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9 Early life

Life is a chemical reaction. All organisms great and small have some form of energy releasing chemical reaction at the core of their living process. Such bioenergetic reactions have a myriad of substrates and products in nature [1, 2] but they all go forward according to the second law of thermodynamics: the conversion of substrates to products, with cell mass as a biological byproduct, releases energy [3]. Some of the energy released can be conserved in chemical form that allows the cell to perform a bit of metabolic work. Life's main energy currency today is adenosine triphosphate (ATP), but there are other "energy-rich" compounds that play an important role in biological energy conservation [4] among them thioesters like acetyl-CoA or reduced ferredoxin [5]. Bioenergetic reactions constitute the main flux of matter and energy through the cell. For an adult human, the core bioenergetic reaction is the oxygen-dependent burning of fats and sugars in our mitochondria. This reaction generates about 1 kg of CO_2 and 0.4 kg of H_2O as end products and about 60–100 kg (roughly a body weight) of ATP per day, per person. For the bacterium *Escherichia coli*, a cell division requires about 60 billion molecules of ATP, corresponding to about 50 body weights of ATP synthesized per cell division [5]. The numbers for an archaeon [6] can be readily calculated from Y_{ATP}, the yield in grams of cell mass per mole ATP consumed, and are very similar to *E. coli* values.

For all forms of life, from archaea and bacteria to humans, when the core bioenergetic reaction stops, so does life. Bioenergetic reactions have been running in a sequence of uninterrupted continuity since the first prokaryotes arose on Earth more than 3.5 billion years ago [7]. During that time, evolution has tinkered mightily with the proteins and cofactors that cells use to harness energy, but not even a force as powerful as evolution can tinker with the second law of thermodynamics. Rather, evolution is the byproduct – the innately creative byproduct – of one long and continuous bioenergetic reaction. When we talk about early life, the title of this chapter, we are basically asking: how did that reaction get started and how did the first cells make a living?

The geochemical record harbors only few and faint traces of the very ancient microbial past that might help to answer such questions or that might bear upon our understanding of the nature of earliest life. Geochemical evidence indicates that methanogenesis goes back about 3.5 billion years [8], approaching the 3.8 billion year old age of the oldest rocks harboring evidence for life [9]. We can be relatively sure that there was no molecular oxygen around at life's origin, because oxygen is a biological product [10]. Beyond that, geological evidence generally tells us much more about the rocks-and-water setting for life's origin than it does about the first

kind of microbes that emerged there [7, 11]. But geochemistry is not the only window we have into the ancient past. The nature of the bioenergetic reactions that the very first microbes used belongs to the subject matter of evolutionary microbiology, so a comparative approach can unearth some insights.

If we want to address the ancestral state of microbial physiology [12], we have to start with a process of elimination, because there are so many possibilities. There are many different kinds of organisms known that use hundreds of different main redox reactions to harness environmentally available energy [1], also among thermophilic habitats [2]. Using common sense and a few simple pruning rules, we can narrow the possibilities.

Biologists have always known that anaerobes are ancient and that anaerobic environments should harbor primitive kinds of bioenergetic reactions [13, 14]. Since life arose in a world without molecular oxygen, the first cells had to be anaerobes. Because eukaryotes arose from a symbiosis of prokaryotes [15–18], the first cells were prokaryotes, not eukaryotes. There are also good reasons to think that biochemistry, and microbial life, started off from CO_2, rather than from some kind of preformed organic soup [19, 20]. Following this reasoning, we can infer that the first organisms were anaerobic, prokaryotic autotrophs. There is nothing new about this approach to the problem of early life, it has a long and robust tradition in biology [13, 14, 21].

We can narrow down the possibilities further. If we accept the reasonable proposition that the deepest branch in the prokaryotic tree of life separates the Bacteria from the Archaea [22–24], the foregoing three criteria place anaerobic autotrophs at the root of the prokaryotic tree. Accordingly, the founder lineages at the base of both the archaeal and the bacterial domains should have the same pathway of CO_2 fixation, namely the one used in by the last universal common ancestor (LUCA). The criterion of CO_2 fixation narrows down the possibilities to a more specific set of candidate ancient lineages for early life. This is because there are at present only six pathways of core CO_2 fixation known [25, 26]: the Calvin cycle, the reductive citric acid cycle, the 3-hydroxypropionate/4-hydroxybutyrate cycle, the dicarboxylate/4-hydroxybutyrate cycle, the 3-hydroxypropionate bi-cycle, and the acetyl-CoA pathway. Yet of those six CO_2 fixation pathways, only the acetyl-CoA – also called the Wood-Ljungdahl (WL) – pathway occurs in both Bacteria and Archaea [25]. For this reason, and for other reasons based on bioenergetic considerations, the simplicity of its chemistry [27, 28], and the prevalence of transition metal catalysis in its main reactions [29], the acetyl-CoA pathway is considered to be the most ancient of the CO_2 fixation pathways [25].

Among the lineages of bacterial and archaeal anaerobes that use the acetyl-CoA pathway, the acetogens and methanogens stand out in particular. This is because in acetogens (Bacteria) and methanogens (Archaea), the acetyl-CoA pathway is not only the main route of CO_2 fixation, it is also integral to energy conservation in that acetogens and methanogens generate their ATP with the help of ion gradients that are generated in the process of reducing CO_2 with electrons from H_2 [30]. The other five pathways of CO_2 fixation do not generate ATP, they consume ATP that is generated via

an independent energy metabolism involving cytochromes and quinones (or quinone analogues). This sets methanogens and acetogens apart from all other autotrophs, as does the circumstance that both among acetogens [31] and among methanogens [6], lineages occur that lack both quinones and cytochromes.

Today, when environmental microbiologists probe ancient anaerobic niches deep in the Earth's crust, they find acetogens (Bacteria) [32–34] and methanogens (Archaea) [35] in addition to sulfate reducers [36], the very same groups that biologists always thought were ancient [13]. Although such anaerobic environments present steep bioenergetic challenges because they harbor so little energy to harness [30, 37, 38], they also are home to the organisms that arguably possess the simplest, and what might be the most primitive, forms of energy metabolism: acetogens and methanogens that lack cytochromes [39].

Given an environmental supply of nitrogen and trace elements, microbial physiology is, at the most basic level, a matter of carbon and energy metabolism. Carbon metabolism points to acetogens and methanogens as ancient and possibly ancestral. What about energy metabolism? Energy metabolism concerns the way(s) in which cells harness environmentally available sources of energy and convert them into chemical forms that are accessible to metabolism. With or without oxygen, life as we know it uses only two basic mechanisms to tap environmentally available energy and harness it as ATP: i) substrate-level phosphorylation and ii) chemiosmotic coupling. Here, critics will be quick to interject "But what about life as we *don't* know it – at the origin of life maybe energy was harnessed differently!" That could be, but for evolutionary biologists the issues are i) how known microbial life forms arose (not ones that we can imagine) and ii) what is the origin of biological energy conversion as it occurs in *life*, the thing that origin of life research is supposed to be explaining. So what are the two mechanisms that cells use to conserve energy?

The first mechanism, substrate level phosphorylation (SLP) is simple: metabolism generates highly reactive phosphate-containing compounds that phosphorylate ADP to make ATP [37, 41]. The energy that is conserved in ATP is then released in a subsequent reaction that can do some chemical work for the cell or allow more sluggish reactions to go forward. There are only a handful of six or eight highly reactive phosphate-containing compounds that are widely used for SLP [37]; they include acyl phosphates like acetyl phosphate, which contains a highly labile ("high energy") mixed anhydride bond. Importantly, the high energy bonds in these highly reactive compounds that fuel SLP are not mined from deposits in the environment. They are generated during conversions of carbon compounds. Their synthesis is driven by environmental sources of chemical energy such as H_2 plus CO_2 (or sugar plus O_2) that are harnessed during synthesis of thermodynamically more favorable end products, like methane and acetate (or water and CO_2).

The second mechanism that cells use to harness energy involves ion gradients and is called chemiosmotic coupling, a mechanism discovered by Peter Mitchell [42]. Here, an exergonic reaction is coupled to the pumping of ions across a membrane

from inside the cell to the outside. The most common ions used for this purpose are protons, rendering the inside of a cell alkaline relative to the outside, but sodium ions are often used in organisms from low energy environments [37]. The energy stored in the ion gradient is then harnessed by a rotor-stator type ATPase to phosphorylate ADP. Even the anaerobic energy misers, methanogens and acetogens, are obligately chemiosmotic. They use an ATPase, but diverge in the mechanism by which they generate their ion gradient [25, 30, 40]. During microbial growth, chemiosmosis and SLP always harness redox energy, the natural tendency of electrons to flow from donors (such as H_2) to acceptors (such as CO_2).

Since Oparin and Haldane [43], scientists long thought that the first free-living cells were pure fermenters, organisms that we know today live from SLP alone. Though all cells use both SLP and chemiosmotic coupling in their overall metabolism (fermenters spend energy gleaned through SLP to make ion gradients for nutrient import), pure fermenters that use SLP as their sole source of energy harnessing are always derived, in the phylogenetic sense, from chemiosmotic forms [44], and fermenters always live from compounds produced by autotrophs, which are always chemiosmotic. That means that whatever the nature of the first free-living cells, they were chemiosmotic – they harnessed ion gradients [45].

But in modern cells, harnessing chemiosmotic potential always requires proteins, posing a chicken-and-egg kind of problem: what kind of energy harnessing allowed genes and proteins to arise? A possibility is that the first prebiotic energy-harnessing reactions involved SLP [41, 46, 47] but that the first free-living cells were chemiosmotic. In that view, life arose where a constant source of reactive carbon compounds was available, but the ability to harness geochemically generated chemiosmotic gradients with the help of proteins was pivotal for the emergence of free-living cells [40]. Where would geochemically generated ion gradients come from? Mike Russell and coworkers suggested that hydrothermal vents could create pH gradients [48–50]. Martin and Russell [50] suggested that those natural pH gradients could have been used by the precursors of the first free-living cells. That is, the ATPase arose in the common ancestor of all prokaryotes, which in turn arose in and inhabited an environment where ion gradients were generated by geochemical processes. That would help explain why the rotor-stator type ATPase is as universal among cells as the ribosome and the genetic code [40, 50], whereas respiratory chains [51, 52] and the many other mechanisms that cells use to generate ion gradients across membranes are as varied as the hundreds of environmental redox couples that modern life forms harness [2].

All this points to two kinds of energy at life's origin: high energy compounds and chemiosmosis. In terms of environments, what on Earth could provide both chemical reactivity and chemiosmotic gradients? Since their discovery, submarine hydrothermal vents have attracted intense interest in this context because they harbor geological manifestations of both kinds of energy that are used by life: chemically reactive compounds [53, 54] and natural proton gradients [48, 49]. In addition, hydrothermal vents reside in the crust and are thus chock full of catalytic transition metals [49, 53].

They furthermore generate vast networks of inorganic microcompartments that i) provide a natural mechanism to concentrate any organic compounds that might have been formed early on, rendering the steep hurdles en route to chemical complexity more readily surmountable [50] and ii) provide a system of naturally formed inorganic territories within which the first replicating systems, once they arose, could have existed and competed in the form of the organic contents of those territories [23].

Indeed, the closer we look at hydrothermal vents, the stronger their similarities with biological energy conversions become [55]. Both chemical reactivity and ion gradients within hydrothermal vents come from the process of serpentinization [56–59]. During serpentinization, seawater circulating through hydrothermal systems reacts with Fe^{2+} in the submarine crust; Fe^{2+} reduces water to H_2, generating up to 50 mM H_2 in vent effluents (and Fe^{3+} in the crust). At the same time, CO_2 is reduced to methane and formate, which occur at 1 mM and 0.1 mM concentrations in the effluent of Lost City (14), a low-temperature hydrothermal vent discovered in 2000 by Deborah Kelly and her colleagues at the University of Washington [60–62]. Serpentinization and its accompanying CO_2 reduction are energy releasing geochemical reactions [58, 59, 63]. And chemiosmosis? The process of serpentinization not only generates a strongly reducing environment at Lost City, it also makes the effluent alkaline [56]. Lost City effluent has a pH of about 10 [58–60, 63], far more alkaline on the inside than ocean water, either now or 4 billion years ago, making these vents naturally chemiosmotic [48, 49]. The natural proton gradients at Lost City are the same in their magnitude and orientation as those in modern autotrophic cells.

A number of ideas about energy-releasing reactions at life's origins have been proposed that have nothing in common with how life actually works. Among such suggestions are pyrite synthesis [64], UV radiation [43], lightning [65], or NiS-based hydrogen generation [66], but none of those processes actually operates in the energy metabolism of modern cells. By contrast, the geochemical synthesis of methane at Lost City [63] and other serpentinizing systems [67], represents a spontaneously occurring geochemical reaction that appears to be homologous to a biological mechanism of energy metabolism: the reduction of CO_2 to methane [55]. That is a strong reason in favour of alkaline hydrothermal vents like Lost City as particularly interesting as models for the origin of early life.

This is especially true when we look closer at the acetyl-CoA pathway in an evolutionary context. The acetyl-CoA pathway consists of two segments: methyl synthesis from H_2 and CO_2 and acetyl synthesis from the methyl moiety and CO [25]. Curiously, the acetyl synthesis segment is highly conserved across the acetogen-methanogen (bacterial-archaeal) divide and entails exclusively transition metals as catalysts. While the methyl synthesis segment is conserved within acetogens and within methanogens, but across the bacterial-archaeal divide the enzymes of the methyl segment are not related, having arisen independently in the common ancestors of the two groups [47]. This circumstance suggests that the acetyl-CoA pathway, while being the most ancient of known CO_2 assimilation pathways, reflects two phases

in early evolution: an ancient phase in a geochemically confined and non-free-living universal common ancestor, in which acetyl thioester synthesis proceeded spontaneously with the help of geochemically supplied methyl groups, and a later phase that reflects the primordial divergence of the bacterial and archaeal stem groups, which independently invented genetically-encoded means to synthesize methyl groups via enzymatic reactions. Sustained spontaneous synthesis of thioesters would be a ready source for acyl phosphates and SLP [41, 46] and it is, at least in principle, possible that processes of that type, as sketched in ▸ Fig. 9.1 **A**, gave rise to genes and proteins [68].

The simplest interpretation of the circumstance that methyl synthesis in acetogens and methanogens differs is that the two ur-lineages of prokaryotes diverged before methyl synthesis had been invented and the pathways arose independently prior to the origin of free-living cells. But the ATPase of bacteria (F-type) and archaea (A-type) is conserved [37], indicating that it was present in their common ancestor as a means of harnessing naturally existing ion gradients and converting their geochemically generated energy into biochemical currency as ATP (▸ Fig. 9.1 **B**). The function of the ATPase required the thickness of a biological lipid bilayer membrane, but those earliest hydrophobic layers need not have been the products of genetically encoded enzymatic pathways, because Fischer-Tropsch type synthesis at hydrothermal vents can generate hydrophobic compounds [58]. Also, membranes are more porous to protons than they are to sodium ions, especially in environments where short chain organic acids abound. Thus, an early event would have been the conversion of the geochemical proton gradient into a sodium gradient, with a simple antiporter [40], as sketched in ▸ Fig. 9.1 **C**. The first ATPases were either sodium-utilizing [69], or more likely promiscuous for protons and sodium, like modern sodium-utilizing ATPases [70]. That leaves as the last step en route to carbon and energy autonomy, the origin of the first pumping reactions. Though some will argue that pumping mechanisms that are dependent upon cytochromes, quinones, and high potential oxidants generated by lightning came first [71], it seems more likely to us that cytochrome-independent pumping mechanisms operating under highly reducing conditions came first. Congruent with our other inferences, so far, there are acetogens and methanogens that have cytochrome-independent pumping mechanisms.

In acetogens that lack cytochromes, *Acetobacterium woodii* [31] serving as an example, ion pumping is achieved with the help of a single protein complex called Rnf [72] that pumps sodium ions from the inside of the cell to the outside in the process of transferring electrons in an exergonic reaction from a low-potential reduced ferredoxin to NAD^+ [73]. The evolution of such a complex would have paved the way to the free-living lifestyle (▸ Fig. 9.1 **E**). Neither cytochromes nor quinones are involved in this pumping. The reduced ferredoxin required at the Rnf reaction is generated with electrons from H_2 with the help of flavin-based electron bifurcation at the iron only hydrogenase used by *A. woodii*: the electron pair of H_2 is split (bifurcated), one electron going energetically uphill to generate the low-potential reduced ferredoxin, the other electron going energetically downhill to NAD^+ [74] so that the overall reaction

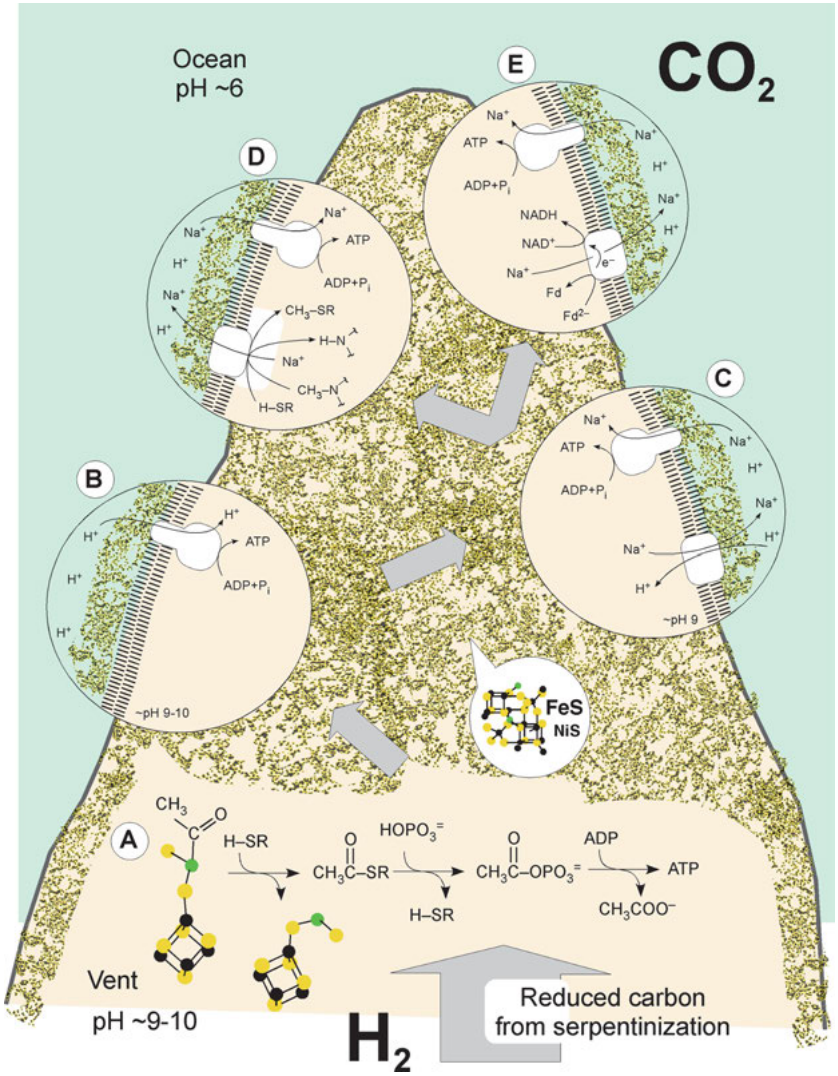

Fig. 9.1. Possible early bioenergetic evolution in an alkaline hydrothermal vent. **A** Substrate-level phosphorylation is thermodynamically feasible under mild hydrothermal conditions [2]. **B** The pH difference between ocean and vent fluids results in a stable, geochemically sustained pH gradient of the polarity and magnitude utilized by modern cells. With the advent of translation and proteins, this could have been harnessed by the universal rotor-stator type ATPase. **C** Membranes are far more permeable to protons than sodium ions. An antiporter, as found in methanogens and acetogens, could convert the geochemical proton gradient into a sodium gradient [40]. The promiscuity of some ATPases for H^+ and Na^+ [70] permits continuity of function. **D**, **E** The origin of biological ion pumping. In primitive methanogens **D**, Na^+ pumping is powered by the exergonic transfer of a methyl group from a nitrogen atom in methanopterin to a sulfur atom in coenzyme M [6]. In primitive acetogens **E**, Na^+ pumping is powered by the exergonic transfer of electrons from iron-sulfur clusters in ferredoxin to NAD^+ [73]. Pumping reactions of this type involve simple chemicals and might be ancient.

is energetically favorable. The NADH generated at the hydrogenase and Rnf steps is reoxidized in the methyl synthesis branch of the acetyl-CoA pathway [30]. In acetogen energy metabolism, the methyl group is excreted as acetate, the end product of energy metabolism.

In methanogens that lack cytochromes, *Methanobacterium marburgensis* being a well-studied example [75], ion pumping is achieved at the MtrA-H methyl transferase complex. The evolution of such a complex would have paved the way to the free-living lifestyle (▸ Fig. 9.1 **D**). MtrA-H catalyzes the exergonic transfer of a methyl group from a nitrogen atom in methyltetrahydromethanopterin to a sulfur atom in coenzyme M [6], the free energy is harnessed by the complex to pump a pair of sodium ions. The resulting methyl-CoM is substrate for the reaction catalyzed by methyl-CoM reductase, which releases methane, the end product of energy metabolism. As with acetogens, neither cytochromes nor quinones (nor quinone analogues like methanophenazine) are involved in the pumping process. In order to synthesize the methyl group, *M. marburgensis* requires low potential reduced ferredoxins. As in the case of *A. woodii*, these are generated from H_2 in a reaction that involves flavin-based electron bifurcation: the electron pair in H_2 is split at a flavin dependent step, one electron going energetically uphill to ferredoxin, the other electron going energetically downhill to the reaction catalysed by heterodisulfide reductase, so that the overall reaction is energetically favorable [75]. Chemically, the sole ion pumping reaction of methanogens that lack cytochromes is a very simple reaction and one that appears to be extremely ancient; it has the attributes of a relic from a phase in early evolution where geochemically synthesized C1 moieties were environmentally available [47].

Metabolism in acetogens and methanogens is furthermore replete with reactions catalyzed by transition metals, such as iron, nickel, molybdenum, or tungsten, another ancient trait that links these groups with geochemical settings where methane and formate, at least, are still being generated today [58, 59, 76].

In talking about early life, we can distinguish three phases. In the first phase, which we can call the generative phase, some spontaneous energy-releasing reaction was going on somewhere in the environment, and this reaction led to the accumulation of reduced carbon compounds, the building blocks of life. This reaction fostered and financed, in the energetic sense, the synthesis of molecular constituents and their organization into the first free-living cells. In the second phase, which we can call emergence, there were free-living cells that were able to foster, by virtue of genetic instructions, their own organization into likenesses of themselves, and energetically finance that organization by tapping environmentally available energy sources with the help of chemical tools that they synthesized by themselves from CO_2, electrons, and nitrogen. That is a way of saying that in early bioenergetic evolution, carbon and energy conversion using only inorganic or spontaneously-formed catalysts was followed by energy conversion using proteins [47]. In the third phase of early life, free-living cells began to diversify and harness environmentally available redox couples.

If acetogens and methanogens are the founders of the bacterial and archaeal lineages, what would be the next evolutionary steps after the origin of free-living cells? The evolution of cytochromes, quinones, and sulfur reduction as manifest in both high and low cytochrome *c*-containing sulfate reducers [77, 78] would be one possible next step [77], not only because they are strict anaerobes [13], but also because many of them can grow autotrophically, using the acetyl-CoA pathway for carbon metabolism and sulfur reduction for energy metabolism. Moreover, the similarity of subunits of the dissimilatory sulfite reductase module (DsrMK) and the quinone membrane-bound oxidoreductase complex (QmoABC) with key enzymes involved in the last step of methanogenesis tightens their close relationship. DsrMK and QmoABC are highly conserved complexes among sulfate reducers and both interact with quinones that have heme *b* in their membranar subunit [77]. Nevertheless, the cytoplasmatic DsrK subunit is homologous to the catalytic HdrD subunit of membranar heterodisulfide reductase HdrED present in methanogens [79] while the soluble QmoA and B subunits have homology with the soluble heterodisulfide reductase subunit HdrA [77]. However it is not yet clear whether archaea and bacteria independently evolved sulfate reduction pathways, or whether one lineage evolved the pathway with subsequent distribution to other lineages. Once standard bioenergetic electron transport chains using cytochromes and quinones were in place, then the evolution of electron accepting complexes, like complex I [52], and various terminal oxidases would follow [80, 81].

It has been suggested that electrochemical gradients at the vent-ocean interface might have been required for early CO_2 reduction [40], but Schuchmann and Müller [82] recently characterized a soluble molybdoenzyme with several FeS clusters from *A. woodii* that reduces CO_2 with H_2 to formate: a hydrogen-dependent carbon dioxide reductase [83]. This is a clear biological demonstration that the concerns expressed by some that there might not be enough energy in H_2 to reduce CO_2 [84, 85] are not founded. The enzymatic reaction shows that CO_2 reduction with H_2 is a matter of limited catalysis, not limited free energy release.

What do phylogenetics say about these issues? Newer trees of early evolution show the archaeal component of the eukaryotes (i.e. information processing systems) branching within the archaea, not as their sisters [17, 86–89]. Furthermore, although those studies – with the exception of one [86] – were aimed specifically at determining the position of the archaeal component of eukaryotes among the archaea, they had to employ bacterial outgroups in order to root the trees. If we look at the position of the bacterial root within the archaeal tree in those analyses, what we see is the nature of the most ancient archaeal lineages emerging from those analyses: methanogens are very close to the root [88], methanogens are on the first branch emerging from the root [17], or the archaeal tree roots within the methanogens, specifically within the hydrogenotrophic forms [86, 87] that lack cytochromes and quinones, as some views of early microbial evolution would have it [13, 39, 40, 46]. Despite many uncertainties [90], the foregoing is one possible route that early life might have taken.

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