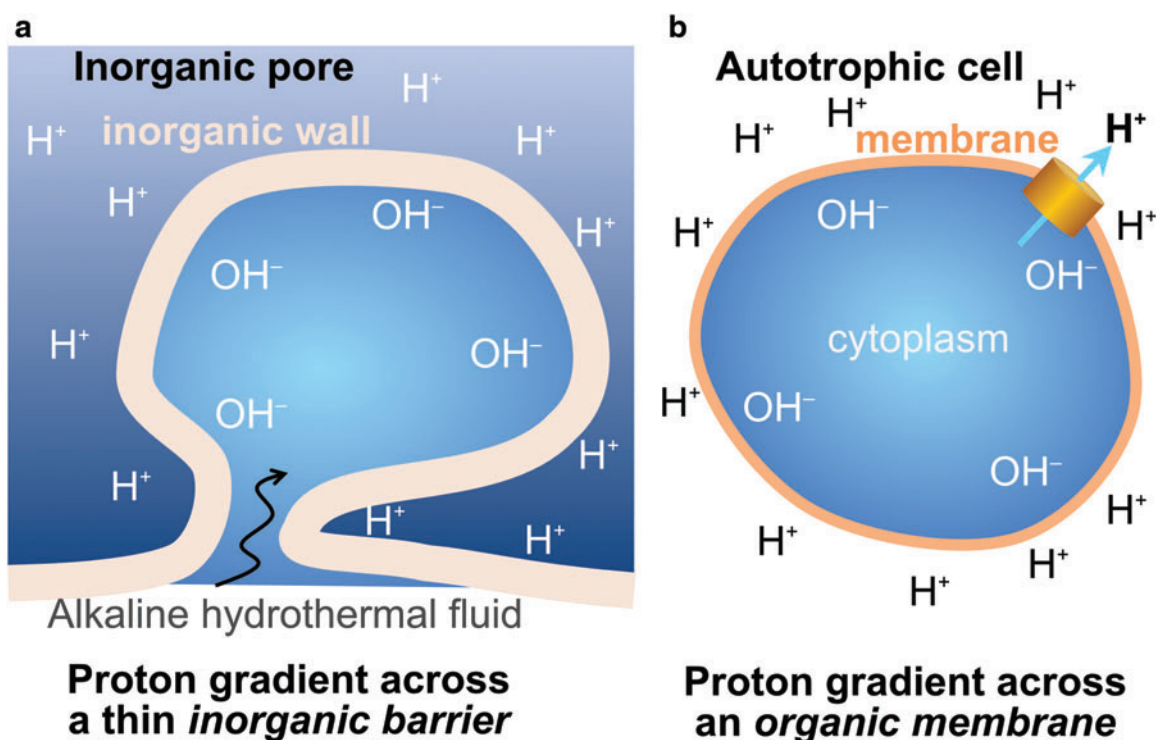


FIG. 1. Vent pores and autotrophic cells have analogous topology. (a) Pores in alkaline hydrothermal vents have relatively thick inorganic walls, which in the Hadean should have incorporated semiconducting Fe(Ni)S minerals such as mackinawite and greigite. These thin barriers separate alkaline (pH 11) hydrothermal fluids from mildly acidic (pH 5–7) ocean waters. The leaky walls do not prevent mixing, but continuous hydrothermal flow and ocean convection maintain steep pH gradients. (b) The topology is precisely analogous in essentially all autotrophic cells including bacteria and archaea. The organic cell membrane (composed of lipids) is much thinner (5 nm vs. 1 μm), but the difference in pH is exactly equivalent in both magnitude (about 3 pH units) and polarity (acidic outside). In the case of autotrophic cells, the proton gradient is continuously generated by membrane pumps, which actively extrude protons (arrow) to maintain the pH difference—the proton-motive force.

1994; Russell and Hall, 1997; Kelley *et al.*, 2005; White *et al.*, 2015). The barriers in vents at Lost City are composed largely of aragonite (CaCO_3) enclosed in metastable brucite ($\text{Mg}(\text{OH})_2$) precipitates, which eventually dissolve on prolonged mixing with seawater (Kelley *et al.*, 2005; Ludwig *et al.*, 2006), while Strytan is composed mostly of saponite (hydroxylated Ca/Mg/Fe aluminosilicates) clay (Martensson *et al.*, 2001; Geptner *et al.*, 2002); neither has any obvious link with cells. But ocean conditions in the Hadean were extremely different from conditions today (Russell and Arndt, 2005; Sleep, 2010; Arndt and Nisbet, 2012) in that they were (i) anoxic and ferruginous and (ii) mildly acidic with high concentrations of CO_2 . Both factors should have made important differences to the chemistry of Hadean vent systems. In particular,

- (1) Ferruginous oceans meant that the vent walls should have incorporated $\text{Fe}(\text{Ni})\text{S}$ minerals, as well as other minerals such as silicates (Russell and Hall, 1997; Russell, 2003), especially in mixed serpentinite/magma-hosted systems such as Rainbow today (Holm and Charlou, 2001). Both molybdate (MoO_4^{2-}) and HS^- ions dissolve in alkaline hydrothermal fluids and, hence, could be provided by serpentinite-hosted systems, as could ammonia (Nitschke and Russell, 2009). Nearby magma-hosted systems such as black smokers provided sources of phosphate, reduced nitrogen, CO_2 (Von Damm, 1990), and divalent metal cations, notably Fe^{2+} and Ni^{2+} . Fe^{2+} is likely to have been present at about $200\ \mu\text{M}$ and Ni^{2+} at about $0.3\ \mu\text{M}$ in Hadean oceans (Pinti, 2005; Sleep *et al.*, 2011; Arndt and Nisbet, 2012; Mloszewska *et al.*, 2012; Keller *et al.*, 2014), but their concentrations would have been higher in the vicinity of magma-hosted systems. $\text{Fe}(\text{Ni})\text{S}$ minerals catalyze redox chemistry (Heinen and Lauwers, 1996; Huber and Wächtershäuser, 1997), and inorganic $\text{Fe}(\text{Ni})\text{S}$ clusters with structures similar to greigite (Fe_3S_4) are still indispensable cofactors for many enzymes required for carbon fixation and energy transduction, especially in ancient pathways such as the acetyl-CoA pathway and the reverse Krebs cycle (Eck and Dayhoff, 1966; Russell and Martin, 2004; Bender *et al.*, 2011). The inorganic barriers bounding pores in Hadean alkaline vents would therefore have contained catalytic $\text{Fe}(\text{Ni})\text{S}$ minerals with similar structures to the FeS cofactors used by modern membrane proteins involved in carbon fixation (Russell and Hall, 1997).
- (2) Anoxic conditions promote organic chemistry, including notably the reaction of H_2 with CO_2 (Shock *et al.*, 1998; Amend and McCollom, 2009; Shock and Canovas, 2010; Amend *et al.*, 2013). That in itself says nothing about the kinetics of this reaction, which are very tardy; but the formation of total cell biomass from H_2 and CO_2 is exergonic under anoxic, alkaline (pH 11) conditions between 25°C and 125°C (Amend and McCollom, 2009)—exactly the temperature range found in alkaline hydrothermal vents. This reduces the problem largely to one of kinetics, rather than thermodynamics. In contrast, under microoxic conditions (equivalent to an O_2 concentration of 0.1% air saturation, or $<1\ \mu\text{M}$), the cost to produce all the monomers

that make up the biomass of a cell from H_2 and CO_2 is approximately 13–15 times more than that required under anoxic conditions (Amend *et al.*, 2013). Importantly, reduction potential does not relate only to oxygen; the Hadean oceans could feasibly have been anoxic but still quite strongly oxidizing if, as some have argued, moderate amounts of NO were produced by atmospheric chemistry (*e.g.*, lightning), sustaining low micromolar concentrations of NO_2^- and NO_3^- in the oceans (Ducluzeau *et al.*, 2009). In the bulk ocean, oxidizing conditions would strongly inhibit organic syntheses, but the thin inorganic barriers between micropores within vents could potentially separate strongly reducing conditions inside pores from oxidizing conditions outside, giving steep redox gradients as well as pH gradients (Russell *et al.*, 2014). Even if these stronger oxidants were wholly reduced by Fe^{2+} in the oceans, the difference in reduction potential between the dominant H_2/H^+ couple in hydrothermal fluids and the CO_2/CH_4 couple in the ocean equates to a favorable 200 mV (Lane, 2014).

- (3) Higher CO_2 concentrations made the early oceans more acidic (Pinti, 2005; Zahnle *et al.*, 2007; Arndt and Nisbet, 2012), increasing the pH difference between ocean and hydrothermal fluids to potentially as much as 6 pH units. The actual CO_2 concentration of the oceans in the Hadean and Archean is unknown but can be constrained roughly to around 10–100 times modern levels by modeling parameters based on the faint young Sun paradox (Sagan and Mullen, 1972; Kasting, 2010; Sleep *et al.*, 2011) and the likely precipitation of dissolved CO_2 as carbonates such as siderite (FeCO_3) (Sleep *et al.*, 2004). The pH of the Hadean oceans is therefore likely to have been 5–7 (Pinti, 2005), compared with ~ 8 today, giving a maximal proton-motive force of around 400 mV (which can be added on to the ~ 200 mV difference in reduction potential between H_2 and CO_2). The acidic pH and higher CO_2 concentration should also have increased carbon availability in alkaline vents, which tend to be carbon-starved in modern systems due to precipitation of calcium carbonate from the alkaline modern oceans (Baross and Hoffman, 1985; Kelley *et al.*, 2002, 2005). In contrast, CO_2 would have been present mostly as HCO_3^- in mildly acidic early oceans, which modeling indicates is the form of carbon most likely to interact with the catalytic FeS surfaces within vents (Roldan *et al.*, 2015).

These differences in ocean chemistry meant that, in the Hadean, alkaline vents operated as natural electrochemical reactors in which warm, alkaline, hydrogen-rich fluids mixed with cool, acidic, CO_2 -rich ocean waters within a labyrinth of catalytic $\text{Fe}(\text{Ni})\text{S}$ micropores (Russell and Hall, 1997; Russell, 2003; Russell *et al.*, 2010). This topological structure, incorporating steep pH and redox gradients across $\text{Fe}(\text{Ni})\text{S}$ barriers, is strikingly analogous to autotrophic cells and specifically to the chemistry of the Wood-Ljungdahl acetyl-CoA pathway, which unites carbon and energy metabolism in both archaea and bacteria (Martin and Russell, 2007; Lane and Martin, 2012; Sousa *et al.*, 2013). The acetyl-CoA pathway depends on the proton-motive force to drive the

reduction of CO_2 by H_2 , using membrane-associated FeS proteins such as ferredoxin (Fuchs, 2011; Poehlein *et al.*, 2012; Buckel and Thauer, 2013). But the key reductive steps in this pathway differ significantly in bacteria and archaea, obscuring the origins of autotrophic carbon fixation in relation to alkaline vents (Sousa and Martin, 2014).

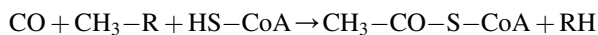
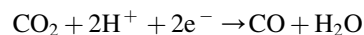
3. Deep Split in the Acetyl-CoA Pathway

There are only six known pathways of carbon fixation across all life (Fuchs, 2011), and a number of good reasons to think that the acetyl-CoA pathway is the most ancient of these (Ferry and House, 2006; Ragsdale and Pierce, 2008; Ferry, 2010; Fuchs, 2011; Martin *et al.*, 2014). First, only the acetyl-CoA pathway is exergonic overall—cells can gain all the carbon and energy needed for growth from this pathway alone; the five other pathways all require an energy input, in the form of ATP, to fix carbon (Fuchs, 2011). Second, the acetyl-CoA pathway is short and noncyclic, taking just a few steps from H_2 and CO_2 to acetyl-CoA, the hub of metabolism in all cells, and indeed in all six pathways of carbon fixation (Martin and Russell, 2007; Fuchs, 2011). That makes it easier to evolve than a complex cycle, with its problems of declining yield at every step (Orgel, 2008). Third, unlike other pathways, a large proportion of the enzymes involved depend on simple Fe(Ni)S cofactors, with structures similar to minerals such as greigite (Cody, 2004; Major *et al.*, 2004; Russell and Martin, 2004; Bender *et al.*, 2011; Kim *et al.*, 2013; Harel *et al.*, 2014) offering a plausible route to get from inorganic catalysts driving abiotic chemistry to genetically encoded enzyme cofactors—which is especially important for a path on which all other metabolism (such as amino acid or nucleotide biosynthesis) is based. Fourth, the pathway depends strictly on chemiosmotic coupling, but in this case the ion electrochemical circuit is extremely simple, lacking the quinones and cytochromes responsible for electron transfer and active ion pumping in the other groups (Thauer *et al.*, 1977, 2008; Buckel and Thauer, 2013; Sousa *et al.*, 2013). Finally, the acetyl-CoA pathway is unique in that it is present in both of the two primary domains of life, the bacteria and the archaea (Fuchs, 2011; Martin *et al.*, 2014), implying it was the only pathway of carbon fixation in the last universal common ancestor (LUCA) (Martin *et al.*, 2014).

But this last statement leads straight to two big problems. First, there is as yet little agreement about the deep branching of the “tree of life” in bacteria and archaea. The two groups that rely on the acetyl-CoA pathway are methanogens (archaea) and acetogens (bacteria), but for every phylogenetic tree that shows these groups as deep branching or ancestral (Ciccarelli *et al.*, 2006; Kelly *et al.*, 2011; Williams *et al.*, 2012; Raymann *et al.*, 2015), some other tree depicts them as secondarily derived (Schoepp-Cothenet *et al.*, 2013; Petitjean *et al.*, 2014; Williams and Embley, 2014). The prevalence of lateral gene transfer between prokaryotes, including across domains, means that the deepest branches of the tree of life may be irresolvable by phylogenetics alone (Martin, 2011). It could be that the acetyl-CoA pathway was indeed the ancestral pathway (Martin *et al.*, 2014), or that it evolved later by convergent evolution as different groups adapted to eke out an existence at the thermodynamic limits (Buckel and Thauer, 2013; Nitschke and Russell, 2013; Schuchmann and

Müller, 2014), or that it evolved in one domain and was acquired in the other domain by lateral gene transfer. The arguments for its priority ultimately rest on the biochemical reasoning outlined above. That leads us to the second problem. These biochemical arguments have an Achilles’ heel. If the acetyl-CoA pathway was genuinely ancestral to both archaea and bacteria, then the basic architecture of the pathway should be conserved in both groups. Yet that is not the case. There are deep phylogenetic differences in the genes required for methyl synthesis in the acetyl-CoA pathway in methanogens and acetogens (Sousa and Martin, 2014), and in the overall “wiring” of the pathways, notably in the process of electron bifurcation (Li *et al.*, 2008; Buckel and Thauer, 2013), and in the membrane proteins involved in active ion pumping (Lane and Martin, 2012; Sojo *et al.*, 2014).

The commonalities and differences in the acetyl-CoA pathway in methanogens and acetogens pose a riddle. Both forms depend on a number of key FeS proteins such as ferredoxin, and both forms employ the recently discovered “trick” of flavin-based electron bifurcation to drive the reduction of a low-potential ferredoxin by H_2 , which is a strongly endergonic reaction (Buckel and Thauer, 2013). In both cases, electron bifurcation employs flavins and Fe(Ni)S proteins (hydrogenases) to split the pair of electrons from H_2 (Buckel and Thauer, 2013). In both cases, the endergonic reduction of ferredoxin is coupled to an exergonic reduction, such that the exergonic reduction drives the endergonic one, with an overall increase in entropy (Nitschke and Russell, 2012; Buckel and Thauer, 2013). There are other similarities, too. In both cases, the acetyl-CoA pathway comprises two converging branches, with one branch generating CO and the other branch forming a methyl group bound to a cofactor (Maden, 1995, 2000). The CO and $-\text{CH}_3$ groups are reacted together by the common Fe(Ni)S enzyme CODH/ACS (carbon monoxide dehydrogenase/acetyl-CoA synthase), which catalyzes the same pair of reactions in methanogens and acetogens:



where R is the cofactor and $\text{CH}_3-\text{CO}-\text{S}-\text{CoA}$ is acetyl-CoA. So there are strong overall similarities that denote common roots of the acetyl-CoA pathway in both domains.

But the differences are equally significant. The enzymes involved in flavin-based electron bifurcation differ in methanogens and acetogens, despite their similarity in overall mechanism; and the enzymes involved in methyl synthesis also differ, as do the pumps that generate electrochemical ion gradients (Martin, 2012; Buckel and Thauer, 2013; Sousa and Martin, 2014). The details here matter.

3.1. The process of methanogenesis

Methanogenesis in archaea has the endergonic reduction of ferredoxin coupled to the exergonic reduction of a heterodisulfide bond to two thiols (Li *et al.*, 2008; Kaster *et al.*, 2011; Buckel and Thauer, 2013). Ferredoxin is then used to reduce CO_2 in the first difficult step of the methyl synthesis branch (Fig. 2a). Energy is conserved at a single site when the methyl group is transferred from one cofactor to another

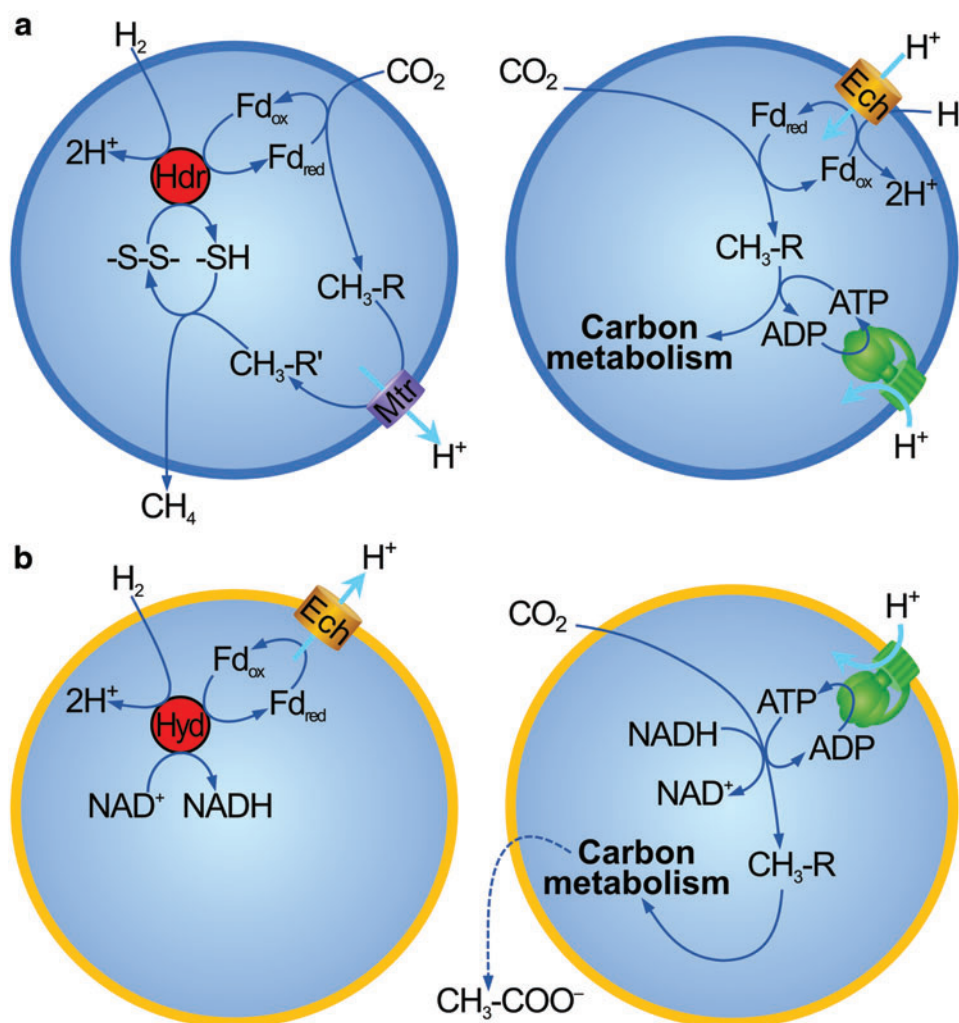


FIG. 2. Schematic depiction of methanogenesis and acetogenesis. **(a)** Methanogenesis. Left panel: Flavin-based electron bifurcation in methanogenesis generates reduced ferredoxin and reduced thiols. Ferredoxin is used to reduce CO_2 to a methyl group; its transfer from one cofactor to another releases enough energy to generate an ion gradient (depicted here as a H^+ gradient for simplicity) via the membrane protein Mtr. Methane is excreted as waste. Right panel: The ion gradient is used to drive ferredoxin reduction directly to form a methyl group, which is reacted with CO to form acetyl-CoA (not shown for simplicity); ATP is synthesized via a standard rotor-stator ATP synthase. Acetyl-CoA and ATP together drive intermediary metabolism. **(b)** Acetogenesis. Left panel: Electron bifurcation in acetogens generates reduced ferredoxin and NADH. Oxidation of ferredoxin drives the extrusion of ions (typically Na^+ but shown here as H^+ for consistency) via the membrane protein Ech or Rnf. Right panel: CO_2 is reduced using NADH and ATP to form a methyl group. This is reacted with CO (not shown) to form acetyl-CoA, which together with ATP is used to drive carbon metabolism. Acetate is excreted to maintain redox balance.

(specifically from an N- to an S-group) by the enzyme methyl transferase (Mtr). The change in conformation of Mtr extrudes one Na^+ ion across the plasma membrane. Finally, the methyl group picks up a pair of electrons from the two thiols formed by electron bifurcation, which regenerates the heterodisulfide bond and releases CH_4 gas as the waste product (Li *et al.*, 2008; Kaster *et al.*, 2011; Buckel and Thauer, 2013). Overall, methanogenesis conserves energy in the form of an electrochemical ion gradient across the membrane, which is to say that the energy released by the generation of methane from H_2 and CO_2 is used to pump ions across a membrane, and nothing else.

While methanogens typically pump Na^+ ions, the Na^+ gradient is wholly interchangeable with H^+ via the Na^+/H^+

antiporter, which appears to be necessary for growth (Surín *et al.*, 2007; Vidová *et al.*, 2011). Having established a proton-motive force in this way, methanogens then use it to drive the direct reduction of ferredoxin by H_2 (Li *et al.*, 2008; Kaster *et al.*, 2011; Buckel and Thauer, 2013). As previously, ferredoxin drives the difficult initial steps of CO_2 reduction, to synthesize a methyl group. But this time (being a direct reduction powered by the proton-motive force, not electron bifurcation) there is no need to regenerate any heterodisulfide bond for redox balance, so the methyl group can be used for the synthesis of acetyl-CoA. This in turn drives intermediary metabolism (Fig. 2a). The ion gradient is also used to power ATP synthesis directly via the ATP synthase. In sum, methanogenesis conserves energy in

the form of a proton gradient—which is provided free in alkaline hydrothermal vents—and uses it to drive both carbon and energy metabolism.

3.2. The process of acetogenesis

Contrast this pathway with acetogenesis in bacteria (Fig. 2b). Here, the difficult reduction of ferredoxin is again achieved by way of flavin-based electron bifurcation (Buckel and Thauer, 2013). In acetogenesis, however, ferredoxin reduction is coupled to the exergonic reduction of NAD^+ to NADH, using a different FeS hydrogenase complex (Poehlein *et al.*, 2012). Ferredoxin is immediately oxidized again, with its electrons transferred onto more NAD^+ , and the energy released drives the extrusion of Na^+ or H^+ ions across the plasma membrane at a single coupling site, Ech (energy-converting hydrogenase) or Rnf (ferredoxin: NAD^+ oxidoreductase) depending on the species (Müller *et al.*, 2008; Buckel and Thauer, 2013). The synthesis of the methyl group in acetogens is achieved via a completely different pathway, driven by ATP and NADH rather than ferredoxin, and catalyzed by enzymes and cofactors that are not related to those in methanogens (Sousa and Martin, 2014). Acetyl-CoA is produced from the same reaction of the methyl group with CO, as in methanogens; but in this case the waste product, formed later on, is acetate. The excretion of acetate drives the whole process in the forward direction, while maintaining redox balance (Buckel and Thauer, 2013). So in acetogens, a proton-motive force is never called upon to reduce ferredoxin directly, but a Na^+ gradient drives ATP synthesis, which in turn powers carbon fixation using NADH as an electron donor.

These profound differences in the acetyl-CoA pathway are confounding but could be explained by three possible hypotheses for the origin of carbon and energy metabolism in alkaline hydrothermal vents. Discriminating between them will demand more data; here, we simply lay out the respective predictions. These predictions have a great deal to say about the deepest divergence in the tree of life, between the two primary domains: the archaea (represented by methanogens) and the bacteria (represented by acetogens).

4. Three Hypotheses on Carbon Fixation in Alkaline Vents

The question is simple: why is the synthesis of methyl groups from CO_2 so different in methanogens and acetogens? The question is also fundamental: everything else depends on carbon fixation. If the process was facile, it ought to have been conserved. Indeed, de Duve's (2005) selective link between geochemistry and biochemistry points to a deep conservation of carbon fixation, yet it does not exist.

4.1. Hypothesis 1 (CH_3SH): No need for genetically encoded methyl synthesis in vents

The simplest hypothesis is that in the vents there was no need for a genetically encoded pathway of methyl synthesis: serpentinization supplied reactive methyl groups in the form of molecules such as methanethiol (CH_3SH , or methyl sulfide) in abundance (Sousa and Martin, 2014). In other words, the reduction of CO_2 was basically a geological

process and at least initially occurred deep beneath the oceanic crust at higher temperatures and pressures. If CH_3SH was indeed plentiful in Hadean vents, there would be no need for cells to evolve a pathway for methyl synthesis until later, when they escaped from the vents; then, presumably, the methanogens and acetogens evolved the methyl synthesis branch independently (Sousa and Martin, 2014), as with several other traits (Sojo *et al.*, 2014; Sousa and Martin, 2014; Sojo, 2015). But the requirement to synthesize CO (via the other branch of the acetyl-CoA pathway) and react it with a methyl group to form acetyl-CoA remained, explaining the conservation of all the rest of the pathway. Biochemical “fossils” suggest that methyl groups might once have been very abundant (Sousa and Martin, 2014). For example, numerous modified bases differ by little more than the position of one methyl group, requiring a large number of dedicated enzymes to transfer these groups from one site to another (Staben and Rabinowitz, 1984; Matthews and Drummond, 1990; Berg *et al.*, 2015). Why? Could it be that methyl groups were once so common that their reactions could barely be constrained, and modern biochemistry simply recapitulates this early abundance? As yet, there is no better explanation.

So is it plausible that Hadean vents could have produced abundant CH_3SH ? Modern alkaline vents produce methane in millimolar amounts (Proskurowski *et al.*, 2006, 2008; Lang *et al.*, 2012), as well as formate and other partially reduced carbon moieties (Proskurowski *et al.*, 2008), apparently reducing CO_2 via geochemically produced H_2 (McCollom, 2013b; McDermott *et al.*, 2015). Methanethiol has also been detected in modern vents (Reeves *et al.*, 2014) and is an important intermediate in microbial ecosystems (Ni *et al.*, 1994; Oremland and Boone, 1994; Lomans *et al.*, 1999, 2002; Moran *et al.*, 2008). However, the main source of CH_3SH in at least some modern vent systems is reported to be thermal decomposition of organics buried deep in the crust, which would make it a product of biochemistry rather than geochemistry (Reeves *et al.*, 2014). On very limited present information, it seems unlikely that CH_3SH is produced in sufficient quantities to drive primordial biochemistry. Nonetheless, it is feasible that CH_3SH was produced more abundantly by the reduction of CO_2 in anoxic Hadean systems; and once formed, it was certainly less likely to be oxidized rapidly in anoxic early vent systems. Or abiotic CH_3SH could be more abundant in as-yet-undiscovered modern systems, or it might be largely consumed today by cells living deeper within the crust, especially given that modern vents are carbon-limited (Bradley *et al.*, 2009). All these are testable predictions, and it is still too early to draw any conclusions.

4.2. Hypothesis 2 (CH_4): Methanotrophy as a starting point

An alternative hypothesis is that methane itself was the source of the necessary methyl groups. The distinction might seem to be a trivial one, but in fact the geological connotations are very different. The argument holds that the acetyl-CoA pathway is not in fact the most ancient pathway of carbon fixation but that it was predated by a pathway that is unknown in modern cells, if not unsupported by some biochemical logic (Nitschke and Russell, 2013). To produce a methyl group from methane demands oxidation, using a

strong oxidant; the oxidation is very endergonic in anoxic conditions. Oxygen is typically the oxidant in modern methanotrophs, though nitrite is used by some anaerobic methanotrophs (Ettwig *et al.*, 2010; Scheller *et al.*, 2010). Each methyl group synthesized requires equimolar quantities of methane and oxidant. Having oxidized methane to a reactive methyl group (indeed beyond to formaldehyde), this putative ancestral cell went on to react it with CO (produced by a standard reduction of CO₂ by ferredoxin via the deeply conserved enzyme CODH/ACS) to form acetyl-CoA (Nitschke and Russell, 2013; Russell *et al.*, 2014). So the reason that methanogens and acetogens do not share the same methyl synthesis pathway is simply that it arose later, independently, in the two groups, as they adapted to a meager living at the thermodynamic limits from H₂ and CO₂ alone.

The predictions of this hypothesis are quite radical. First, the Hadean oceans (at least in the vicinity of the vents) must have been fairly oxidizing—not oxygenated, but containing oxidants such as NO or its degradation products, NO₂[−] and NO₃[−]. These can be produced by atmospheric chemistry involving lightning, meteorite impact plumes, or volcanic degassing. Their concentration could feasibly have reached the low micromolar range (Ducluzeau *et al.*, 2009), although this is still about 1000-fold lower than the concentration of CH₄ and, hence, must have been limiting for methyl synthesis. The actual ocean concentration of NO₂[−] and NO₃[−] would depend on the rate of reduction by Fe²⁺ in ferruginous oceans (Summers and Chang, 1993), relative to reduction by FeS surfaces in hydrothermal systems (Summers, 2005), neither of which is known. If the flux of the oceans through the subsurface and hydrothermal systems was as fast in the Hadean as it is today—taking just thousands of years (German and Von Damm 2006)—then most oxidized forms of nitrogen would be reduced to ammonia (Brandes *et al.*, 1998). Other oxidants used by associations of anaerobic methane oxidizers include sulfate and disulfide disproportionation (Joye, 2012), and methane-metabolizing biofilms associated with Lost City carbonate structures can both synthesize and anaerobically oxidize methane (Brazelton *et al.*, 2011). However, such sulfate-oxidizing associations operate very close to thermodynamic equilibrium (Thauer, 2011), so it seems unlikely that sulfate could oxidize methane abiotically.

If Hadean oceans did indeed contain low micromolar amounts of NO₂[−] and NO₃[−], then alkaline vents would have harbored steep redox gradients as well as pH gradients. That would theoretically provide much greater electrochemical power, adding potentially an extra 800 mV to the charge available to drive carbon and energy metabolism (about 600 mV in the absence of those strong oxidants) (Nitschke and Russell, 2013; Russell *et al.*, 2014). The problem is that these oxidizing conditions strongly oppose further organic syntheses, such as amino acid biosynthesis, which requires 13- to 15-fold more energy input under microoxic conditions, as noted earlier (Amend *et al.*, 2013). The only way this could work would be if organic syntheses occurred under strongly reducing conditions, in the H₂-rich alkaline pores, whereas the oxidation of methane occurred outside the barrier, in more oxidizing conditions (Russell *et al.*, 2014). Mixing would have had to be extremely restricted for steep redox gradients to be maintained across short distances, yet somehow the CO and the −CH₃ groups would still have to be brought together on the “inside,” to drive

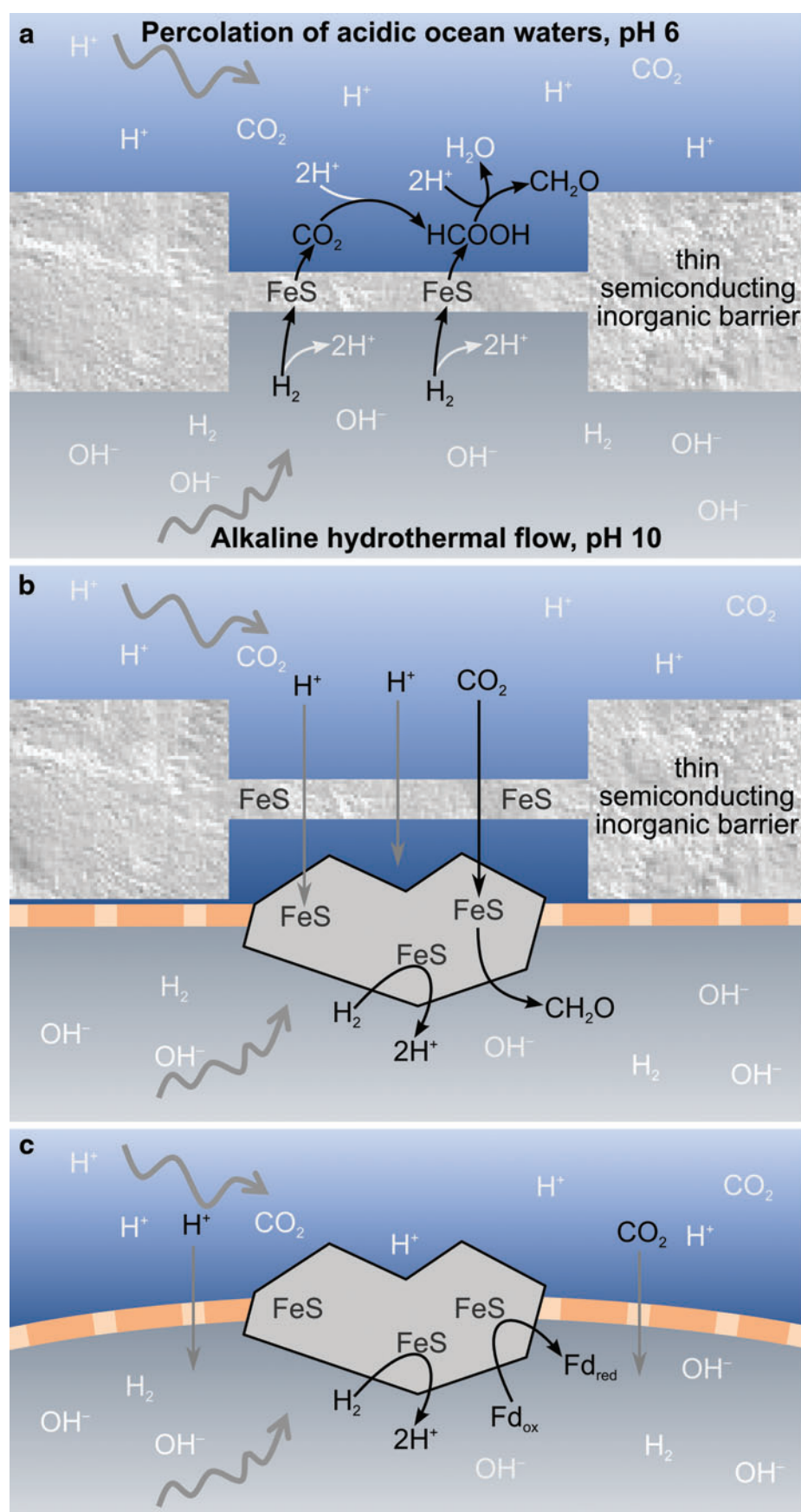
carbon and energy metabolism. At the least, this imposes a tight set of requirements on the barriers in a naturally compartmentalized system, which we suspect might be too exacting. But these are again testable predictions that can be (and are being) addressed in the lab.

4.3. Hypothesis 3 (CO₂): Direct reduction of CO₂ with H₂ at the vent-ocean interface

The third hypothesis is that CO₂ was reduced directly by H₂ at the vent-ocean interface, with this tardy reaction being driven by Fe(Ni)S mineral catalysts and natural proton gradients in a manner exactly analogous (and implicitly homologous) to acetyl-CoA synthesis in modern methanogens (Herschy *et al.*, 2014; Lane, 2014). The key point here is that the process of methanogenesis itself does no more than generate an electrochemical proton gradient, which is provided for free in alkaline hydrothermal vents. Methanogens use their H⁺ gradient to drive the direct reduction of ferredoxin via another Fe(Ni)S protein associated with the membrane, Ech (energy-converting hydrogenase). Ferredoxin in turn drives the reduction of CO₂ to acetyl-CoA (Ferry and House, 2006). This is critical. Acetyl-CoA is readily phosphorylated to form acetyl phosphate (AcP), which functions as an energy currency in its own right, and can phosphorylate ADP to ATP (Thauer *et al.*, 1977). So, in principle, a proton gradient could drive both carbon and energy metabolism via the formation of acetyl-CoA and acetyl phosphate, without the need to use a rotor-stator ATP synthase, so long as cells do not have to consume energy in pumping protons (which is unnecessary, given a free proton gradient).

The question is this: could a natural proton gradient of about 3–5 pH units, across thin inorganic barriers containing Fe(Ni)S minerals, drive CO₂ reduction in the absence of organic membranes or enzymes? In theory, the answer is yes, as the reduction potential of H₂, CO₂, and FeS minerals depends on pH, according to the Nernst equation (Lane and Martin, 2012; Herschy *et al.*, 2014; Lane, 2014). The reduction potential of the H₂/H⁺ couple falls to around −660 mV at pH 11, making H₂ far more reducing, whereas the reduction potential of the CO₂/CH₂O couple (the difficult initial four-electron reduction to formaldehyde) rises to about −520 mV at pH 6, making it easier to reduce CO₂ (Lane and Martin, 2012; Herschy *et al.*, 2014; Lane, 2014). The structure of the vent pores should theoretically facilitate this reduction, as H₂ at pH 11 is brought into sharp juxtaposition with CO₂ at pH 6, across thin, semiconducting Fe(Ni)S walls (Fig. 3a). Mixing of hydrothermal fluids and ocean water elsewhere in the microporous labyrinth of the vent should prevent a buildup of charge on the barriers (Sojo *et al.*, 2014). Experiments from several different labs suggest that H₂ can indeed reduce CO₂ to make formaldehyde, acetate, and pyruvate (Herschy *et al.*, 2014; Yamaguchi *et al.*, 2014; Roldan *et al.*, 2015), and that this difficult reduction can be enhanced by modulating the electrical potential, corroborating these predictions (Yamaguchi *et al.*, 2014; Roldan *et al.*, 2015). Yields were low, however, potentially reflecting the low pressure of benchtop systems, which limits the solubility of H₂, but also the instability of formaldehyde in acid; formaldehyde easily breaks down to reduce H⁺ to H₂, and indeed electrons transferred across the

FIG. 3. Possible mechanism of vectorial CO_2 reduction across vent pores. **(a)** H_2 is more reducing in alkaline solution; its reduction potential falls to -600 mV at pH 10 and -660 mV at pH 11. In contrast, CO_2 is more easily reduced in acidic solution, the reduction potential of the $\text{CO}_2/\text{CH}_2\text{O}$ redox couple being about -520 mV at pH 6. These two phases are juxtaposed in alkaline hydrothermal vents, in which thin, semiconducting $\text{Fe}(\text{Ni})\text{S}$ barriers separate hydrothermal fluids from ocean waters. Continuous flow and mixing of the two phases elsewhere in the system should prevent the accumulation of charge on the barriers. However, note that CH_2O is formed in the acid phase, where it is vulnerable to oxidation. Organics are likely to accumulate slowly over time within the vent by thermophoresis until they produce protocell-like structures with polypeptides and fatty acids. **(b)** A leaky protocell, lacking genes and proteins but with a naturally precipitated fatty acid membrane (dashed) and polypeptides composed of amino acids such as cysteine that chelate the surfaces of FeS minerals. These organic membranes facilitate the juxtaposition of acid and alkaline phases and potentially promote the reduction of CO_2 to organics, here shown symbolically as CH_2O , directly within the protocell. **(c)** Protocells are robust structures that can potentially detach from the FeS barriers and interface directly with the two different phases in vents, allowing faster flux of H^+ across membranes, here shown powering the reduction of a proto-ferredoxin (Fd). Fd_{ox} , ferredoxin; Fd_{red} , reduced ferredoxin.



FeS barrier are as likely to reduce H^+ to H_2 as they are CO_2 to CH_2O , depending on the relative concentrations (Yamaguchi *et al.*, 2014). Mg^{2+} ions are reported to facilitate the reduction of CO_2 rather than H^+ (Yamaguchi *et al.*, 2014).

More experimental work needs to be done, but in principle it is feasible that CO_2 could be reduced by H_2 , in the presence of a proton-motive force across FeS barriers, to form reactive thioesters such as methyl thioacetate or thioacetate (Herschy *et al.*, 2014). We should stress that these reductions take place within the labyrinth of micropores inside the vent, which potentially retains and concentrates any organics, and not in the bulk ocean. Formation of reactive thioesters could in turn drive abiotic carbon and energy metabolism, prefiguring the metabolism of methanogens. This hypothesis is pleasing in that it explains what we are setting out to explain: why CO_2 is the form of carbon most commonly assimilated in living cells—and why carbon assimilation is always driven by ion gradients, either directly or indirectly (all autotrophs are obligately chemiosmotic).

But this scenario does not as yet explain why the synthesis of methyl groups is so different in acetogens. The answer could relate to the fact that methanogenesis and acetogenesis, as processes, merely power active ion pumping, whereas in the vents natural proton gradients rendered active pumping unnecessary (Lane, 2014). The distinction is critical because pumping carries a major energy cost, consuming at least 40 times as many resources (by weight) as carbon fixation (Thauer *et al.*, 2008; Lane and Martin, 2012). In vents, supported by natural proton gradients, this high energetic cost is averted; hence, carbon fixation via the acetyl-CoA pathway is nowhere near thermodynamic limits, and there is no requirement for steeper redox gradients to drive carbon fixation (Lane, 2015). Seen from this perspective, the origin of pumping now becomes a major drain on carbon and energy resources. Cells could only escape from alkaline vents when they became able to generate their own electrochemical ion gradients, at a high cost. We propose that the deep differences in carbon fixation between methanogens and acetogens can be explained by two separate origins of active pumping.

5. The Origin of Pumping in Methanogens and Acetogens

Dependence on natural proton gradients in vents is far from straightforward. Neither hydrothermal fluids nor seawater carries a charge; the charges on OH^- and H^+ , respectively, are offset by charges on other ions such as Na^+ and Cl^- . However, unlike H^+ , these ions are roughly balanced in both fluids and disperse less rapidly than H^+ . When H^+ diffuses down a natural concentration gradient, every H^+ that crosses a barrier transfers a positive charge. Likewise, every e^- that crosses the barrier transfers a negative charge, so the same principles apply. This generates an electrical charge on the barrier, which opposes further flux unless the charge is removed again (Nicholls and Ferguson, 2013; Sojo *et al.*, 2014). Assuming that transfer of H^+ ions down a natural concentration gradient initially drove CO_2 reduction (Fig. 3a), the only way to prevent charge building up on inorganic barriers would be by mixing elsewhere in the system— H^+ that enters compartments must leave again quickly, by flux and diffusion. Ultimately, mixing of hydrothermal fluids and ocean waters

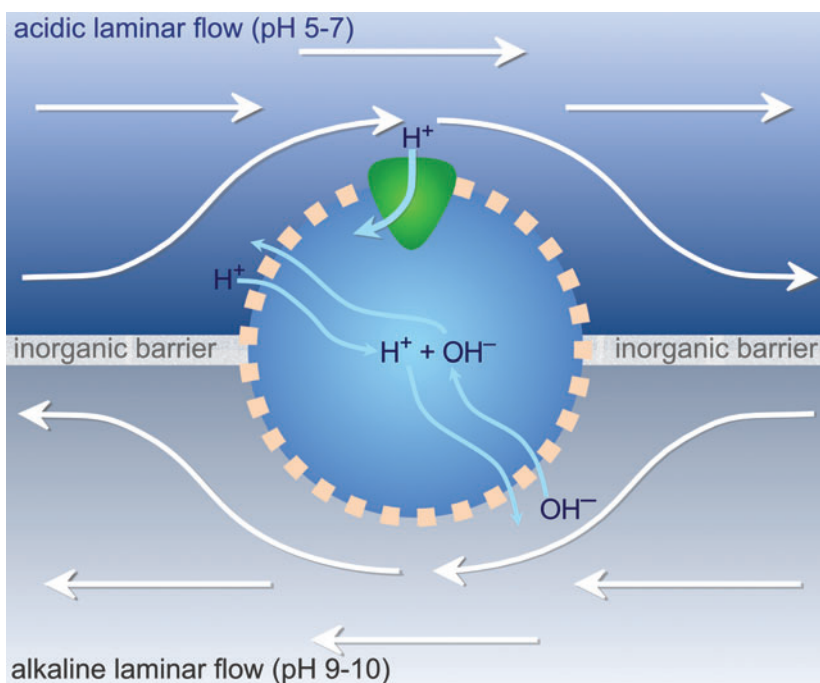
within the vent prevents accumulation of charge and permits the continuous flux of protons (Sojo *et al.*, 2014).

The corollary of this requirement is that the first organic protocells living on natural proton gradients in alkaline vents must also have been very leaky to protons (Lane and Martin, 2012; Sojo *et al.*, 2014). If pH differences across barriers broke down the kinetic barrier to the reduction of CO_2 , the organics formed should have been favored thermodynamically and so would almost certainly include amino acids and fatty acids (Amend *et al.*, 2013). Above a threshold concentration—possibly attained by thermophoresis (Duhr and Braun, 2006; Baaske *et al.*, 2007; Herschy *et al.*, 2014)—fatty acids should spontaneously form vesicles (Budin *et al.*, 2009). Amino acids such as cysteine would presumably have chelated FeS clusters, as they still do, giving a structure similar to the active center of Ech, which would potentially associate with the membrane phase (Fig. 3b). This in turn could have sped the reduction of CO_2 within vesicles, driving growth and ultimately forming a population of organic protocells (Fig. 3c). We have shown that natural proton gradients acting across lipid membranes could power all carbon and energy metabolism, right up to the evolution of genes and proteins in LUCA—but only if their lipid membranes remained leaky to protons throughout (Sojo *et al.*, 2014). Protons entering the cell must be able to leave again, or the electrical charge would prevent any further growth (Fig. 4).

The requirement for leaky protocells makes the origins of pumping even more paradoxical: simply pumping protons across leaky membranes gives little benefit (Sojo *et al.*, 2014). Several steps appear to be required before pumping pays dividends, in particular the evolution of a Na^+/H^+ antiporter (or SPAP), which enabled populations of early cells to colonize different regions of the vent (because a SPAP adds a biochemical sodium gradient to a geochemical proton gradient, giving more power to each proton translocated; Sojo *et al.*, 2014). Only when a SPAP had evolved did it pay to pump protons across a membrane, and only when pumping was advantageous was there any benefit to lowering membrane permeability, tightening the leaky primordial membranes to protons (Sojo *et al.*, 2014). The opposite stereochemistry of glycerol phosphate headgroups in the membrane lipids of archaea and bacteria (Koga *et al.*, 1998)—which cannot be explained by selection (Sojo, 2015)—implies that phospholipid membranes arose independently in archaea and bacteria, arguably from two distinct populations of cells living on natural proton gradients (Martin and Russell, 2003; Sojo *et al.*, 2014; Sojo, 2015). If so, and if the evolution of ion-tight membranes was contingent on the prior invention of active pumping, could the evolution of pumping have occurred independently as well? That seems to be the most likely possibility, because the differences between methanogens and acetogens can be explained by the distinct ways in which they “wire up” electron bifurcation to ion pumping (Fig. 5).

If we assume that the ancestral metabolism of cells was analogous to methanogens, where natural proton gradients drove the reduction of ferredoxin via Ech (Fig. 5a), what would have been the simplest pump to evolve? The *simplest* pump required no inventions at all. It already existed: Ech, the energy-converting hydrogenase (Hedderich, 2004). Methanogens use the inward flux of H^+ to reduce

FIG. 4. H^+ flux through a leaky protocell in a natural proton gradient. A hypothetical protocell with a semipermeable membrane sits at the interface between an alkaline and an acidic fluid. The fluids are continuously replenished and otherwise separated by an inorganic barrier. Hydroxide ions (OH^-) can flow into the protocell from the alkaline side by simple diffusion across the membrane, with protons (H^+) entering in a similar manner from the acidic side. Other ions (Na^+ , K^+ , Cl^- , not shown) diffuse similarly, as a function of their permeability, charge, and respective internal and external concentrations on each side. Inside the protocell, H^+ and OH^- can neutralize into water or leave toward either side. Internal pH thus depends on the water equilibrium and relative influxes of each ion. A protein capable of exploiting the natural proton gradient sits on the acidic side, allowing energy assimilation via ATP production or carbon assimilation via CO_2 fixation (not shown). Reproduced with permission from Sojo *et al.* (2014).



ferredoxin (Buckel and Thauer, 2013), but some acetogens simply reverse the direction of ion flux, using the energy of ferredoxin oxidation to pump ions outward across the membrane (Buckel and Thauer, 2013). Like most membrane channels and enzymes, Ech is spontaneously re-

versible. So there is no magic about this reversal, but it must have created an immediate problem. Ferredoxin had until now been used to reduce CO_2 to a methyl group, driving all carbon and energy metabolism; now it was being put to another use—active pumping (Fig. 5c). That led

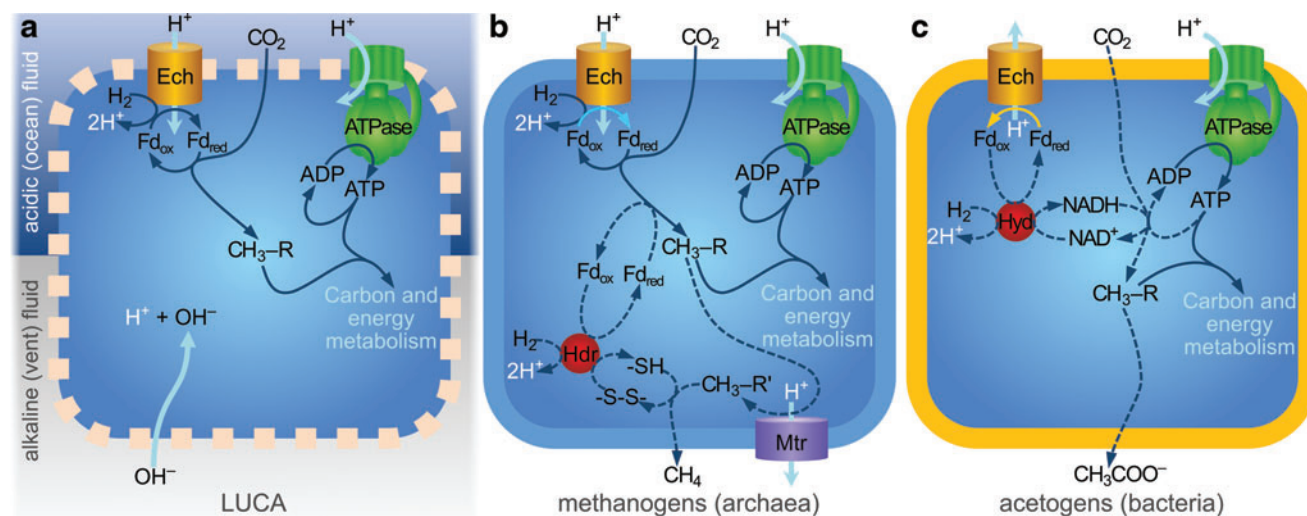


FIG. 5. Proposed evolution of pumping in archaea and bacteria. (a) Proposed carbon and energy metabolism powered by natural proton gradients in an ancestral protocell with leaky membrane. Ech, energy-converting hydrogenase; Fd_{ox} , ferredoxin; Fd_{red} , reduced ferredoxin. For simplicity, this figure only depicts the $-CH_3$ branch of a postulated ancestral acetyl-CoA pathway. R is one of a number of cofactors that differ between methanogens and acetogens. The direction of proton flow through Ech is critical and differs in (c). (b) Simplified carbon and energy metabolism of methanogens (archaea). Ech reduces ferredoxin using a proton gradient as in (a), but there is now a requirement to pump ions to regenerate membrane potential. This is achieved by electron bifurcation, using H_2 to simultaneously reduce ferredoxin and a heterodisulfide ($-S-S-$). Methanogenesis regenerates membrane potential via a new ion pump (Mtr), which may have evolved from a Na^+/H^+ antiporter. (c) Simplified carbon and energy metabolism of acetogens (bacteria). Ech reverses, oxidizing ferredoxin to generate membrane potential. There is now a need to regenerate reduced ferredoxin, achieved via a distinct form of electron bifurcation that uses H_2 to simultaneously reduce ferredoxin and NAD^+ . However, acetogens can no longer use ferredoxin to reduce CO_2 hence had to evolve a new pathway of carbon metabolism, using ATP and NADH in place of ferredoxin. New pathways of energy metabolism are depicted with dotted lines.

to two shortfalls. First, there was no longer any ferredoxin to reduce CO_2 , so the ancestral pathway of methyl synthesis had to be replaced. But oxidation of ferredoxin did generate NADH (Buckel and Thauer, 2013), and the newly generated ion gradient drove ATP synthesis. This is precisely how acetogens reduce CO_2 to form a methyl group: they use NADH and ATP (Buckel and Thauer, 2013). This can be seen as a pleasingly novel pathway that made use of the resources that were present. The second shortfall was in the reduction of ferredoxin itself. These cells, the ancestors of acetogens, could no longer use the proton gradient to reduce ferredoxin but instead evolved a form of flavin-based electron bifurcation. That draws on a large number of FeS proteins (so the basic “kit” was already present) to split the pair of electrons from H_2 , generating both ferredoxin and more NADH (Buckel and Thauer, 2013). Overall, this means that acetogens use flavin-based electron bifurcation to drive both ion pumping and a novel pathway of methyl synthesis (Mtr). We should note that many acetogens use a ferredoxin:NAD⁺ oxidoreductase (Rnf) as their ion pump in place of Ech, but these two protein complexes are functionally equivalent and relatively simple (Buckel and Thauer, 2013), so it is easy to imagine one replacing another.

So what did the methanogens do? They went on doing what they always did, drawing on the proton-motive force to reduce ferredoxin via Ech (Fig. 5b). But that meant they had to evolve a new pump, glean enough energy from somewhere to power the active extrusion of ions. That somewhere turned out to be the transfer of a methyl group—which was already being synthesized *en route* to acetyl-CoA by the ancestral pathway—onto a novel cofactor (Li *et al.*, 2008; Kaster *et al.*, 2011; Poehlein *et al.*, 2012). This transfer is catalyzed by the enzyme Mtr, which appears to have evolved from a membrane antiporter (Harms *et al.*, 1995), again requisitioning a preexisting protein. The conformational change of Mtr extrudes a Na^+ ion across the membrane, which as noted earlier is converted into a H^+ gradient via the Na^+/H^+ antiporter (SPAP). While the transfer of a methyl group conserves energy in the form of an ion gradient, it is no longer capable of forming the high-energy intermediate acetyl-CoA. Instead, it picks up a final pair of electrons and is released as methane (Buckel and Thauer, 2013). That final pair of electrons comes from H_2 , but not directly. Methanogens requisitioned more Fe(Ni)S proteins to develop their own form of flavin-based electron bifurcation, in which the endergonic reduction of ferredoxin is driven by the exergonic reduction of a heterodisulfide group, to form two thiols. These thiols, in turn, pass on their electrons to the energetically depleted methyl group, to generate the waste product methane (Buckel and Thauer, 2013).

If this reasoning is correct, then the deep difference in methyl synthesis between methanogens and acetogens is explained simply in terms of a binary decision over the direction of proton flux through Ech. Methanogens have an inward flux that drives ferredoxin reduction, whereas acetogens reverse that flow, driving an outward flux by ferredoxin oxidation. We appreciate that this scheme is speculative, but it is testable both in the lab and by phylogenetics. If correct, it implies that ancestral metabolism is preserved in the carbon fixation pathways of methanogens, and this is again testable in the lab.

6. Conclusions

Carbon dioxide has been the dominant volatile form of carbon on Earth over the last 4 billion years (Trail *et al.*, 2011) and is the form of carbon assimilated by essentially all autotrophs (Goldschmidt, 1952; Stetter, 2006; Fuchs, 2011). However, the chemical reduction of CO_2 is difficult, and CO_2 has been disregarded in most experimental work on the origin of life in favor of more reactive, but less plausible, forms of carbon, from CO to formamide to cyanide (Oro *et al.*, 1959; Hulett *et al.*, 1971; Hennet *et al.*, 1992; Huber and Wächtershäuser, 1997; Saladino *et al.*, 2012). While this abiotic chemistry has been very successful, culminating in the difficult synthesis of activated nucleotides (Powner *et al.*, 2009), it bears little resemblance to the pathways, catalysts, and substrates actually used by living cells. There is therefore a serious disconnect between abiotic chemistry and biochemistry.

Biochemistry is fundamentally vectorial (Mitchell, 1957, 1961, 1966): all autotrophic cells draw on electrochemical differences in ion concentration across membranes to power CO_2 reduction (Maden, 1995; Stetter, 2006; Lane *et al.*, 2010; Fuchs, 2011). The most ancient pathway of carbon fixation is arguably the acetyl-CoA pathway, which uses H_2 to reduce CO_2 , driven by Fe(Ni)S proteins and H^+ gradients (Maden, 1995; Ragsdale and Pierce, 2008; Sousa *et al.*, 2013). Remarkably analogous conditions are found in alkaline hydrothermal vents, right down to the magnitude and polarity of H^+ gradients across thin inorganic barriers (Lane *et al.*, 2010). The reduction of CO_2 by H_2 to form organics is exergonic under alkaline hydrothermal conditions, but the reaction is notoriously tardy. While the acetyl-CoA pathway would seem to be a helpful guide to autotrophic origins in alkaline vents, deep differences in methyl synthesis between methanogens (archaea) and acetogens (bacteria) confound facile interpretation of how CO_2 was first reduced by H_2 .

By considering the mechanisms of flavin-based electron bifurcation (Buckel and Thauer, 2013), we suggest that the primordial pathway of carbon fixation prefigured that which is still used by methanogens today. H^+ gradients across barriers, and later lipid membranes, drove ferredoxin reduction, which powered the reduction of CO_2 to CO and a methyl group, and thence to acetyl-CoA. We propose a vectorial basis for CO_2 reduction to simple thioesters and acetyl phosphate under abiotic, alkaline hydrothermal conditions. From this early biochemistry, we infer a novel basis for the independent origins of active pumping in archaea and bacteria.

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Abbreviations Used

Ech = energy-converting hydrogenase

LUCA = the last universal common ancestor

Mtr = methyl transferase

Rnf = ferredoxin:NAD⁺ oxidoreductase

SPAP = Na⁺/H⁺ antiporter