The Origin of Membrane Bioenergetics

Nick Lane[1],* and William F. Martin[2]
[1]Research Department of Genetics, Evolution and Environment, University College London, Darwin Building, Gower Street, London WC1E 6BT, UK
[2]Institute of Molecular Evolution, Heinrich-Heine-Universität, Universitätsstr.1, Building 26.13.01, 40225 Düsseldorf, Germany
*Correspondence: nick.lane@ucl.ac.uk
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Harnessing energy as ion gradients across membranes is as universal as the genetic code. We leverage new insights into anaerobe metabolism to propose geochemical origins that account for the ubiquity of chemiosmotic coupling, and Na^+/H^+ transporters in particular. Natural proton gradients acting across thin FeS walls within alkaline hydrothermal vents could drive carbon assimilation, leading to the emergence of protocells within vent pores. Protocell membranes that were initially leaky would eventually become less permeable, forcing cells dependent on natural H^+ gradients to pump Na^+ ions. Our hypothesis accounts for the Na^+/H^+ promiscuity of bioenergetic proteins, as well as the deep divergence between bacteria and archaea.

Introduction

The use of ion gradients over membranes for energy conservation, as in chemiosmotic coupling, is as universal as the genetic code itself, yet its origins are obscure. Insofar as phylogenetics can give any indication of the deepest branches of a "tree of life," autotrophic, chemiosmotic cells invariably cluster at its base (Say and Fuchs, 2010; Stetter, 2006; Maden, 1995). Although there is little doubt that the last universal common ancestor (LUCA) was chemiosmotic with a membrane-bound ATP synthase (Mulkidjanian et al., 2007), how proton and sodium pumping across membranes arose has rarely been addressed. The issue harbors several severe evolutionary problems, but important clues to the early evolution of energy conservation are emerging from biochemical studies of methanogens and acetogens that live from the reduction of CO_2, using electrons from H_2 (Fuchs, 2011; Kaster et al., 2011; Buckel and Thauer, 2012).

Many methanogens grow from the mildly exergonic reaction of $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$. To do so, they make about 1 mol of methane per 1.3 g of cells (Thauer et al., 2008). This means that the flux of H_2 and CO_2 to CH_4 and H_2O that sustains cells is about 40 times greater, by mass, than the yield (the mass of cell material formed). Similar values can be calculated for bacteria such as *E. coli*, based on estimated rates of ATP synthesis per cell division. Both methanogens and *E. coli* turn over ∼50–55 billion ATP molecules per division (Thauer et al., 2008; Phillips and Milo, 2009), 50–100 times each cell's mass. Life is not so much a reaction as a side reaction of the cell's core bioenergetic process. These figures are for modern cells with evolutionarily refined enzymes. Before the advent of enzymes, flux through life's initial main energy-releasing reaction was, by necessity, less specifically channeled toward cell material (or its building blocks) than today. For any amount of RNA-like bases to form spontaneously via prebiotic chemistry—a central pillar of the RNA world concept (Joyce, 2002)—or to double in mass through replication, the excess of waste product versus biomass must have been closer to 40,000:1, orders of magnitude greater

than the 40-fold excess of modern methanogens. For lack of true specificity in its original catalysts, early biochemistry required much more carbon and energy flux than modern cells.

Despite life's almost boundless diversity, there are only two ways that living systems conserve energy in the form of ATP: (1) chemiosmotic coupling via membrane-integral ATP synthases and (2) substrate-level phosphorylations (SLPs), in which soluble enzymes phosphorylate ADP during catalysis of a highly exergonic reaction. Today, all energy that biological systems use is ultimately harnessed through chemiosmotic coupling across membranes because all SLPs use substrates generated by chemiosmotic organisms. But membrane bioenergetics requires proteins capable of both generating and tapping a gradient. These proteins include some of the most astonishing nanodevices known, notably the ATP synthase, an energy-conserving rotary motor. The ATP synthase was a product of long selection during the early phases of evolution, but like only 30 or so other proteins, it is as universal as the ribosome, and it displays the same deep phylogenetic split between archaea and bacteria (Mulkidjanian et al., 2007). Hence, it was present in the last common ancestor. This raises the first evolutionary chicken-and-egg problem: protein synthesis consumes 75% of a cell's ATP budget (Harold, 1986), and the ATP pool is ultimately replenished by proteins that harness chemiosmotic gradients. But if energy conserved by proteins is needed to make proteins, where did the energy come from that gave rise to the first proteins?

Naturally reactive chemical environments can, in principle, cut this Gordian knot. Shock and colleagues (Shock et al., 1998; Shock and Canovas, 2010; Amend and McCollom, 2009) have shown that sustained disequilibrium at submarine hydrothermal vents interfacing with ocean water generates conditions that thermodynamically favor the synthesis of life's building blocks, amino acids in particular, from H_2, CO_2, and NH_4^+. Russell and colleagues (Russell et al., 1993; Russell and Hall, 1997) have argued that the process of serpentinization at alkaline hydrothermal vents (see Box 1) generates natural proton gradients of the magnitude and orientation used by modern cells. Such vents

are stable over timescales of 30,000 years and more (Kelley et al., 2002) and would have been common on the early Earth (Arndt and Nisbet, 2012).
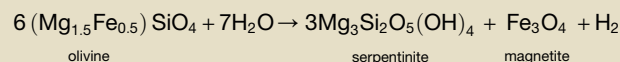
But the devil is in the details, and proton gradients harbor their own specific problems when it comes to early energy harnessing. Although modern membranes are relatively impermeable to protons and Na^+, the first membranes were almost certainly leaky to small ions, especially protons (Pohorille and Deamer, 2009; Mulkidjanian et al., 2012). If we embrace the chemical environment presented by alkaline hydrothermal vents, small organic acids like acetate would have been abundant (Shock and Canovas, 2010). By traversing the membrane in protonated form, organic acids dissipate proton gradients, but not Na^+ gradients. For these reasons, prokaryotes that inhabit such environments today tend to exhibit Na^+ bioenergetics (Buckel and Thauer, 2012). Yet neither serpentinization nor any other currently known process on the early Earth would have readily generated dynamic Na^+ gradients. Thus, the alkaline vent theory, although rich in stable sources of chemical energy, might seem headed to a dead end when it comes to specific mechanisms that would allow the early evolution of biological energy harnessing.

But now, modern anaerobic autotrophic prokaryotes that live from H_2 and CO_2—acetogens and methanogens—are beginning to relinquish their bioenergetic secrets, and these fall into place with alkaline vents in a way that could hardly be more unexpected. The newly discovered process of flavin-based electron bifurcation (see Box 2) (Herrmann et al., 2008; Li et al., 2008; Kaster et al., 2011; Buckel and Thauer, 2012; Schuchmann and Müller, 2012) reveals how these cells reduce CO_2 with electrons from H_2, even though the midpoint potential of H_2 makes the reaction look impossible. Electron bifurcation provides a mechanism for the synthesis of low potential ($\sim$–500mV) ferredoxins capable of reducing CO_2. This mechanism involves soluble enzymes and Na^+/H^+ gradients over membranes, thereby providing important insights into the possible chemistry of CO_2 reduction before the advent of protein-based chemiosmotic harnessing (Figure 1). It also returns reduced ferredoxin, long thought to be one of the most ancient of all proteins because of its FeS centers (Eck and Dayhoff, 1966), to the foreground of thoughts on ancient biological energy conservation. In fact, electron bifurcation reveals the FeS clusters of reduced ferredoxin to be a biological energy currency chemically simpler and more ancient than ATP itself (Buckel and Thauer, 2012).

Here, we outline (1) how the energy required for the origin of life is provided abundantly at alkaline hydrothermal vents, in a form essentially identical to that used by modern cells; (2) how natural proton gradients could drive abiotic electron flux from H_2 to CO_2 to generate organic molecules in a manner closely analogous to modern anaerobes living in similar environments; and (3) why the requirement for active pumping threatened a bioenergetic "crisis," as membranes tightened off to protons. We propose that this crisis was averted through the combination of H^+ and Na^+ energetics, producing a bottleneck through which only cells with promiscuous H^+/Na^+ membrane bioenergetics could pass. These considerations potentially explain the universality of chemiosmotic coupling, the early divergence of archaea and bacteria, and the phylogeny of key bioenergetic proteins.

Box 1. Serpentinization

Serpentinization is important in the context of biochemical origins because it is the source of electrons for reducing CO_2 in hydrothermal systems. At the high pressures and moderately high temperatures of the deep ocean crust, minerals with low SiO_2 content such as olivine react with water to form a hydroxylated mineral, serpentinite, and 10–20 mM concentrations of H_2, dissolved in alkaline fluids (Sleep et al., 2004). Proskurowski et al. (2008) write the serpentinization reaction as:

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

   olivine   serpentinite   magnetite

Serpentinization occurs when rocks derived from the upper mantle (rich in olivine) are exposed to ocean water, which percolates down fractures several km to react with rocks beneath the sea floor. This exothermic reaction, combined with geothermal heat, warms the circulating fluid to $\sim$150°C, generating a buoyant alkaline (pH 9–11, note magnesium hydroxide in the above equation) mineral-laden hydrothermal fluid, originally sourced from the ocean, that rises up to the sea floor and exhales at 70–90°C.

At Lost City, the exhalate precipitates into large spires (<60 m) of microporous minerals consisting of calcium magnesium carbonate (Kelley et al., 2001, 2005). The thin mineral walls thereof (100 nm to 5 μm in diameter) form osmotic barriers that separate warm H_2-rich alkaline fluids from cooler, more oxidized ocean waters (Kelley et al., 2001, 2005). Reduced, warm, alkaline fluids percolate continually through the labyrinths of micropores, sustaining thermal, redox, and pH gradients within the vents. Secondary convection in the adjacent ocean waters guarantees a steady supply of CO_2 and other solutes to the mound's margins. At the interface with Fe^{2+}-containing oceans (Arndt and Nisbet, 2012), the hydrothermal mounds on the early Earth would not have been carbonate spires as at Lost City today but would have been rich in transition metal sulfides instead.

Biochemistry Descended from Alkaline Hydrothermal Vents

A variety of geochemical settings for the origin of life have been proposed (Baross and Hoffman, 1985; Wächtershäuser, 1988; Russell et al., 1993), but there are compelling reasons to favor alkaline hydrothermal vents as the most likely site of the transition from geochemistry to life. The two most important reasons are their sustained far-from-equilibrium conditions and their basic similarities with the carbon and energy metabolism of autotrophic cells. Such conditions are found at modern alkaline hydrothermal vents, such as the Lost City Hydrothermal Field, which is the best known example of its kind (Kelley et al., 2001).

The origin of life required an environment that provided a high enough energy (enthalpy) flux to maintain a low-entropy state (Morowitz, 1968). The low-entropy state of living cells can only be maintained if counterbalanced by an even larger decrease in enthalpy, so the resultant change in free energy remains negative ($\Delta G = \Delta H - T\Delta S$). Thus, life requires a continuous and high input of energy. These considerations mitigate against many settings for life's origin, notably high-entropy, low-enthalpy systems such as primordial soup (whether formed by lightning strikes, UV radiation, or the delivery of organics from space), as well as microcompartmentalized systems not continually

replenished in chemically active precursors, such as ice or pumice. Thermodynamic considerations do not rule out volcanic vents (black smokers) a priori, but other factors make them less likely than alkaline vents. Specifically, volcanic vents have a much shorter life span than alkaline vents, in the order of decades as opposed to >30,000 years for Lost City (Kelley et al., 2002); their temperatures are much higher, above 250°C, where carbon is stable as CO_2 (Shock et al., 1998; Miller and Bada, 1988), as opposed to the life-compatible range of 50–90°C for Lost City; and they are very acidic in pH, typically pH ~1. This is a value that modern cell contents never see, as opposed to pH ~9–10 at Lost City, a value not far from that of an active mitochondrial matrix, pH ~8 or above.

Far-from-equilibrium conditions in alkaline hydrothermal vents satisfy thermodynamic constraints and provide continuously reactive chemical environments. Alkaline vents, currently typified by Lost City, are not volcanic but are formed by the geological process of serpentinization (Box 1), which is the source of abundant H_2 in their hydrothermal effluents (Proskurowski et al., 2008). Thermodynamic calculations show that the synthesis of cell biomass, including amino acids, bases, sugars, and lipids, from H_2, CO_2, and trace NH_4^+ is exothermic under alkaline hydrothermal conditions (pH 9, 50–125°C, H_2 concentrations in the mM range, etc); the reactions provide, in principle, both the reduced carbon and energy needed for life (Amend and McCollom, 2009). These calculations are based on geochemically plausible conditions, measurable in alkaline hydrothermal vents today and reasonable for early Earth settings. They are also supported by recent experiments showing that sugars, bases, carboxylic acids, and amino acids can be formed from the simple C_1 compound formamide by using mineral catalysts under alkaline hydrothermal conditions (Saladino et al., 2012).

The second reason to favor alkaline vents as reactors for life is the striking overall similarity between the chemistry at alkaline hydrothermal vents on the one hand and the core carbon and energy metabolism of modern methanogens and acetogens on the other. No other geochemical setting comes as close to bridging the gap between inorganic and biological chemistry (Martin and Russell, 2007). At the time when life started, atmospheric CO_2 concentrations were probably up to 1,000-fold above present levels (i.e., 0.1–1 bar; Zahnle et al., 2007), and molecular oxygen was absent, giving a very different ocean chemistry from today. High CO_2 made the oceans mildly acidic (pH 5.5–6) compared with pH 8 today, which, in the absence of O_2, allowed reduced transition metals, most significantly Fe^{2+} and Ni^{2+}, to accumulate in the early oceans (Arndt and Nisbet, 2012). These metals, exhaled from volcanic vents (possibly nearby), gave rise to mineral precipitates at alkaline vents, the chimneys of which likely constituted a mixture of silicates, clays, carbonates, and sulfides (Martin et al., 2008). At a Lost-City-type vent in an early-Earth setting, this chemistry delivers catalytic Fe(Ni)S minerals laced with Mo, W, and other transition metals from the alkaline fluids.

Alkaline vents prefigure membrane bioenergetics, as they provide natural proton gradients across thin inorganic walls, as well as redox gradients, with reduced gases (notably H_2) on the inside and oxidized gases (notably CO_2) on the outside (Russell and Hall, 1997). An ocean pH of 5.5–6 and hydrothermal fluid

pH of 9–11 give a proton gradient of ~3–5 pH units, which is equivalent to a proton-motive force of 150–300mV, with the outside acidic, positively charged, and oxidized relative to the inside. This gradient is identical in polarity and is remarkably similar in range of both pH and potential to modern autotrophic cells. In our view—and given the near universality of proton gradients across life—this is no coincidence (Lane et al., 2010).

This overall geochemical setting not only has broad and general similarity to the chemical and energetic processes of life, but it also has specific and detailed similarity—in our view, homology—to carbon and energy metabolism in autotrophs that live from reducing CO_2 with electrons from H_2—acetogens and methanogens. This immediately raises an important question of fundamental nature: how can CO_2 be reduced by H_2, given that the reduction potential of the CO_2 /HCOOH couple ($E_0' = -430$mV) is below that of the $2H^+/H_2$ couple ($E_0' = -414$mV) and that the formate/formaldehyde couple is even lower ($E_0' = -580$mV)? This difficulty is hardly trivial, and it prompted Wächtershäuser (1988) to surmise that it was impossible for life to have started from H_2 and CO_2 for that very reason. But methanogens and acetogens make a living from the reduction of CO_2 with H_2. Only now are microbiologists beginning to understand what tricks microbes use to make the "impossible" possible. The key is a newly recognized process called flavin-based electron bifurcation (Herrmann et al., 2008; Li et al., 2008; Kaster et al., 2011; Buckel and Thauer, 2012). It is elegant, widespread, and provides anaerobic autotrophs with a means to synthesize the key to their CO_2 fixation—reduced low-potential ferredoxins.

Electron Bifurcation and Ion Gradients

Among anaerobic autotrophs (cells that satisfy their carbon needs from CO_2 alone), only two kinds of microbes are known that also harness energy by reducing CO_2 with electrons from H_2: acetogens and methanogens. When growing on H_2, acetogens generate their ATP via chemiosmotic coupling, using the reaction

$$4H_2 + 2HCO_3^- + H^+ \rightarrow CH_3COO^- + 2H_2O$$
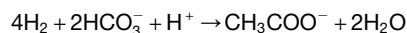
with $\Delta G^{o\prime} = -104.6$ kJ·mol^{-1} (Fuchs, 1986). All acetogens characterized so far are eubacteria, belonging to the clostridias. Methanogens also generate their ATP via chemiosmotic coupling using the reaction

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$
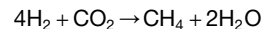
with $\Delta G^{o\prime} = -131$ kJ·mol^{-1} (Thauer et al., 2008). A geochemical variant of the methanogenic reaction occurs in the Earth's crust at Lost City, the effluent of which contains about 1 mM methane of abiotic origin (Proskurowski et al., 2008; Lang et al., 2010), a hitherto unique example of geochemical and biochemical homology. All known methanogens are archaebacteria. Acetogens and methanogens are strict anaerobes; their carbon assimilation entails the acetyl-CoA pathway, a linear pathway of CO_2 fixation that—similar to the situation for hydrothermal vent conditions mentioned above (Amend and McCollom, 2009)—releases energy while generating cell mass rather than

The reduction of CO_2 by H_2 to methane or acetate is exergonic overall under a wide range of conditions but requires overcoming a thermodynamic barrier (Maden, 2000). This is achieved by reducing low-potential ferredoxins with the help of flavin-based electron bifurcation (Buckel and Thauer, 2012).

Flavin-based electron bifurcation couples the endergonic reduction of a low-potential ferredoxin by using electrons derived from H_2 to the exergonic reduction of a high-potential acceptor. In methanogenesis, the high-potential acceptor is the heterodisulfide CoM-S-S-CoB (Kaster et al., 2011); in acetogenesis, the high-potential acceptor is NAD^+ (Poehlein et al., 2012; Schuchmann and Müller, 2012). The energy of the exergonic reaction is conserved in the currency of reduced low-potential ferredoxin, which, in contrast to the starting reductant H_2, is capable of reducing CO_2. In both acetogens and methanogens, CO_2 is reduced stepwise to a methyl group. In methanogens lacking cytochromes, the coupling site is a methyltransferase (Mtr) whose reaction is sufficiently exergonic to drive the extrusion of ions (Na^+ or H^+) across the membrane, conserving energy as chemiosmotic potential (Thauer et al., 2008). In *Acetobacterium woodii*, which lacks cytochromes, the coupling site of Na^+ pumping resides in Rnf, which reduces NAD with electrons from low-potential ferredoxin (Poehlein et al., 2012). In both groups, CO_2 is the terminal electron acceptor, being released as methane or the methyl moiety of acetate (CH_3COO^-).

The membrane potential generated is used for ATP synthesis (via an ATP synthase) and carbon assimilation. Methanogens can reduce ferredoxin with electrons from H_2 via the energy-converting hydrogenase (Ech), a membrane protein that harnesses the ion gradient generated by methanogenesis. When ferredoxin is reduced by Ech, a portion of the ion gradient is spent (Fuchs, 2011; Figure 2). In acetogenesis, acetyl-CoA synthesis consumes and generates one ATP, so there is no net ATP gain, but ATP synthesized via Na^+ pumping comes into play, permitting net carbon assimilation as acetyl-CoA.

The acetyl-CoA pathway is regarded as the most ancient of known CO_2 fixation pathways (Fuchs, 2011; Ferry, 2010) and is replete in FeS and Fe(Ni)S proteins (Bender et al., 2011). The similarities and differences in its manifestations in acetogens and methanogens suggest that the basic chemistry of transition metal-catalyzed methyl synthesis is more ancient than the nonhomologous enzymes of these pathways, which arose in the world of genes and proteins (Martin, 2012). Abiogenic methane and formate synthesis at Lost City would attest to the feasibility and antiquity of geochemical methyl synthesis (Proskurowski et al., 2008). We posit that flavin-based electron bifurcation arose in vents as membranes began tightening to Na^+ and H^+, independently in acetogens and methanogens, albeit in both cases drawing on a similar subset of homologous proteins, notably CO dehydrogenase, acetyl-CoA synthase, ferredoxin, and soluble hydrogenases (see Figure 2).

requiring energy input. Even to the level of the "energy-rich" thioester, the reaction

$$2CO_2 + 4H_2 + CoASH \rightarrow CH_3COSCoA + 3H_2O$$
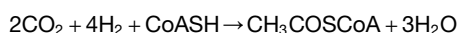
is exergonic with an estimated of $\Delta G^{o\prime}$ = −59.2 kJ/mol (Fuchs, 2011).

Among acetogens and methanogens, the energetically simplest and arguably most ancient (Martin, 2012) species have a single ion coupling site and lack quinones and cytochromes.

They reduce CO_2 by using a low-potential ferredoxin via flavin-based electron bifurcation (see Box 2). The key point is that electron flux to methane and acetate is used purely to generate membrane potential, which is harnessed for both carbon assimilation and ATP synthesis via the acetyl CoA pathway. As alkaline vents already possess ion gradients across thin inorganic walls, methanogenesis and acetogenesis in fact reconstitute what alkaline vents provide for free. Could a natural proton-motive force be tapped abiotically to drive carbon flux toward organic synthesis in a manner analogous to acetogens or methanogens?

The pH-dependent midpoint reduction potential of many ferredoxins offers clues as to how CO_2 might be reduced by H_2. The reduction potential (E_h) falls with increasing pH (Corrado et al., 1996) by $\sim$60mV per pH unit, according to the Nernst equation. This means that ferredoxin is at its most reducing under alkaline conditions and is itself most easily reduced under acidic conditions. Ferredoxins contain FeS clusters that are similar in structure to FeS minerals likely found in early alkaline vents, notably mackinawite and greigite (Russell and Martin, 2004). The reduction potential of FeS proteins depends in part on the protonation of amino acid residues as well as sulfides in the FeS clusters themselves (Chen et al., 2002). Less is known about the reduction potential of FeS minerals, but the first FeS mineral to precipitate, disordered mackinawite, protonates on the surface sulfide residues with an isoelectric point of pH 7.5 (Wolthers et al., 2005). These protonations and deprotonations are quantitatively important, as disordered mackinawite has a high surface area of about 350 m^2 g^{-1}, with a total reactive-site density of 4.0 sites nm^{-2} (Wolthers et al., 2005). The E_h of freshly precipitated mackinawite at pH 7.5 is $\sim$−300mV (Chaves et al., 2011). Given these properties, it is plausible that the reduction potential of disordered mackinawite could fall under alkaline conditions to the point that it could reduce CO_2 to CO ($E_0\prime$ = −520mV), HCOOH ($E_0\prime$ = −430mV), or formaldehyde (HCOOH + $2H^+$ + $2e^-$ ↔ HCHO + H_2O, $E_0\prime$ = −580mV).

Thus, like ferredoxin, FeS minerals could facilitate the reduction of CO_2 by H_2 under natural proton gradients (Figure 1). The reduction potentials of H_2 and CO_2 also vary with pH; H_2 is most reducing in alkaline conditions, and CO_2 is most easily reduced in acidic conditions. Because CO_2 is replenished in the acidic ocean phase as CO_2 or bicarbonate (pH 5.5, high reduction potential) and H_2 is replenished in the alkaline hydrothermal phase (pH 9, low reduction potential), there should be a transfer of electrons across thin semiconducting FeS walls from H_2 to CO_2, lowering the thermodynamic barrier, and so driving organic carbon assimilation in vents. Such a reduction is made possible by the fact that this system is naturally compartmentalized, with different reduction potentials acting on opposite sides of thin, semiconducting FeS walls.

A second possible factor in the reduction of CO_2 to CO, formate, or formaldehyde might be Mo^{4+} (as a dithiolene supplied in alkaline hydrothermal solutions). This can be oxidized via a two-electron reaction to Mo^{6+} (Nitschke and Russell, 2009). The transient Mo^{5+} intermediate is strongly reducing, with an E_h of −355mV, and the reduction potential of the Mo^{5+}/Mo^{6+} couple is pH dependent in proteins falling below −600mV at pH 11
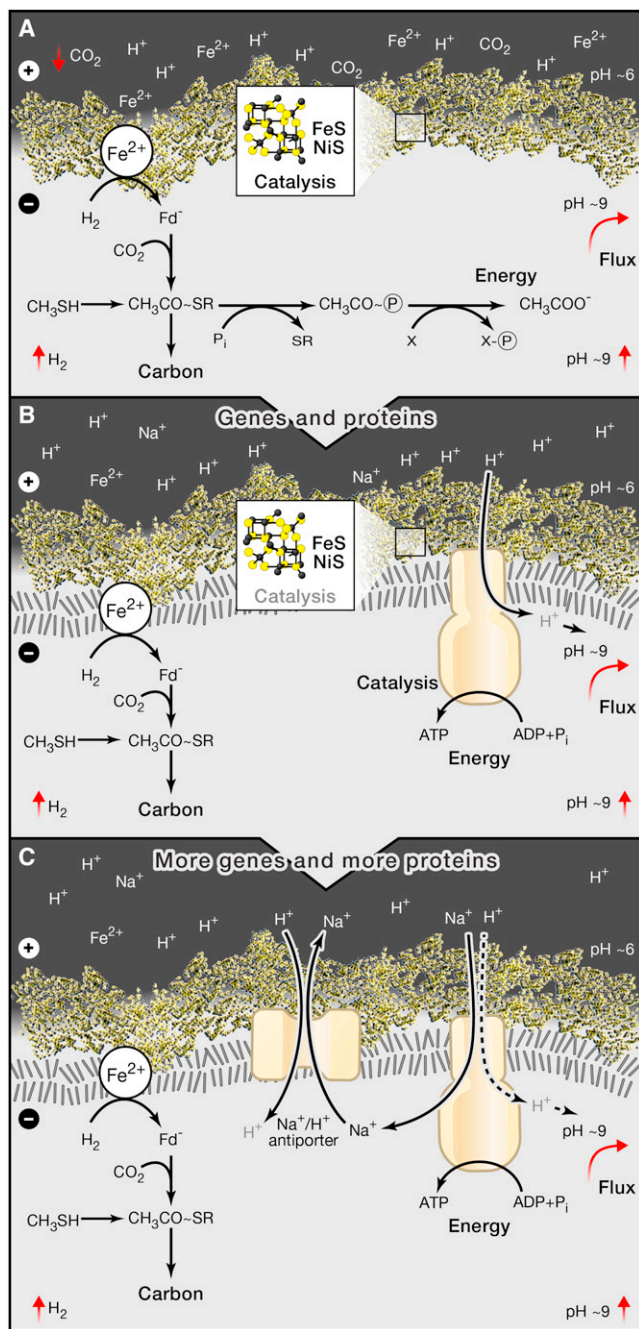
Figure 1. Possible Stages in Early Bioenergetic Evolution

(A) Fe^{2+}-dependent CO_2 reduction by H_2 and organic synthesis. Critical energy currencies are Fe^{2+}, reduced FeS minerals similar to the catalytic FeS clusters in ferredoxin (Fd^-), thioesters, and acyl phosphates. A continuous flux of ~pH 9 hydrothermal effluent is indicated, as are positively and negatively charged compartments. For visual clarity, CO_2 reduction is not shown associated with the inorganic wall (see text).

(B) From the thermodynamic standpoint (Amend and McCollom, 2009), the energetic configuration outlined in (A) could support the origin of genes, proteins, and a proto-membrane. So long as methyl moieties are provided continuously (via CO_2 reduction at the vent or serpentinization), net carbon and energy gain via acetyl thioesters is possible (see Martin and Russell, 2007). The ion-gradient-harnessing ATP synthase is universal, but no ion pumping machinery is, suggesting that the ability to harness the proton gradient at an

(Barber and Siegel, 1982), which is low enough to reduce CO_2 to formate or formaldehyde.

How these factors might act to reduce CO_2 geochemically is still speculation, but three points are worth making. First, Lost City contains methane of geochemical origin (Proskurowski et al., 2008; Lang et al., 2010); somehow CO_2 is being geochemically reduced. Second, the enzymes involved in the reduction of CO_2 in acetogens and methanogens are replete with FeS and Fe(Ni)S centers (Bender et al., 2011) and typically require pterin cofactors containing Mo or W (Nitschke and Russell, 2009). The transition metals themselves are the critical catalysts that transfer electrons; enzymes speed up these transfers and modulate reduction potential, but the transition metal sulfide cofactors, not the amino acid side chain moieties, provide the catalysis. Third, CO_2 is most readily reduced two electrons at a time; hence, the $1e^-$ reductions of the Fe^{2+}/Fe^{3+} couple in FeS minerals must be coordinated with the $2e^-$ reductions of the $CO_2/HCOO^-$ couple. The Mo^{4+}/Mo^{6+} couple might facilitate this switch.

It is possible that the origin of life depended upon organic syntheses—for example, chemically accessible methyl groups—that might have that required high pressure and moderately high temperature conditions provided in the crust during serpentinization; the 1 mM abiogenic methane in Lost City attests to the carbon-reducing abilities of that geochemical process. It is, however, also possible that life-relevant CO_2 reduction occurred almost solely at the vent ocean interface. There are no reports of laboratory experiments to indicate that CO_2 reduction with Fe^{2+} is facile, but Heinen and Lauwers (1996) showed that it is possible. We suggest that the critical factor that could potentially modulate midpoint potentials of mineral reductants between –300 and –600mV required to reduce CO_2 is pH. Natural proton gradients across inorganic walls containing FeS, Fe(Ni)S, and MoS_2 could theoretically drive the reduction of CO_2 by H_2 to organic carbon by lowering the thermodynamic barrier to their reaction, thereby driving the thermodynamically favorable accumulation of biologically relevant molecules, including amino acids, bases, sugars and lipids. Once formed, organics can be concentrated many thousands-fold by temperature gradients (thermophoresis) within the microporous labyrinth, facilitating polymerization of amino acids and nucleotides, precipitation of lipids, and, ultimately, cycles of replication (Baaske et al., 2007; Mast and Braun, 2010).

Thus, one can, in principle, envisage the origins of genes, proteins, and natural selection in alkaline vent systems, but this is not our current focus; salient aspects are discussed elsewhere (Martin and Russell, 2003, 2007; Koonin and Martin, 2005; Branciamore et al., 2009). The significant point here is that natural proton gradients can, in principle, drive the beginnings of an

alkaline hydrothermal vent is older than any biochemical machinery that could generate a gradient with a chemistry specified by genes. Continuous hydrothermal flux maintains pH 9 on the inside of the vent-ocean interface.

(C) Early membranes would not have been tight to protons, but a H^+/Na^+ antiporter could transduce a free proton gradient into a Na^+ gradient, tightening coupling. This would not require a mutational shift in substrate specificity, as the methanogen ATPase is promiscuous for H^+ and Na^+ (Schlegel et al., 2012). The H^+/Na^+ antiporter, present in modern methanogens (Surín et al., 2007) and acetogens (V. Müller, personal communication), converts H^+ into Na^+ currency with essentially no energetic cost.

anabolic biochemistry, eventually forming protocells within the vent pores. By protocells, we mean the organic contents occupying inorganic compartments, lined partially or completely with leaky organic membranes. In early stages, we envisage networks of inorganic compartments lined distally to the ocean with leaky organic membranes but proximally contiguous with vent effluent. The first organic membranes were presumably composed of spontaneously phase-separated alkanes, hydrophobic amino acids and peptides, fatty acids, and other amphiphiles. At a later protocellular stage, membrane lipids and proteins became genetically encoded, and cell-like structures were beginning to seal off within vent pores. The deep differences between archaebacterial and eubacterial membranes imply divergence even within the vents.

At all stages, protocells were using both organic membranes and inorganic walls to help harness the geochemical chemiosmotic potential. But to escape from the vents as independent free-living cells requires a switch from relying on natural proton gradients to forming true cells capable of actively generating ion gradients on their own. The problems involved are counterintuitive and suggest further intriguing parallels with methanogens and acetogens.

The Origin of Active Ion Pumping

In modern cells, membrane bioenergetics depends on the impermeability of membranes to H^+ or Na^+. In vents, neither thin inorganic walls nor the first leaky organic membranes could retain an electrochemical potential for long. Nonetheless, and critically, the percolation of alkaline fluids and ocean water through labyrinthine microcompartments continually juxtaposes solutions of different pH and reduction potential, maintaining proton and redox gradients despite the leakiness of the walls and membranes.

In this setting, protocells with lower membrane permeability should have prospered. As organic membranes became less permeable to small ions, proton flow would by necessity be funneled increasingly through membrane proteins such as Ech (see Box 2), enhancing the reduction of early ferredoxins and speeding carbon assimilation. At a later stage, the ATP synthase would also require relatively impermeable—well coupled—organic membranes to function. Although the soluble ATPase and membrane domains of the ATP synthase are homologous to bacterial RNA helicase and translocase enzymes, respectively, which may have played an earlier role (Mulkidjanian et al., 2007), the universality of chemiosmotic ATP synthesis in archaebacteria and eubacteria, combined with the high energy requirements of early cells, strongly suggests that the ATP synthase arose in vents as a product of natural selection acting on genes and proteins, along with other nanomachines such as ribosomes. Crucially, however, the presence of natural proton gradients across organic membranes means that the ATP synthase could function long before the origin of active ion-pumping systems that work to generate ion gradients (Figure 2).

While improving coupling, decreasing membrane permeability led to a precarious energy crisis. The problem is that a continuous flow of protons through proteins such as Ech and the ATP synthase can only be sustained if the protons entering the cell are removed again. Otherwise, the system swiftly equilibrates,

dissipating the proton-motive force. With a discontinuous or semipermeable membrane, this is no problem; the flux of protons from the ocean is neutralized by the flux of OH^- ions in hydrothermal fluids to maintain the proton-motive force (Figure 1). As soon as the membrane becomes impermeable, however, sealing off as a cell-like vesicle, the hydrothermal flux ceases, and protons accumulate within, equilibrating the inside and outside, dissipating the proton-motive force. Unless the protocell can pump these protons out again, regenerating the proton gradient actively with the help of another energy source, it will equilibrate with the environment. Thus, protocells with permeable membranes should survive perfectly well in vents because they can take advantage of the natural proton-motive force; but cells with genetically encoded membranes that have become impermeable to protons should die unless they can find a way to eliminate protons accumulating within by pumping them out again to regenerate the proton gradient. Active pumping is of course a prerequisite for leaving the vents at all but harbors serious problems of its own.

Either the cell must "invent" a proton pump as soon as the membrane becomes impermeable, or else it must evolve one while the membrane is still leaky to protons. "Immediate invention" is obviously unlikely. But if a proton pump were to evolve before the membrane had become impermeable to protons, then protons would need to be pumped out against a 1,000-fold natural gradient, which quickly dissipates back through the membrane anyway (hardly an option). Assuming hydrothermal fluids were alkaline ($\geq$ pH 9), the internal proton concentration would be $\leq$1 nM. Machinery pumping protons against that gradient would need to be extremely sophisticated while offering no immediate advantage.

What is worse, in energetic terms, pumping is very costly. Modern methanogens produce 40 times more methane than biomass just to generate ion gradients. When equivalent gradients are provided for free by the vents, protocells are bathed in an abundant supply of energy. The crisis comes when available redox energy has to be diverted from reducing CO_2 (carbon metabolism) toward pumping (energy metabolism). The transition to active pumping drastically reduces the energy available for synthesizing biomass. Assuming that the earliest proton pumps were energetically inefficient (as they had yet to be evolutionarily refined) and that the membranes they were acting over were still permeable to protons, the energetic costs must have been colossal, the evolutionary challenges severe, and the advantages very limited—merely the regeneration of a proton gradient that already exists in vents. Thus, protocells with membranes that have become impermeable to protons face a seemingly insurmountable bioenergetic crisis. Such protocells would surely have been outcompeted in vents by protocells with more proton-permeable membranes that never relinquished their energetic dependence on natural proton gradients, but such dependent entities are energetically tied to the vents and ultimately died with them. So how did these first evolving systems escape the energy crisis imparted by proton-tight membranes?

Taking our cue once again from the biochemistry of methanogens and acetogens, we propose that the answer lies in the differential permeability of membranes to small ions. Primordial
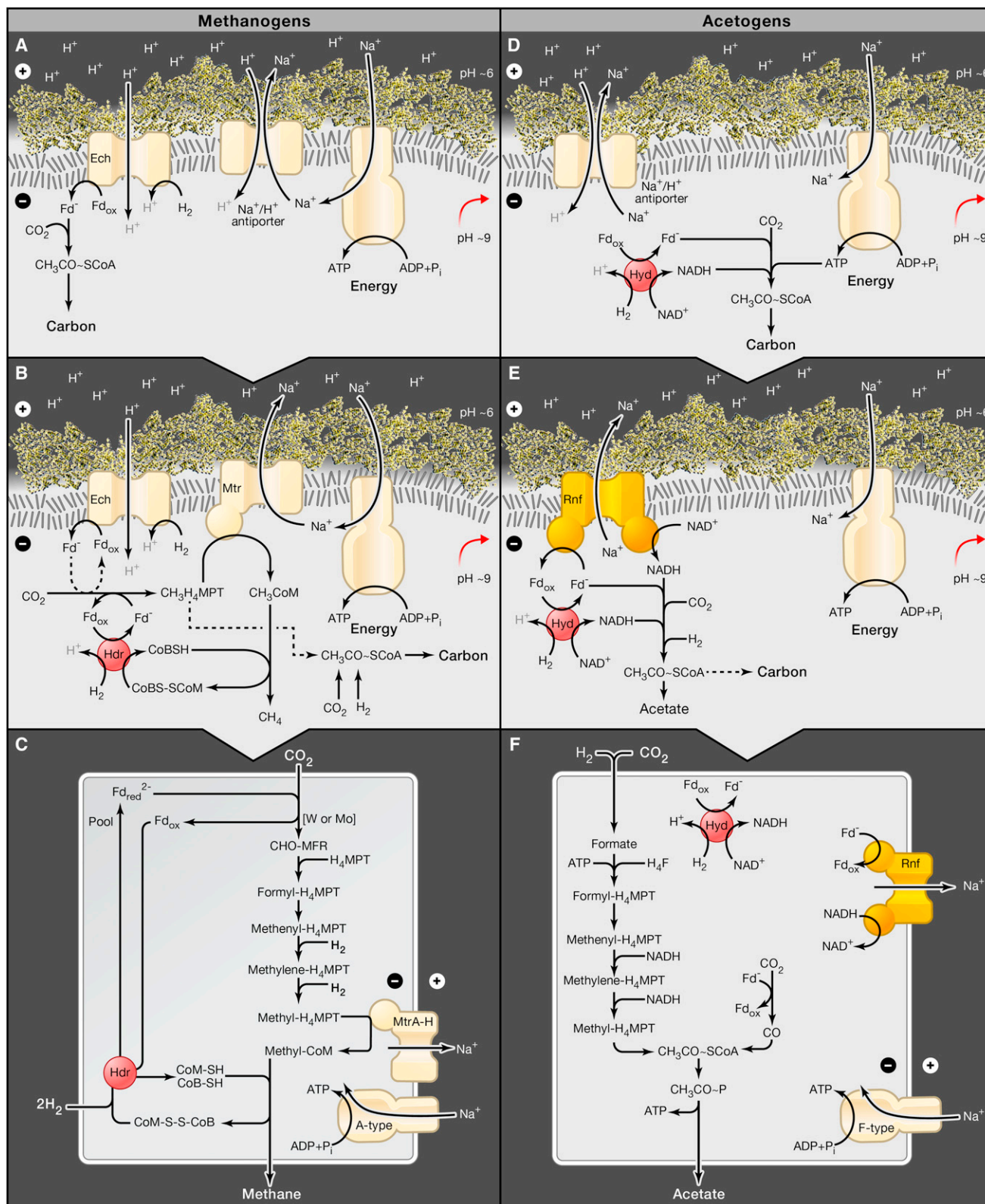
Figure 2. Possible Divergence of Acetyl CoA Pathway in Methanogens and Acetogens

(A–F) Methanogens (A–C) and acetogens (D–F) share several proteins of carbon and energy metabolism (ferredoxin, acetyl CoA synthase, carbon monoxide dehydrogenase, soluble hydrogenases, and the ATPase), but their enzymes of methyl synthesis are unrelated, suggesting that geochemical methyl synthesis

membranes in vents would have become impermeable to Na^+ before they became impermeable to protons. Even today, liposomes composed of bacterial lipids differ in their permeability to Na^+ and H^+, especially at high temperatures (Na^+ and H^+ permeability increases by one to two orders of magnitude between 20 and 80°C; van de Vossenberg et al., 1995). The permeability coefficient for protons ranges from 10^{-10} to 10^{-9} cm s^{-1}. In contrast, Na^+ permeability is much lower, in the order of 10^{-13} to 10^{-11} cm s^{-1} (van de Vossenberg et al., 1995). Thus, modern bacterial membranes are two to three orders of magnitude more permeable to H^+ than to Na^+, and this was probably more marked as the earliest membranes were first becoming impermeable to Na^+. In a membrane that is impermeable to Na^+, but not to H^+, a continuous flow of protons could power a Na^+ efflux via a simple Na^+/H^+ antiporter, as exists in many cells, including modern methanogens (Surín et al., 2007). The passage of protons through such an antiporter would not dissipate the proton-motive force, as hydrothermal flux would continue to supply OH^- ions that neutralized protons inside. A proton-driven antiporter in a semipermeable membrane would therefore transduce a geochemical H^+ gradient into a biochemical Na^+ gradient, offering immediate benefits in terms of improved coupling.

This process is energetically free for protocells that harness it, as it is powered by geothermal proton gradients. As a bonus, a simple Na^+/H^+ antiporter could also explain Na^+ balance (and Na^+/K^+ ratio) in modern cells, which usually have low (~10 mM) intracellular [Na^+] relative to the oceans (~475 mM). If this circumstance is a physiological fossil of early metabolism (Mulkidjanian et al., 2012), it could be readily explained by the action of an antiporter driven by natural proton gradients, which could optimize intracellular ion balance for enzyme function.

Thus, a simple Na^+/H^+ antiporter in protocells within vent pores would produce Na^+ gradients. The great advantage of an H^+-coupled Na^+ pump is that the proteins required for Na^+ bioenergetics could adapt to the larger ion before membranes tightened off to protons, at zero energetic cost. Natural proton gradients could therefore give rise to Na^+-coupled energetics from H^+-coupled energetics. Protocells that remained strictly proton dependent would fall victim to the energy crisis induced by proton-tight membranes. In contrast, cells that had already evolved H^+/Na^+ energetics would thrive with proton-tight membranes, as the Na^+ circuit already existed.

The promiscuous behavior of ion channels in cells living from the H_2/CO_2 couple today might be relics of this solution to the energy crisis. Methanogens such as *Methanothermobacter thermautotrophicus* rely on both H^+ and Na^+ gradients, modulated by an H^+/Na^+ antiporter (Surín et al., 2007). In *Methanosarcina acetivorans*, the ATP synthase has an equal affinity for Na^+ and H^+, translocated concurrently to drive ATP synthesis (Schlegel et al., 2012). This promiscuity could also explain why Na^+-motive and H^+-motive ATP synthases are interleaved in phylogenetic trees (Mulkidjanian et al., 2008), making it difficult to infer whether Na^+ or H^+-coupled energetics arose first (Lane et al., 2010). Other bioenergetically crucial membrane proteins in methanogens and acetogens, notably Ech and Rnf, are also apparently promiscuous for Na^+ and H^+ (Buckel and Thauer, 2012). Even complex I (NADH dehydrogenase) displays intriguing Na^+/H^+ promiscuity (Batista et al., 2012).

The simplest mechanism for the origin of ion pumping is to reverse processes that already existed. Rather than reducing ferredoxin by using membrane potential via proteins such as Ech, cells could drive the extrusion of ions by ferredoxin oxidation. *Acetobacterium woodii*, for example, couples a single Na^+ pump (Rnf), powered by Fd^{2-} oxidation, to ATP synthesis via a Na^+/H^+-motive ATP synthase (Poehlein et al., 2012). Reduced ferredoxin is now generated via electron bifurcation, as discussed in Box 2, requisitioning enzymes (FeNi hydrogenases) and cofactors (NAD$^+$) that already existed (Figure 2). Methanogens call on several of the same players (Figure 2) and likewise employ electron bifurcation for ferredoxin reduction. The primary sodium pump in this case, the methyl transferase (Mtr) again contains subunits related to antiporters (Harms et al., 1995). When actively pumping, acetogens and methanogens are energetically at the limits of feasibility (albeit have doubling times measured in minutes or hours), yet they offer the simplest solution to the pumping problem by using single coupling sites already involved in Na^+/H^+ circuits, minimizing the need for de novo invention.

Only when cells mastered Na^+ pumping with an energetic efficiency stipulated by thermodynamics and only when they were able to generate their ion gradient with a chemistry fully specified by genes would they have been free to escape from the vents. Two separate escapes would readily explain the early divergence of archaea and bacteria, with their very different cell membranes and walls (Martin and Russell, 2003; Koonin and

(Proskurowski et al., 2008; Lang et al., 2010) predated the advent of genes and proteins and further suggesting a divergence of eubacterial and archaebacterial lineages after the origin of the ATP synthase but before the origin of free living cells (see text). In methanogens (A), the energy-conserving hydrogenase (Ech), which consumes part of the membrane potential to provide reduced ferredoxin for carbon metabolism, may have played an early bioenergetic role. In the presence of a geochemical proton gradient, Ech would be an inexhaustible source of reduced ferredoxin from H_2, channeling the methanogen lineage down a path in which Fd^-, rather than ATP, becomes the central energy currency in carbon and energy metabolism. (B) In modern methanogens lacking cytochromes (Kaster et al., 2011), electron bifurcation at heterodisulfide reductase (Hdr) is the main source of Fd^-. The endergonic reduction of ferredoxin is coupled to the exergonic reduction of the heterodisulfide CoB-S-S-CoM, which is reoxidized at the methane synthesis step. The methyl transferase (Mtr) reaction in this branch is sufficiently exergonic to pump ions (with no other ancestral candidate for that role) in *Methanothermobacter marburgensis* (Kaster et al., 2011), whose energy metabolism is shown in (C). Net carbon assimilation in methanogens that lack cytochromes consumes a portion of the Na^+ gradient by Ech (not shown) for Fd reduction, leading to net acetyl-CoA accumulation (Kaster et al., 2011). (D) In acetogens, the methyl group in acetate serves as the reduced end product of energy metabolism. The trimeric, bifurcating hydrogenase (Hyd) (Schuchmann and Müller, 2012) becomes the main source of Fd^-, with the exergonic reaction to drive Fd reduction at bifurcation being NAD$^+$ reduction, requiring NADH-oxidizing steps in methyl synthesis (a difference to methanogens) for redox balance. A Na^+-utilizing ATPase supports methyl synthesis, allowing net acetyl-CoA accumulation. (E) Fd-dependent Na^+-pumping via Rnf generates a Na^+ gradient during the synthesis of acetate from H_2 and CO_2. (F) Energy metabolism of *Acetobacterium woodii* (Poehlein et al., 2012), an acetogen that lacks cytochromes. Net carbon assimilation requires investing a portion of ATP-synthase-derived ATP to acetyl CoA synthesis. The electron bifurcating enzymes (Buckel and Thauer, 2012) central to carbon and energy metabolism in methanogens and acetogens, Hdr (Kaster et al., 2011) and Hyd (Poehlein et al., 2012), respectively, are indicated in red.

Martin, 2005); two separate solutions to the pumping paradox could also explain the deep differences between acetogenesis and methanogenesis, which are chemically similar pathways that nonetheless have little biochemistry in common (Martin and Russell, 2007).

Remarkably, nearly 4 billion years of innovations never led to the replacement of the universal ATP synthase by a better protein, nor did cells ever fundamentally alter the primordial electrochemical basis of membrane bioenergetics. Bacterial respiratory complex I, for example, is homologous to Ech, with the addition of quinone-binding domains. Both Ech and complex I contain subunits that are homologous to the Na^+/H^+ antiporter and to soluble FeNi hydrogenases of the type discussed here (Marreiros et al., 2012). A reasonable interpretation is that complex I arose through the addition of quinone-binding domains to Ech (Marreiros et al., 2012; Hedderich, 2004), which is consistent with the fact that Ech is widespread among eubacteria as well as among archaebacteria. Independent origins of both quinone (White, 2004) and heme (Storbeck et al., 2010) biosynthesis in archaebacteria and eubacteria support the view that more elaborate respiratory chains containing both quinones and cytochromes evolved after Ech and ferredoxin-based membrane bioenergetics. Many respiratory proteins are assembled from a redox protein "construction kit" (Baymann et al., 2003) and are easily passed around by lateral gene transfer; the early acquisition of quinones and cytochromes may even have enabled the early radiation of eubacteria and archaebacteria. Both groups evolved access to the hundreds of redox couples known to support life via membrane bioenergetics. Even electron bifurcation arose a second time, involving quinones and cytochrome b complexes in the respiratory Q cycle (Mitchell, 1975). In light of such diversity, acetogens and methanogens that lack quinones and cytochromes stand out more than ever as simply construed strict anaerobes living from gases (H_2, CO_2, N_2, NH_3, and H_2S) present in early vents and with cofactor requirements comprising phosphate and a few metals.

Conclusions

Dependence on membrane bioenergetics is as universal as the genetic code. As the mechanisms of energy conservation in methanogens and acetogens have come into focus in recent years, so too have the contours of a possible path from rocks and water to biological ion pumping and energy conservation via the rotor-stator ATP synthase. The great sophistication of modern chemiosmotic coupling, combined with the difficulties involved in tightening off early membranes to small ions, especially protons, has led to much skepticism that ion gradients over membranes could have helped meet the energy requirements for the origin of life. Yet we have described here how natural proton gradients in alkaline hydrothermal vents could have supported organic carbon flux through the pH-dependent reduction potential of Fe(Ni)S minerals, as happens in modern FeS proteins, notably ferredoxin. The tightening of early membranes to small ions appears to have forced the combination of H^+ and Na^+ energetics, as seen in many of the cells living in similar environments today, because protocells that remained dependent on proton gradients alone could not make transition to the free-living state. Finally, the origins of Na^+ pumping required no mechanistically groundbreaking genetic innovations, just a protein, an antiporter that transduced a geochemical gradient (H^+) into a biochemical one (Na^+). The high energy demands for early life, the membrane bioenergetics of cells today, the antiquity of transition metal catalysis, and the sources of power that were abundantly available on the early Earth together suggest that the processes of biochemical energy conservation and geological energy dissipation at alkaline hydrothermal vents are homologous.

REFERENCES

Amend, J.P., and McCollom, T.M. (2009). Energetics of biomolecule synthesis on early earth. In Chemical Evolution II: From the Origins of Life to Modern Society, L. Zaikowski, J.M. Friedrich, and S.R. Seidel, eds. (Washington, DC: American Chemical Society), pp. 63–94.

Arndt, N., and Nisbet, E. (2012). Processes on the young earth and the habitats of early life. Annu. Rev. Earth Planet. Sci. 40, 521–549.

Baaske, P., Weinert, F.M., Duhr, S., Lemke, K.H., Russell, M.J., and Braun, D. (2007). Extreme accumulation of nucleotides in simulated hydrothermal pore systems. Proc. Natl. Acad. Sci. USA 104, 9346–9351.

Barber, M.J., and Siegel, L.M. (1982). Oxidation-reduction potentials of molybdenum, flavin, and iron-sulfur centers in milk xanthine oxidase: variation with pH. Biochemistry 21, 1638–1647.

Baross, J.A., and Hoffman, S.E. (1985). Submarine hydrothermal vents and associated gradient environments as sites for the origin and evolution of life. Orig. Life Evol. Biosph. 15, 327–345.

Baymann, F., Lebrun, E., Brugna, M., Schoepp-Cothenet, B., Giudici-Orticoni, M.-T., and Nitschke, W. (2003). The redox protein construction kit; Pre-last universal common ancestor evolution of energy-conserving enzymes. Philos. Trans. R. Soc. Lond. B Biol. Sci. 358, 267–274.

Batista, A.P., Marreiros, B.C., and Pereira, M.M. (2012). The role of proton and sodium ions in energy transduction by respiratory complex I. IUBMB Life 64, 492–498.

Bender, G., Pierce, E., Hill, J.A., Darty, J.E., and Ragsdale, S.W. (2011). Metal centers in the anaerobic microbial metabolism of CO and CO_2. Metallomics 3, 797–815.

Branciamore, S., Gallori, E., Szathmáry, E., and Czárán, T. (2009). The origin of life: chemical evolution of a metabolic system in a mineral honeycomb? J. Mol. Evol. 69, 458–469.

Buckel, W., and Thauer, R.K. (2012). Energy conservation via electron bifurcating ferredoxin reduction and proton/Na^+ translocating ferredoxin oxidation (Biochim. Biophys. Acta). Published online July 15, 2012. http://dx.doi.org/10.1016/j.bbabio.2012.07.002.

Chaves, M.R.M., Valsaraj, K., DeLaune, R.D., Gambrell, R.P., and Buchler, P.M. (2011). Modification of mackinawite with L-cysteine: Synthesis, characterization, and implications to mercury immobilization in sediment. In Sediment Transport, S.S. Ginsberg, ed. (New York: InTech), pp. 313–334.

Chen, K., Bonagura, C.A., Tilley, G.J., McEvoy, J.P., Jung, Y.S., Armstrong, F.A., Stout, C.D., and Burgess, B.K. (2002). Crystal structures of ferredoxin variants exhibiting large changes in [Fe-S] reduction potential. Nat. Struct. Biol. 9, 188–192.

Corrado, M.E., Aliverti, A., Zanetti, G., and Mayhew, S.G. (1996). Analysis of the oxidation-reduction potentials of recombinant ferredoxin-NADP$^+$ reductase from spinach chloroplasts. Eur. J. Biochem. *239*, 662–667.

Eck, R.V., and Dayhoff, M.O. (1966). Evolution of the structure of ferredoxin based on living relics of primitive amino Acid sequences. Science *152*, 363–366.

Ferry, J.G. (2010). How to make a living by exhaling methane. Annu. Rev. Microbiol. *64*, 453–473.

Fuchs, G. (1986). CO_2 fixation in acetogenic bacteria: variations on a theme. FEMS Microbiol. Lett. *39*, 181–213.

Fuchs, G. (2011). Alternative pathways of carbon dioxide fixation: insights into the early evolution of life? Annu. Rev. Microbiol. *65*, 631–658.

Harms, U., Weiss, D.S., Gärtner, P., Linder, D., and Thauer, R.K. (1995). The energy conserving N5-methyltetrahydromethanopterin:coenzyme M methyltransferase complex from *Methanobacterium thermoautotrophicum* is composed of eight different subunits. Eur. J. Biochem. *228*, 640–648.

Harold, F.M. (1986). The Vital Force: A Study of Bioenergetics (New York: W.H. Freeman).

Hedderich, R. (2004). Energy-converting [NiFe] hydrogenases from archaea and extremophiles: ancestors of complex I. J. Bioenerg. Biomembr. *36*, 65–75.

Heinen, W., and Lauwers, A.M. (1996). Organic sulfur compounds resulting from the interaction of iron sulfide, hydrogen sulfide and carbon dioxide in an anaerobic aqueous environment. Orig. Life Evol. Biosph. *26*, 131–150.

Herrmann, G., Jayamani, E., Mai, G., and Buckel, W. (2008). Energy conservation via electron-transferring flavoprotein in anaerobic bacteria. J. Bacteriol. *190*, 784–791.

Joyce, G.F. (2002). The antiquity of RNA-based evolution. Nature *418*, 214–221.

Kaster, A.-K., Moll, J., Parey, K., and Thauer, R.K. (2011). Coupling of ferredoxin and heterodisulfide reduction via electron bifurcation in hydrogenotrophic methanogenic archaea. Proc. Natl. Acad. Sci. USA *108*, 2981–2986.

Kelley, D.S., Karson, J.A., Blackman, D.K., Früh-Green, G.L., Butterfield, D.A., Lilley, M.D., Olson, E.J., Schrenk, M.O., Roe, K.K., Lebon, G.T., and Rivizzigno, P.; AT3-60 Shipboard Party (2001). An off-axis hydrothermal vent field near the Mid-Atlantic Ridge at 30 degrees N. Nature *412*, 145–149.

Kelley, D.S., Baross, J.A., and Delaney, J.R. (2002). Volcanoes, fluids, and life at mid-ocean ridge spreading centers. Annu. Rev. Earth Planet. Sci. *30*, 385–491.

Kelley, D.S., Karson, J.A., Früh-Green, G.L., Yoerger, D.R., Shank, T.M., Butterfield, D.A., Hayes, J.M., Schrenk, M.O., Olson, E.J., Proskurowski, G., et al. (2005). A serpentinite-hosted ecosystem: the Lost City hydrothermal field. Science *307*, 1428–1434.

Koonin, E.V., and Martin, W. (2005). On the origin of genomes and cells within inorganic compartments. Trends Genet. *21*, 647–654.

Lane, N., Allen, J.F., and Martin, W. (2010). How did LUCA make a living? Chemiosmosis in the origin of life. Bioessays *32*, 271–280.

Lang, S.Q., Butterfield, D.A., Schulte, M., Kelley, D.S., and Lilley, M.D. (2010). Elevated concentrations of formate, acetate and dissolved organic carbon found at the Lost City hydrothermal field. Geochim. Cosmochim. Acta *74*, 941–952.

Li, F., Hinderberger, J., Seedorf, H., Zhang, J., Buckel, W., and Thauer, R.K. (2008). Coupled ferredoxin and crotonyl coenzyme A (CoA) reduction with NADH catalyzed by the butyryl-CoA dehydrogenase/Etf complex from *Clostridium kluyveri*. J. Bacteriol. *190*, 843–850.

Maden, B.E.H. (1995). No soup for starters? Autotrophy and the origins of metabolism. Trends Biochem. Sci. *20*, 337–341.

Maden, B.E.H. (2000). Tetrahydrofolate and tetrahydromethanopterin compared: functionally distinct carriers in C1 metabolism. Biochem. J. *350*, 609–629.

Mast, C.B., and Braun, D. (2010). Thermal trap for DNA replication. Phys. Rev. Lett. *104*, 188102.

Marreiros, B.C., Batista, A.P., Duarte, A.M.S., and Pereira, M.M. (2012). A missing link between complex I and group 4 membrane-bound [NiFe] hydrogenases. Biochim. Biophys. Acta. Published online September 19. http://dx.doi.org/10.1016/j.bbabio.2012.09.012.

Martin, W.F. (2012). Hydrogen, metals, bifurcating electrons, and proton gradients: the early evolution of biological energy conservation. FEBS Lett. *586*, 485–493.

Martin, W., and Russell, M.J. (2003). On the origins of cells: a hypothesis for the evolutionary transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from prokaryotes to nucleated cells. Philos. Trans. R. Soc. Lond. B Biol. Sci. *358*, 59–83, discussion 83–85.

Martin, W., and Russell, M.J. (2007). On the origin of biochemistry at an alkaline hydrothermal vent. Philos. Trans. R. Soc. Lond. B Biol. Sci. *362*, 1887–1925.

Martin, W., Baross, J., Kelley, D., and Russell, M.J. (2008). Hydrothermal vents and the origin of life. Nat. Rev. Microbiol. *6*, 805–814.

Miller, S.L., and Bada, J.L. (1988). Submarine hot springs and the origin of life. Nature *334*, 609–611.

Mitchell, P. (1975). Protonmotive redox mechanism of the cytochrome b–c1 complex in the respiratory chain: protonmotive ubiqiuinone cycle. FEBS Lett. *56*, 1–6.

Morowitz, H.J. (1968). Energy Flow in Biology (New York: Academic Press).

Mulkidjanian, A.Y., Makarova, K.S., Galperin, M.Y., and Koonin, E.V. (2007). Inventing the dynamo machine: the evolution of the F-type and V-type ATPases. Nat. Rev. Microbiol. *5*, 892–899.

Mulkidjanian, A.Y., Galperin, M.Y., Makarova, K.S., Wolf, Y.I., and Koonin, E.V. (2008). Evolutionary primacy of sodium bioenergetics. Biol. Direct *3*, 13.

Mulkidjanian, A.Y., Bychkov, A.Y., Dibrova, D.V., Galperin, M.Y., and Koonin, E.V. (2012). Origin of first cells at terrestrial, anoxic geothermal fields. Proc. Natl. Acad. Sci. USA *109*, E821–E830.

Nitschke, W., and Russell, M.J. (2009). Hydrothermal focusing of chemical and chemiosmotic energy, supported by delivery of catalytic Fe, Ni, Mo/W, Co, S and Se, forced life to emerge. J. Mol. Evol. *69*, 481–496.

Phillips, R., and Milo, R. (2009). A feeling for the numbers in biology. Proc. Natl. Acad. Sci. USA *106*, 21465–21471.

Poehlein, A., Schmidt, S., Kaster, A.-K., Goenrich, M., Vollmers, J., Thürmer, A., Bertsch, J., Schuchmann, K., Voigt, B., Hecker, M., et al. (2012). An ancient pathway combining carbon dioxide fixation with the generation and utilization of a sodium ion gradient for ATP synthesis. PLoS ONE *7*, e33439.

Pohorille, A., and Deamer, D.W. (2009). Self-assembly and function of primitive cell membranes. Res. Microbiol. *160*, 449–456.

Proskurowski, G., Lilley, M.D., Seewald, J.S., Früh-Green, G.L., Olson, E.J., Lupton, J.E., Sylva, S.P., and Kelley, D.S. (2008). Abiogenic hydrocarbon production at lost city hydrothermal field. Science *319*, 604–607.

Russell, M.J., and Hall, A.J. (1997). The emergence of life from iron monosulphide bubbles at a submarine hydrothermal redox and pH front. J. Geol. Soc. London *154*, 377–402.

Russell, M.J., and Martin, W. (2004). The rocky roots of the acetyl-CoA pathway. Trends Biochem. Sci. *29*, 358–363.

Russell, M.J., Daniel, R.M., and Hall, A. (1993). On the emergence of life via catalytic iron-sulphide membranes. Terra Nova *5*, 343–347.

Saladino, R., Crestini, C., Pino, S., Costanzo, G., and Di Mauro, E. (2012). Formamide and the origin of life. Phys. Life Rev. *9*, 84–104.

Say, R.F., and Fuchs, G. (2010). Fructose 1,6-bisphosphate aldolase/phosphatase may be an ancestral gluconeogenic enzyme. Nature *464*, 1077–1081.

Schlegel, K., Leone, V., Faraldo-Gómez, J.D., and Müller, V. (2012). Promiscuous archaeal ATP synthase concurrently coupled to Na$^+$ and H$^+$ translocation. Proc. Natl. Acad. Sci. USA *109*, 947–952.

Schuchmann, K., and Müller, V. (2012). A bacterial electron-bifurcating hydrogenase. J. Biol. Chem. *287*, 31165–31171.

Shock, E., and Canovas, P. (2010). The potential for abiotic organic synthesis and biosynthesis at seafloor hydrothermal systems. Geofluids *10*, 161–192.

Shock, E.L., McCollom, T., and Schulte, M.D. (1998). The emergence of metabolism from within hydrothermal systems. In Thermophiles: the Keys to Molecular Evolution and the Origin of Life, J. Wiegel and M.W.W. Adams, eds. (Washington, DC: Taylor and Francis), pp. 59–76.

Sleep, N.H., Meibom, A., Fridriksson, T., Coleman, R.G., and Bird, D.K. (2004). H_2-rich fluids from serpentinization: geochemical and biotic implications. Proc. Natl. Acad. Sci. USA *101*, 12818–12823.

Stetter, K.O. (2006). Hyperthermophiles in the history of life. Philos. Trans. R. Soc. Lond. B Biol. Sci. *361*, 1837–1842, discussion 1842–1843.

Storbeck, S., Rolfes, S., Raux-Deery, E., Warren, M.J., Jahn, D., and Layer, G. (2010). A novel pathway for the biosynthesis of heme in archaea: genome-based bioinformatic predictions and experimental evidence. Archaea. Published online December 13, 2010. http://dx.doi.org/10.1155/2010/175050.

Surín, S., Cubonová, L., Majerník, A.I., McDermott, P., Chong, J.P.J., and Smigán, P. (2007). Isolation and characterization of an amiloride-resistant mutant of *Methanothermobacter thermautotrophicus* possessing a defective Na^+/H^+ antiport. FEMS Microbiol. Lett. *269*, 301–308.

Thauer, R.K., Kaster, A.-K., Seedorf, H., Buckel, W., and Hedderich, R. (2008). Methanogenic archaea: ecologically relevant differences in energy conservation. Nat. Rev. Microbiol. *6*, 579–591.

van de Vossenberg, J.L.C.M., Ubbink-Kok, T., Elferink, M.G.L., Driessen, A.J.M., and Konings, W.N. (1995). Ion permeability of the cytoplasmic membrane limits the maximum growth temperature of bacteria and archaea. Mol. Microbiol. *18*, 925–932.

Wächtershäuser, G. (1988). Pyrite formation, the first energy source for life: a hypothesis. Syst. Appl. Microbiol. *10*, 207–210.

White, R.H. (2004). L-Aspartate semialdehyde and a 6-deoxy-5-ketohexose 1-phosphate are the precursors to the aromatic amino acids in *Methanocaldococcus jannaschii*. Biochemistry *43*, 7618–7627.

Wolthers, M., Charlet, L., van der Linde, P.R., Rickard, S., and van der Weijden, C.H. (2005). Surface chemistry of disordered mackinawite (FeS). Geochim. Cosmochim. Acta *69*, 3469–3481.

Zahnle, K., Arndt, N., Cockell, C., Halliday, A., Nisbet, E., Selsis, F., and Sleep, N.H. (2007). Emergence of a habitable planet. Space Sci. Rev. *129*, 35–78.